Abstracts of the 2016 TTS Congress August 18-23, 2016, Hongkong

320.1

Potential kidney donors with asymptomatic microscopic hematuria: histopathological findings & outcomes

Ehab Hammad, Aleid Ahassan, Tariq Ali, Ibrahim Aehab, Almanea Hadeel.
Kidney & Pancreas Transplantation, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia.

Background: Microscopic hematuria is not uncommon in potential kidney donors. After excluding urological causes, most of these donors require kidney biopsy to exclude glomerular lesions. We evaluated biopsy findings among donors with isolated microscopic hematuria and assessed short-term outcomes of their prospective recipients.
Methods: kidney donors between January 2010 and January 2015 who had microscopic hematuria which was not due to urological causes were included in the study. All of them underwent native kidney biopsies which were examined by light microscopy, immunofluorescence and electron microscopy. Donor characteristics pre-donation were compared between abnormal biopsy and normal biopsy groups. Short-term outcomes of our donors were compared to 27 age & sex matched non hematuric donors 3 month post donation.
Results: Out of 750 donors, 27donors (3.6%) were found to have isolated microscopic hematuria (IMH) and underwent kidney biopsy. Mean age of these donors was 32.6±8.Nineteen donors had 1+ hematuria, 6 donors had 2 + and 2 donors had 3+ hematuria or more in urine dipstick. All of them had a minimum of 3 red blood cells/high power filed on urine microscopy. Eight (29.6%) biopsies showed histopathological abnormalities of which, thin basement membrane disease (n=6, 22.2%) was the commonest cause followed by IgA nephropathy (n=2, 7.4 %). Donors with abnormal biopsy findings were excluded from donation. The remaining donors (n=19, 70.4%) had no significant pathology and were permitted to donate. Except for 2donors (7.4%) who were excluded due to other medical/recipient related issues. There was no significant difference in short-term outcomes between hematuric donors accepted for donation and non hematuric donors group 3 month post donation. The recipients of kidneys from donors with microscopic hematuria with no histopathological abnormalities had a mean SBP of 121 ± 9.5, mean DBP 74 ±8.5 and mean serum creatinine of (75.6µmol/l±21.3) at three month follow up. Their last urine analysis confirmed absence of hematuria in 6 of them, 3 showed +ve dipstick and microscopic hematuria.
Conclusions: Our study showed that 29.6 % of our donors with isolated microscopic hematuria had abnormal histopathology on renal biopsy. Isolated asymptomatic microscopic hematuria justifies extensive work up including renal biopsy to identify donors who may have underlying renal pathology.

320.2

Comparison of the rate of decline of GFR and 1. 5 and 10 year outcomes of live kidney donors – UK cohort study

Nithya Krishnan[1], Lisa Bradbury[2], Graham Lipkin[3], Neil Raymond[1].
[1]Renal Unit, University Hospitals Coventry & Warwickshire NHS Trust, Coventry, United Kingdom; [2]Statistics, National Health Services Blood & Transplant, Bristol, United Kingdom; [3]Renal Unit, University Hospitals Birmingham, Birmingham, United Kingdom.

It is important to have a better understanding of the long term outcomes and risks of kidney donation. We compared the rate of decline of GFR levels and 1, 5 and 10 year outcomes of live kidney donors. From January 1, 2001 until December 31, 2013 inclusive, all live kidney donors in the U.K, were included in the study. December 31, 2014 was considered the study end, meaning that all patients had at least one year of follow-up. A total of 9750 live donor records were available; out of which 9229 had GFR measured at baseline.
The baseline GFR was divided into 3 groups – 1. Meeting the British transplant guidelines' recommended levels (n= 9047). 2. Up to 5 mls/min/m2 less than the recommended levels (n=121) and 3. More than 5 mls/min/m2 less than the recommended levels (n=52). The mean (M) and standard deviation (SD) of the rate of decline of GFR (mls/min/m2) in years 1, 5 & 10 for group 1, 2 and 3 are 35.37 (15.21), 7.65 (3.6) and 4.34(2.02) ; 19.80 (11.36). 3.03 (1.8) 1.46 (0.63); 6.41(22.58). 0.46 (5.62), 0.75 (0.21) respectively. Using 1 year GFR as baseline the rate of change was +0.63 (2.55) in 5 and -0.07 (1.73) at 10 years. Morbidity outcomes at years 1, 5 and 10 were not significantly different between the 3 groups . None of the 173 donors in groups 2 & 3 had died during the 10 year follow-up period in comparison to 48 in Group 1.
Analysing by dividing group 1 into 10 year age bands from <30 to 70+, showed a decline in GFR of 34.4(17.9) to 25(13.4) in 1, 7.1(3.7) to 6.4(2.5) in 5 and 4.1(2) to 3.9 (0.8) in 10 years. Using GFR at year 1 as baseline, showed an increase of 0.9(3) to 0.8 (2.2) in 5 and a very minimal decline about in 10 years. Adjusted Multiple linear logistic regression analysis showed that that females had a lower rate of decline at 1 year, -0.83 (-1.49 to 0.18), P=0.013. but the effect was not significant at year 5 or 10.
Thus, there is a significant decline in GFR at 1 year, more so for men. However, the rate of decline after 1 year is almost similar to that of the general population. Though the rate of decline is lesser in lower baseline groups the numbers are too small to draw definitive conclusions.

320.3

Acute operative complications and 1, 5, & 10 year outcomes in living donors by type of live donor nephrectomy

Nithya Krishnan[1], Lisa Bradbury[2], Neil Raymond[1].
[1]Renal Unit, University Hospitals Coventry & Warwickshire NHS Trust, Coventry, United Kingdom; [2]Statistics, National Health Services Blood & Transplant, Bristol, United Kingdom.

Introduction: Living kidney donation has significantly improved recipient and graft survival world wide. With a move to increase these numbers further, it becomes mandatory to have a better understanding of the short and long term outcomes and risks of kidney donation.
Aim: To investigate occurrence of acute operative complications and 1,5,& 10 year outcomes in living donors by type of nephrectomy
Methods: National Health Service and Blood and Transplant, U.K (NHSBT), obtains informed consent from all patients undergoing a transplant in the UK for continuing data collection and subsequent analyses. The study protocol was reviewed and passed by the Renal Registry (RR) projects advisory group, UK. From January 1, 2001 until December 31, 2013 inclusive, all live kidney donors were included in the study. No formal sample size estimate was produced for the study; all eligible patients records were used. December 31, 2014 was considered the study end, meaning that all patients had at least one year of follow-up. Datasets, based on regular returns from individual transplant centres across the UK, were obtained from NHSBT.
Results: A total of 9750 live donor records were available. Nephrectomy type was available for 9602 donors; Open 3132 (33%), Pure laproscopic - 3886 (38%) and hand assisted laproscopic– 2802 (29%). We analysed the incidence of operative complications; splenectomy, reoperation required, organ perforation, operative haemorrhage, pneumonia, pneumothorax, pulmonary embolism, wound infection, DVT, other complications and a combined variable for any one or more complication.
Statistically significant differences was were noted for the incidence of reoperation (41/2794, P=0.018), organ perforation (44/2802, P=0.032), wound Infection (60/ 2796, P=0.018), other complication (323/2793, P<0.0001), any one or more complications (402/2802 P<0.0001) in the hand assisted laproscopic group when compared to the other 2 groups. Incidence of operative haemorrhage (64/3122, P<0.0001) and pneumothorax (46/3130, P<0.0001) were higher in open nephrectomies. Pure laproscopic nephrectomy yielded the least complications.
One, 5 and 10 year outcomes were analysed using logistic regression methods to facilitate adjustment for donor age, gender and operative complications. At one year, there was significantly more wound pain in the open group in comparison to both the laparoscopic groups P=0.001. Incisional and operation related hernia was more in the hand assisted laparoscopic group P=0.048. Other operation related conditions was more in the laparoscopic groups, especially pure, compared to the open group P=0.024. At five years (n= 6011) wound pain remained significant with more wound pain in open nephrectomy group: 9/2814 vs. 0/ 2282 P=0.007. At 10 years (n=1863) there were only 2 donors with wound pain, both in the open group. Also all laparoscopic procedures were “pure”, as the hand assisted procedures only started in 2007 in the U.K.
Conclusions: Though there were small numbers of complications overall, significant differences were identified with most complications seen in hand assisted laproscopic nephrectomies and least in pure laproscopic operations. Wound pain remained significant in long term outcomes and was mainly related to open nephrectomy.

320.4

The Percentage of CD19 positive cells in lymphocytes can predict acute antibody-mediated rejection after administration of rituximab in ABO-incompatible kidney transplantation

Takayuki Hirose, Daiki Iwami, Hajime Sasaki, Haruka Higuchi, Ken Morita, Nobuo Shinohara.
Renal and Genitourinary Surgery, Hokkaido University, Sapporo, Japan.

Introduction: Administration of rituximab to ABO-incompatible kidney transplant recipients has been recognized as effective and safe. Although a single dose of rituximab at 375 mg/m^2 is known to lead to B-cell depletion, the dosage of immunosuppressants should be reduced because it may cause excessive immunosuppression and the risk of infection[1],[2]. However, the appropriate dosage and whether the B-cell population should be monitored after administration remain to be determined.
Materials and Methods: Forty-four consecutive ABO-incompatible kidney recipients (28 men and 16 women) were enrolled in this retrospective study. They were treated with rituximab (65–400 mg/body) 2 to 4 weeks before transplantation, followed by plasmapheresis and intravenous immunoglobulin as desensitization therapy. They were given basiliximab as induction therapy and maintained with triple immunosuppressants. The mean age at transplantation was 50.3 ± 15.4 years, and the mean pretransplant dialysis period was 2.8 ± 3.8 years. The percentage of CD19-positive cells in lymphocytes (%CD19) and CD19-positive cell count after administration of rituximab were examined just before transplantation. These recipients were divided into 2 groups according to pretransplant %CD19 as follows: low %CD19 group, <1% (n = 34) and high %CD19 group, ≥1% (n = 10). The relationship between %CD19 and the incidence of acute antibody-mediated rejection (AAMR) was evaluated.
Results: AAMR was observed in 6 recipients whose pretransplant %CD19 were significantly higher than that of recipients without AAMR (3.1% vs 0.6%; $p = 0.004$). Neither CD19$^+$ cell count nor rituximab dosage significantly changed according to AAMR incidence. The incidence of AAMR was significantly higher in the high %CD19 group than in the low %CD19 group (40.0% vs 5.9%, $p = 0.006$). One of 2 recipients who developed AAMR in the low %CD19 group had not been treated with plasmapheresis because of low anti-ABO IgG titer before desensitization. Serum creatinine level was significantly higher in the high %CD19 group than in the low %CD19 group (1.64 ± 0.16 mg/dL vs 1.22 ± 0.06 mg/dL, $p = 0.005$), although all rejection cases were resolved by anti-rejection therapy and no graft loss occurred.

	High %CD19 (n=10)	Low %CD19 (n=34)	*p* value
AMR (%; n)	40.0 % (n=4)	5.9% (n=2)	0.006 *
S-Cr (mg/dl)	1.64 ± 0.16	1.22 ± 0.06	0.005 †

Table. Comparison of graft outcome between high %CD19 group and low %CD19 group
High %CD19 group: %CD19 of lymphocytes ≥1%, low %CD19 group: %CD19 <1%
*: Chi-square, †: *t*-test

Conclusion: High levels of %CD19 after rituximab administration in ABO-incompatible kidney transplant recipients may indicate insufficient depletion of B cells that can lead to AAMR. Measurement of %CD19 may be useful identifying patients who need additional administration of rituximab.
References:
[1] Vo A, Pharm.D. P., Lukovsky M, et al. Rituximab and Intravenous Immune Globulin for Desensitization during Renal Transplantation. *New Engl J Medicine* 2008;359.
[2] Toki D, Ishida H, Horita S, et al. Impact of low-dose rituximab on splenic B cellsin ABO-incompatible renal transplant recipients. *Transplantation International* 2009;447-454

320.5

ABO incompatible renal transplantation without splenectomy or anti-CD20 treatment: Comparison with ABO compatible renal transplant

Amit Sharma[1], Harinderjit S.Gill[1], Apra Kalra[2], Priyadarshi Ranjan[3], Divya Singh[4], Rupinderjit Kaur[1], Monika Rana[1], Neha Bahari[1].
[1]Nephrology, Dialysis and Kidney Transplant, Fortis Hospital, Mohali, Punjab, India; [2]Transfusion Medicine, Fortis Hospital, Mohali, Punjab, India; [3]Urology and Kidney Transplant Surgery, Fortis Hospital, Mohali, Punjab, India; [4]Radiodiagnosis, Prime Diagnostic and Heart Institute, Chandigarh, India.

Introduction: ABO-incompatible (ABOi) renal transplant has diminished the gap between demand and supply of kidneys for patients with end stage renal disease (ESRD). It involves preconditioning the patient by lowering the isoagglutinin titers and preventing further rise. The study was conducted to assess the feasibility of performing ABOi renal transplant without the use of Anti CD20 antibody or splenectomy and comparison of outcome with ABO compatible (ABOc) transplants.
Methods: This study was conducted from January 2014 to August 2015. A total of 116 patients were divided into two groups based on ABOi or ABOc donors. Patients received Tacrolimus, Mycophenolate mofetil and Prednisolone, started 10 days (ABOi) and 2 days (ABOc) prior to transplant. Plasmapheresis and intravenous immunoglobulin was given until isoagglutinin titer <1:4 was obtained on the date of transplantation in ABOi cases. All patients received induction immunosuppression: Thymoglobulin (1 mg/kg/day for 3 days) for ABOi and ABOc with HLA mismatch >3/6 and Basiliximab 20 mg (2 doses) in remaining. In ABOi transplant, isoagglutinin titers were regularly monitored postoperatively and plasmapheresis was done preemptively if they increased to >1:8 in first week post transplant and >1:16 in 2nd and 3rd week post transplant). Patient and allograft survival; 1-, 3-, 6-, 9-, 12-, and 18-month renal function; infectious complications; and incidence of rejection were evaluated.
Results: 75% of the ABOi and 81% of ABOc recipients were males. Mean±Standard Deviation (SD) age of the ABOi recipients was 40.12±11.59 years (39.79±14.41 years in ABOc). Median isoagglutinin titer at start was 1:32 (1:16 to 1:256). Mean plasmapheresis required were 3. In ABOi group, Mean±SD creatinine levels were 1.26±0.29 mg/dL at 6 months, 1.34±0.37 mg/dL at 12 months and 1.36±0.33 mg/dL at 18 months (1.24±0.36 mg/dL, 1.21±0.33 mg/dL and 1.23±0.34 mg/dL respectively in ABOc group).

Table 1: Patient Characteristics of ABO incompatible (ABOi) and compatible (ABOc) transplant groups

		ABOi (n=16)	ABOc (n=100)
Donor	Age (Mean ±SD) in years	48.25 ± 13.14	48.9 ± 11.73
	Females	43.75%	61%
Recipient	Age (Mean ±SD) in years	40.12 ± 11.59	39.79 ± 14.41
	Females	25%	19%
S. Creatinine (Mean ±SD) mg%	At discharge	1.37 ± .46	1.33 ± .48
	At 1 month	1.25 ± .30	1.26 ± .35
	At 3 month	1.26 ± .30	1.24 ± .35
	At 6 month	1.26 ± .29	1.24 ± .36
	At 9 month	1.34 ± .37	1.21 ± .33
	At 12 month	1.36 ± .33	1.23 ± .34
	At 18 month	1.47 ± .34	1.26 ± .35
Graft Survival At 1 year		87.5%	97%
Patient survival At 1 year		87.5%	97%
Rejection episodes	AMR	12.5% (2/16)	0%
	ACR	6.25% (1/16)	1%
Infections	UTI in first 12 weeks	0%	9%
	Parvovirus	0%	2%
	Fungal Septicemia	12.5% (2/16)	2%
	CMV	0%	2%

Discussion: No significant difference between post transplant serum creatinine observed in the 2 groups. In ABOi group, 3 episodes of antibody mediated rejection (AMR) & 1 acute cellular rejection (ACR) occured which were successfully treated. No AMR seen in ABOc group. Two patients succumbed to fungal sepsis in th ABOi group.
Conclusions: This study suggests that successful ABOi renal transplantation is possible without the use of splenectomy or Anti-CD20 treatment but AMR episodes as well as fungal sepsis are significantly high in the ABOi group.
References:
[1] Flint SM, Walker RG, Hogan C, et al. Successful ABO-Incompatible Kidney Transplantation with Antibody Removal and Standard Immunosuppression. Am J Transplant 2011;11:1016-24.
[2] Segev DL, Simpkins CE, Warren DS, et al. ABO Incompatible High-Titer Renal Transplantation without Splenectomy or Anti-CD20 Treatment. Am J Transplant 2005;5:2570-5.

320.6

HLA identical renal transplants. Immunologically privileged why do they fail?

S. Adibul Hasan Rizvi[1], Muhammad Mubarak[2], Tahir Aziz[3], Ejaz Ahmed[3], Fazal Akhtar[3], Mirza Naqi Zafar[2], Anwar Naqvi[1].
[1]Urology and Transplantation, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [2]Pathology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [3]Nephrology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan.

Objectives: HLA identical transplant are immunologically privileged and thus have the best graft survival in both deceased and living donor transplants. Superior survival yet the graft fail. This paper describes the causes of graft failure in HLA identical transplants in the getting of a develop country.
Patient and Methods: Between 1992 and 2012, 3749 live related transplant were performed at our center. Of these 3749, 756 (20%) were HLA identical transplants. Immunosuppression was by triple drug regimen CyA/AZA or Tacrolimus/MMF with steroid. All dysfunction were confirmed by biopsy. Each recipient is followed life long where all treatment is provided free including immunosuppression drugs.
Result: In the follow-up period of 1 to 20 years, 160 (21%) of the 756 identical graft were lost with 5, 10, 15 years survival rates of 85%, 68% and 60% respectively. A comparison of those who lost grafts (Group A) (n=160) vs those who maintained function (Group B) (n=596) showed that in Group A donors were older 35±9 vs 32±9 (p0.001), more female 46% vs 33% (p0.031), more recipients were female 25% vs 18% (p0.03) and hypertensive 60% vs 49% (<p0.04), acute rejection rates were higher 13% vs 4.8% (p0.001), increased cyclosporine toxicity in biopsy 14% vs 11% (p0.20) and high recurrence of original disease 9% vs 4% (p0.03) as compared to Group B. Of the 160 grafts lost 24% were functioning but lost due to death of recipient, 7% lost due to recurrence, 7% lost due to infection in the graft, 6% lost due to acute rejection and 50% were lost due to chronic rejection.
Conclusion: HLA identical grafts have superior survival due to immunological privilege. Unfortunately 30% grafts are lost due to infection and 7% due to recurrence of disease. Old age of donors, female gender and hypertension and cyclosporine toxicity are the other causes leading to chronic graft loss.

320.7

Retroperitoneal versus intraperitoneal hand assisted laparoscopic donor nephrectomy: a case matched study

Zubir Ahmed, Pankaj Chandak, Raphael Uwechue, Nicos Kessaris, NIzam Mamode.
Transplantation, Guy's Hospital, London, United Kingdom.

Introduction: Although hand assisted laparoscopic donor nephrectomy has become a standard of care for living kidney procurement in most centres it is not clear if a retroperitoneal approach offers any advantage over an intraperitoneal approach. Recent RCT data has been published on the subject but the results have been biased by small patient numbers and significant demographic and operative heterogeneity between groups. We therefore conducted a case matched study of retroperitoneal versus intraperitoneal HALDN.
Methods: 61 retroperitoneal HALDNs were matched with 2:1 with 122 intraperitoneal HALDNs for age, sex, laterality, multiplicity of arteries and obesity status (BMI > 30) using a propensity matching score approach using Stata SE 12. Outcomes assessed were Clavien grade 2, 3 and 4 complications, infective episodes, readmission, need for reoperation, postoperative hospital stay, creatinine change at Day 30 and development of incisional hernia.
Results: The mean age of the cohort was 44.1 years (SD11) with 52.5% female. The mean length of surgery was greater in the retroperitoneal cohort (225 mins v 200 mins, p=0.001). Hospital stay (4.2 v 3.9 days p=0.11) and readmission rates (9.1 v 9.6% p=0.12) were similar. Incidence of postoperative surgical site infection (9.9% v 10.1% p=0.1) and the occurrence of minor complications (Clavien 2) were also equivalent (16.3 v 17.3% p=0.45). The abdominal reoperation rate was lower in the retroperitoneal group (1.6% v 3.3% p=0.009) as was the rate of incisional hernia development at one year (0% v 5% p=0.08). Day 2 peak CRP levels were less in the retroperitoneal group (111 v 123 p=0.035). Creatinine rise at day 30 was also similar (+34 v +37 p =0.35).
Discussion: Retroperitoneal HALDN has similar minor complication outcomes compared to the intraperitoneal approach with similar lengths of hospital stay and readmission rates. However there is evidence from this study that it may exhibit a reduced systemic inflammatory response (as measured by Day 2 CRP) and also reduce more serious surgical complications and thereby reduce the need for reoperation.

320.8

Different sensitivity of rituximab-treatment to B-cells between ABO-incompatible kidney and liver transplantation

Asuka Tanaka, Hiroshi Morimoto, Kentaro Ide, Yuka Tanaka, Kohei Ishiyama, Masahiro Ohira, Hiroyuki Tahara, Hideki Ohdan.
Department of Surgery, Division of Frontier Medical Science, Programs for Biomedical Research, Hiroshima University, Hiroshima, Japan.

The use of ABO-incompatible (ABO-I) donor organs is a possible solution for the shortage of donor organs for transplantation; however, naturally occurring antibodies (Abs) against blood group A or B (A/B) carbohydrate determinants in sera are a major impediment to achieving successful transplantation. Therefore, in ABO-I transplant, plasmapheresis or plasma exchange, splenectomy, and/or anti-B-cell immunosuppressant treatment are widely adopted and improved graft and patient survivals to the level comparable to ABO-compatible (ABO-C). In addition to these modalities, the prophylactic use of rituximab, a monoclonal chimeric human-murine anti-CD20 Ab that depletes B-cells by complement-dependent cytotoxicity (CDC), Ab-dependent cell-mediated cytotoxicity (ADCC), and stimulation of apoptosis, is currently indispensable to achieving successful ABO-I KT and LT. A desensitization protocol with rituximab is currently widely used for kidney transplantation (KT) and liver transplantation (LT) across the ABO-I barrier. However, it remains to be elucidated whether rituximab is equally effective for B-cell and T-cell immune responses in both KT and LT recipients. To clarify these effects of rituximab, we enrolled 46 KT recipients (18 ABO-I recipients 28 ABO-C recipients) and 77 LT recipients (14 ABO-I recipients ABO-C recipients) in this study. Both ABO-I KT and LT recipients were preconditioned prior to surgery with a common desensitization protocol that consisted of a single dose of rituximab and subsequent daily internal use of calcineurin inhibitor (CNI) and mycophenolate mofetil (MMF). The basic immunosuppressive regimen after KT comprised CsA, MMF, and methylprednisolone (MP) with gradually tapering doses, and Basiliximab was administered at the time of transplantation and on postoperative day 4. After LT, the basic immunosuppressive regimen comprised TAC and MP at gradually tapering doses. Only in ABO-I LT, MMF is added to the regimen. The proportion of peripheral blood B-cells was determined at the perioperative period. T-cell responses to allo stimulation were evaluated by a mixed lymphocyte reaction (MLR) assay. One week after rituximab administration, the proportion of peripheral B-cells in all of the ABO-I KT recipients decreased below 0.1%. In contrast, that in some of the ABO-I LT recipients remained above 0.1% at this time point. Eventually, B-cells were undetectable in both groups by week 2. And, B-cells remained below the detection limit throughout the first year in the ABO-I KT recipients, whereas they reappeared in the periphery after 6 months in the ABO-I LT recipients. Although the difference was not statistically significant, during the first year after transplant, alloreactive T-cell responses tended to be lower than those in the ABO-C group.
In conclusion,this study indicates that rituximab has differing B-cell sensitivity between KT and LT recipients and a minimal effect on the alloreactive T-cell responses in KT and LT recipients.

320.9

Immunosuppressive myeloid cells in patients given combined kidney and hematopoietic cell transplants in a tolerance protocol with TLI and ATG conditioning

Kent Jensen[1], John Scandling[3], Stephan Busque[2], Judy Shizuru[4], Robert Lowsky[4], Ani Saraswathula[1], Kevin Sheehan[4], Asha Shori[2], Edgar Engleman[5], Samuel Strober[1].
[1]Medicine - Immunology & Rheumatology, Stanford University School of Medicine, Stanford, CA, United States; [2]Medicine - Surgery, Stanford University School of Medicine, Stanford, CA, United States; [3]Medicine - Nephrology, Stanford University School of Medicine, Stanford, CA, United States; [4]Medicine - Blood and Marrow Transplantation, Stanford University School of Medicine, Stanford, CA, United States; [5]Medicine - Pathology, Stanford University School of Medicine, Stanford, CA, United States.

In preclinical murine models of mixed chimerism and tolerance induction, combined organ and hematopoietic cell transplantation using total lymphoid irradiation (TLI) and anti-thymocyte globulin (ATG) conditioning results in changes in the percentages of innate and adaptive immune cells. These changes include an increase in CD11b+Gr-1+MHCII- myeloid derived suppressor cells (MDSCs), and CD8+CD11c+MHCII+ dendritic cells (DCs), as well as a decrease in T and B cells. Murine MDSCs and DCs exhibited increased production of arginase-1 and IDO respectively, suppressed the MLR, and were required for induction of tolerance. Here, we studied the changes in myeloid cells in the blood of 10 patients given combined HLA matched or mismatched kidney and hematopoietic cell transplants following TLI and ATG conditioning. We found that in the first 4 months post transplant, there was a profound depletion of T cells in the blood of HLA matched and mismatched patients but no significant change in the absolute cell number of Lin-CD11bhiDRlo MDSCs, CD11bhiCD14+DR+monocytes, or CD11bloCD14-DR+ DCs. The ratio of myeloid to T cells significantly changed to favor the myeloid cells during the first four months post transplant, but returned to pre-transplant values between 6 to 12 months post transplant. Similar changes were found in patients regardless of whether they developed mixed chimerism. In addition, the myeloid cells developed a significantly increased ability to suppress the activation and proliferation of autologous, purified, anti-CD3/-CD28 microbead-stimulated, pre-transplant T cells when co-cultured in vitro. The suppression was most pronounced when pre-transplant versus early (1.5-4 month) post transplant MDSCs were compared. MDSC suppression was associated with increased production of arginase-1. The suppressive capacity of the myeloid cells returned to pre-transplant values 6 to 12 months post transplant. Subsets of DCs were studied to compare the percentages of pre-transplant myeloid versus plasmacytoid DCs among all DCs to percentages measured at serial time points through 24 months post transplant. Since the balance of myeloid DC subsets changed in the preclinical model following transplantation to favor the CD8+ DC subset, we also determined the percentages of myeloid DC subsets (CD16+, CD1c+ and CD141+). There were no significant changes in the percentage of myeloid versus plasmacytoid DCs or among subsets of myeloid DCs after transplantation in our patients. In conclusion, there was a transient change in the balance of T cells and myeloid cells in the blood of our patients following transplantation to favor the myeloid cells. The latter cells developed immune suppressive activity that promotes tolerance to the transplanted kidney in a manner that mirror our preclinical murine studies.

320.10

Treatment of large plasma volumes using specific immunoadsorption to desensitize ABO-incompatible kidney-transplant candidates

Lionel Rostaing[1,5,6], Asma Allal[1], Arnaud Del Bello[1], Federico Sallusto[2], Laure Esposito[1], Nicolas Doumerc[2], Bénédicte Debiol[3], Audrey Delas[4], Xavier Game[2,6], Nassim Kamar[1,6,7].
[1]Néphrologie, Dialyse et Transplantation d'Organes, CHU Rangueil, Toulouse, France; [2]Urologie, Andrologie et Transplantation d'Organes , CHU Rangueil, Toulouse, France; [3]Etablissement Français du Sang de Midi-Pyrénées, CHU Purpan, Toulouse, France; [4]Laboratoire d'Histologie, CHU Rangueil, Toulouse, France; [5]INSERM U563 - IFR -BMT , CHU Purpan, Toulouse, France; [6]Université Toulouse III - Paul Sabatier, Faculté de Médecine de Rangueil , Toulouse, France; [7]INSERM U1043 - IFR-BMT, CHU Purpan, Toulouse, France.

Background: ABO-incompatible (ABOi) kidney transplantation has very good long-term results, i.e., similar to those observed for living-kidney ABO-compatible transplantation. This is because patients are desensitized at pretransplant using apheresis and rituximab therapy, associated with tacrolimus-based immunosuppression.
Methods: This prospective single-center study evaluated 12 consecutive patients (6 males), aged 40 (23–59) years. Incompatibilities were A into 0 (8), B into 0 (3), and AB into 0 (1). Efficacy of a single, pre-transplant (Day –1), specific immunoadsorption session was evaluated using Glycosorb® columns (anti-A or anti-B; Glycorex Sweden) to treat large volumes of plasma, i.e., up to 18 L. Pretransplant desensitization relied on rituximab (D –30), tacrolimus, mycophenolic acid, and steroids (all started on D –13), and a single session of specific immunoadsorption on D –1. For every patient, immunoadsorption was coupled in tandem with a hemodialysis session.
Results: Overall, 15 L (11–18) of plasma were treated per patient, i.e., 0.2 (0.11–0.36 L/kg). Isoagglutinin titers were 1/16 (1/5–1/64) before the procedure, decreasing after 6 hours to 1/5 (1/1–1/16; p=0.008), and to 1/2 (1/1–1/8; p=0.05) at completion of the session. The next day, i.e., the day of transplantation, there was no rebound of isoagglutinins [1/4 (1/1–1/5); p=ns]. The procedure was well tolerated with no side effects and no significant changes in hemoglobin level, platelet counts, fibrinogen, or albumin levels.
Conclusion: For ABOi kidney transplantation, a single, longer, specific immunoadsorption session was very efficient at 1 day pre-transplantation with no rebound. However, these results should be confirmed when isoagglutinin titers are higher (≥120).

320.11

"Paired Kidney Exchange" program – single Czech institution experience with 50 paired transplants since 2011

Jiri Fronek[1], Libor Janousek[1], Renata Zamecnikova[1], Pavel Chromy[3], Ondrej Viklicky[2], Tomas Marada[1].
[1]Transplant Surgery, IKEM, Prague, Czech Republic; [2]Department of Nephrology, IKEM, Prague, Czech Republic; [3]IT Department, IKEM, Prague, Czech Republic.

Introduction: Kidney paired donation has been at first performed at our institution in 2003. Until 2011 only four 2-way exchanges were performed. Since 2011 the kidney exchange started at our institution as coordinated program. All the incompatible pairs are collected prospectively in the database. The matching run is performed every three monts with on average 20 pairs included for matching.
Methods: There were in total 58 paired live kidney transplants (KTx) performed in Czech since 2003, of those 50 since 2011, those we assessed. There were five 2-way, three 3-way, two 4-way, two 5-way, one 6-way and one 7-way domino kidney paired exchanges performed, two altruistic samaritan donors entered the scheme. There were 9 cases of re-transplant, of those seven second, one third and one forth KTx. Two surgeons perfomed all the transplants, one did all the mininvasive nephrectomies using Hand Assisted Retroperitoneoscopic (HARS) live donor nephrectomy technique.
Results: Mean recipient age was 46.4 years (SD 10.9), mean SCr one month after transplant was 123 umol/l (SD 41), equivalent of 1.39 mg/dl (SD 0.46). There was one case of delayed graft function due to early rejection observed. The program did help some 50 patients so far.
Conclusions: Kidney paired donation program can be run with success even at single institution, this limits some of the highly sensitised patients as well as blood group 0 recipients. Also, the bigger is the group for matching, the higher might bet the number of transplants. Possible cooperation within European centres would help to treat more patients with the best treatment modality – live donor kidney transplantation.

320.12

Management of plasma cells rich acute rejections- role of proteasome inhibitors

Khawar Abbas[1], Tahir Aziz[2], Mirza Naqi Zafar[3], Muhammed Mubarak[4], Adib ul Hassan Rizvi[5].
[1]Transplant Immunology, Sindh Institute Of Urlogy and transplantation, Karachi, Pakistan; [2]Nephrology, Sindh Institute Of Urlogy and transplantation, Karachi, Pakistan; [3]Transplant Immunology, Sindh Institute Of Urlogy and transplantation, Karachi, Pakistan; [4]Histopathology, Sindh Institute Of Urlogy and transplantation, Karachi, Pakistan; [5]Urology, Sindh Institute Of Urlogy and transplantation, Karachi, Pakistan.

Introduction: Plasma cells acute rejection (PCAR) appears to be poorly responsive to anti-rejection therapy and showing poor prognosis for survival of the renal allograft. This paper describes outcome of PCAR after proteasome inhibition therapy.
Patients and Method: Plasma cell acute rejection (PCAR) was diagnosed using Banff 2007 classification of renal allograft pathology and its revision including Banff 2013 update. Ten patients received proteasome inhibition based therapy (1.3mg/m2) for their PCAR. Each patient's also received two doses of Rituximab (375mg/m2), 14 doses of anti -thymocyte globulin (ATG) (1.6mg/kg) and 10 session of Plasmapheresis. Historic pre-transplant and sera at PCAR were tested for the presence of human leukocyte antigen (HLA) antibodies for HLA class I and Class II by Luminex (one Lambda, Canoga Park, CA, USA)according to manufacturer's instruction. We used the value of 1000 mean fluorescence intensity (MFI) as a cutoff for positive results.
Results: PCAR had had occurred 3.1±2.55 yr after transplant. The percentage of plasma cells was 47.8 ±12.2. Donor specific antibodies (DSAs) were found in all 10 cases, Class I in 20% and class II in 80%. Mean Post treatment serum creatinine improved from 4.90±2.75 to 3.30±1.58 mg/dl. The mean HLA class I and class II DSA MFI decreased from 4018± 126.57 to 706±430 and 5355±2921 to 1332±1203 respectively. One and two year graft survival was 90%.
Conclusion: Proteasome based therapy is effective in patients with PCAR, reducing both plasma cells and DSA with improvements in serum creatinine. In all 10% of the grafts were lost within the first year of PCAR.

320.13

The importance of renal biopsy in candidate living donors with proteinuria

Handan Özdemir[1], Gökce Özdemir[1], Aysen Terzi[1], Nurhan Özdemir[2], Merih Tepeoglu[1], Gökhan Moray[3], Mehmet Haberal[3].
[1]Pathology, Baskent University, Ankara, Turkey; [2]Nephrology, Baskent University, Ankara, Turkey; [3]Transplant Surgery, Baskent University, Ankara, Turkey.

Due to organ shortage and difficulties for availability of cadaveric donors, living donor transplantation is an important choice for having allograft. Whereas donors with hematuria and proteinuria consist of complex living donors and these donors requires careful evaluation for future renal risk.
The aim of this study is two-fold; first to investigate the importance of proteinuria in the determination of renal pathology in donors, and second to find out the importance of renal biopsy in the selection of donor for transplantation.
Only 679 donors whom were underwent donor evaluation process between 2000 and 2013 were included in the study. Among 679 donors only 70 showed proteinuria. Serial 24-hour urine protein and microalbumin were examined in all these donors and all of them underwent renal biopsy. Renal biopsies were examined under light, immunofluorescence and electron microscopy.
The mean 24-hour urine protein and microalbumin were found 438±302 mg/day and 103,6±235 mg/day respectively in 70 cases. Among 70 cases with proteinuria, only 21 (30%) had chance to become a donor after biopsy (Group 1). Biopsy findings of these 21 cases were found to be nonspecific. Remaining 49 cases were taken out of donor list because of their biopsy and/or clinical findings (Group 2). In group 2, the biopsy diagnosis was FSGS in 17, IgA nephropathy in 8, MPGN in 4, IgM nephropathy in 3, and tubulointerstitial nephritis in 2, hypertensive nephropathy in 2, membranous glomerulonephritis in 1, minimal change disease in 1, lupus nephritis in 1 and nonspecific biopsy findings in 10 cases. A significant difference was found between group 1 (284,8±114 mg/day) and group 2 (503±333 mg/day) in regards of 24-hour urine protein ($p<0.01$). In addition significant differences was noted between group 1 (34,2±59,2 mg/day) and group 2 (143±275,4 mg/dl) in regards of 24-hour urine microalbumin ($p<0.01$). Statistically no significant difference was found between two groups in regards of creatinine clearance. Only 16 patients also showed microscopic hematuria in addition to proteinuria. All 16 cases were included to group 2.
In conclusion proteinuria should be assessed as a standard part of the donor evaluation process. We suggested that proteinuria greater than 300 mg is a marker of glomerular pathology and renal disease. We also concluded that microalbuminuria determination may be a more reliable marker of renal disease and the presence of microalbuminuria should preclude donation.

321.1

Risk of acute cardiovascular complications following pancreas transplantation

Jim Kim[1], Joshua Schulman-Marcus[2], Anthony C.Watkins[1], Thangamani Muthukumar[2], Dmitriy Feldman[2], Rajesh Swaminathan[2], Luke Kim[2], Choli Hartono[2].
[1]Surgery, Weill Cornell Medicine, New York, United States;
[2]Medicine, Weill Cornell Medicine, New York, United States.

Introduction: Type 1 diabetes mellitus (T1DM) and chronic kidney disease (CKD) are known independent risk factors for cardiovascular (CV) disease. To date, the association between in-hospital CV complications following pancreas and kidney transplants has primarily been described only in smaller single-center studies. We therefore analyzed a large nationally representative database to assess composite CV morbidities in patients who had a simultaneous pancreas and kidney transplant (SPK) compared with pancreas transplant alone (PTA), and pancreas after kidney transplant (PAK). Our aim is to gain insight into early CV complications following pancreas transplantation and in patients who have CKD and end-stage renal disease (ESRD).
Materials and Methods: We conducted a serial, cross-sectional analysis of the Nationwide Inpatient Sample (NIS) for patients who underwent pancreas transplantation in the US from 2003 to 2012 (weighted n = 13,399). Multivariate logistic regression analysis was performed to examine the odds of CV complications as well as in-hospital mortality in SPK (weighted n = 8,613) compared to PTA and PAK (weighted n = 4,786).
Results: After adjusting for age, sex, race and co-morbidities, SPK patients had an overall increased risk of CV complications versus either PTA or PAK; OR = 1.38 [95% CI (1.14-1.68), p < 0.001]. Subgroup analysis demonstrated an equivalent risk of myocardial infarction, but a six-fold increased risk for stroke following SPK (see Figure). Atrial fibrillation/flutter and congestive heart failure were also increased after SPK.

	Univariate Analysis		Multivariate Analysis	
Complications	Odds Ratio [95% C.I.]	P value	Odds Ratio [95% C.I.]	P value
Stroke	4.65 [1.78-12.12]	0.002	**6.45 [2.15-19.11]**	**0.001**
Myocardial infarction	1.21 [0.92-1.60]	0.17	0.99 [0.68-1.44]	0.96
Atrial fibrillation/ flutter	0.95 [0.69-1.32]	0.78	1.62 [1.05-2.50]	0.03
Congestive heart failure	1.56 [1.24-1.95]	<0.001	1.33 [1.01-1.75]	0.04
All-cause CV complications	1.44 [1.22-1.69]	< 0.001	**1.38 [1.14-1.68]**	**0.001**
Death	0.40 [0.30-0.53]	<0.001	0.59 [0.40-0.871]	0.01

Discussion: Pancreas transplantation restores long-term physiologic control of euglycemia in patients with T1DM and subsequently leads to improvements in secondary complications, including CV disease. SPK and PAK are potential therapeutic options for Type 1 diabetics who have concomitant ESRD. To our knowledge, this study represents the largest pooled analysis of pancreas transplants examining differences in postoperative CV complications, namely the increased risk in patients undergoing pancreas transplant in conjunction with a kidney transplant (SPK) versus those who did not have ESRD at the time of their pancreas transplant (PTA and PAK). Undoubtedly, patient selection differences exist between the groups, but given the overlapping comorbidities, the effects of uremia appear to be a significant risk factor for CV complications following pancreas transplantation. We report multiple novel findings, including a six-fold increased risk for stroke following SPK versus pancreas alone (PTA+PAK).

Conclusion: Despite rigorous pretransplant evaluation, acute complications from CV disease may still cause considerable morbidity and mortality. ESRD is a significant risk factor for all-cause CV morbidity in patients undergoing pancreas transplantation. Further research is needed to elucidate the underlying mechanisms and to identify potential preventative measures.

321.2

Postoperative monitoring and diagnosis of common surgical complications following pancreas transplantation by microdialysis catheters

Gisle Kjøsen[1], Rune Horneland[2], Ole Øyen[2], Einar M.Aandahl[2,4], Kari G.Dahl[2], Andreas Barratt-Due[1,5], Sören Pischke[1,5], Gisli Björn Bergmann[1], Tor Inge Tønnessen[1,3], Håkon Haugaa[1].
[1]Dept. of Anaesthesiology, Oslo University Hospital, Oslo, Norway; [2]Dept. of Transplantation Medicine, Section for Transplant Surgery, Oslo University Hospital, Oslo, Norway; [3]Institute of Clinical Medicine, Oslo University Hospital, Oslo, Norway; [4]The Biotechnology Institute, Oslo University Hospital, Oslo, Norway; [5]Dept. of Immunology and Transfusion Medicine, Oslo University Hospital, Oslo, Norway.

Introduction: The complication rate after pancreas transplantation (PTx) is very high, with venous thromboses, bleeding and enteric anastomosis leakages being the most commonly occurring events that lead to early graft loss. There is currently a lack of reliable methods to uncover complications at an early stage. By the time the blood sugar increases, the grafts are in most cases irreversibly damaged, and can only infrequently be saved. In an ongoing study, we investigate whether microdialysis catheters are able to detect complications post-PTx. In this case report we describe the successful detection of venous thrombi and anastomotic leakage by microdialysis catheters.
Method: Four diabetic patients underwent technically similar solitary pancreas transplantations. A common aortic patch with both the coeliac trunk and the superior mesenteric artery was anastomosed "end-to-side" to the common iliac artery on the right side and an elongated portal vein was anastomosed "end-to-side" to the inferior caval vein. The enteric anastomosis was performed by a side-to-side duodeno-dudenostomy. At the end of surgery, two microdialysis catheters were placed on the anterior and posterior pancreatic surface. We measured glucose, lactate, pyruvate and glycerol bedside, sampled every 1-2 hours postoperatively.
Results: All patients had uncomplicated initial postoperative courses. On the third postoperative day however, we experienced elevations in lactate and lactate/pyruvate ratio in two patients. Doppler ultrasounds showed well-circulated grafts, but supplemental CT scans revealed venous thrombi in both patients. They underwent immediate angiographic intervention, and the thrombi were successfully removed. The patients were discharged on the 14th and 10th postoperative day, respectively. They have both had normal control CT scans after discharge, and remain insulin-free nine months post-PTx.

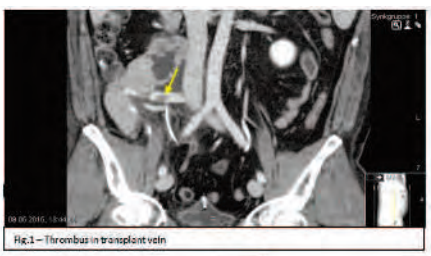


Fig.1 – Thrombus in transplant vein

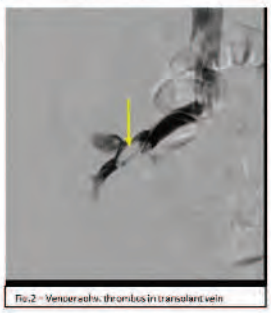


Fig.2 – Venous echo, thrombus in transplant vein

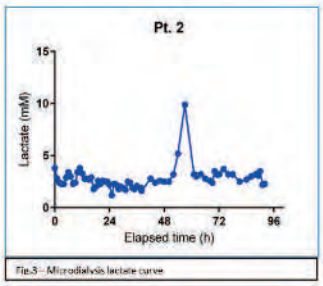
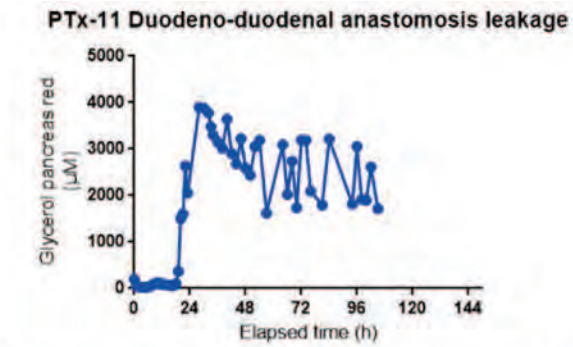
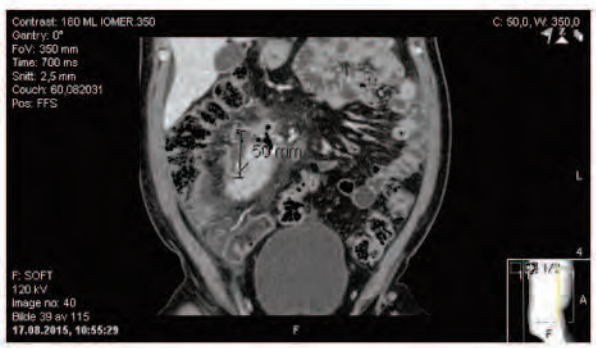


Fig.3 – Microdialysis lactate curve

Another patient had a 36-fold increase in microdialysis glycerol 20 hours postoperatively, but apart from this remained clinically well. He was discharged on the 10th postoperative day, but was readmitted on the 16th postoperative day with an abscess in relation to the enteric anastomosis. He was discharged after 5 days of IV antibiotics, without any intervention.
The last patient had elevated microdialysis glycerol in excess of 1000µm from the first measurement. This was accompanied by an amylase count of 21000 U/L measured in drainage fluid. He was otherwise clinically well, and was transferred to the medical ward on postoperative day 8. Six days later signs of infection developed, and a CT showed a peripancreatic abscess. The abscess was treated by percutaneous, ultrasound-guided drainage and IV antibiotics. The patient was discharged on the 28th postoperative day.





Conclusion: Monitoring pancreas transplants with microdialysis catheters appear to detect early complications like anastomotic leakage and ischemia at an early stage, and may potentially improve graft survival.

321.3

Longitudinal assessment of oral glucose tolerance test parameters for the prediction of pancreas transplant dysfunction

Emmanuel Morelon[1], Pascaline Alix[4], Fanny Buron[1], Sophie Reffet[2], Maria Brunet[1], Olivier Thaunat[1], Ricardo Codas[1], Lionel Badet[1], Fitsum Guebre[3].
[1]Transplantation, Hospices Civils de Lyon, Lyon, France;
[2]Endocrinology, Hospices Civils de Lyon, Pierre Bénite, France;
[3]Nephrology and Transplantation, CHU Grenoble, Grenoble, France;
[4]Nephrology, Hospices Civils de Lyon, Lyon, France.

Introduction: In patients with type 1 diabetes and end-stage renal disease, simultaneous pancreas-kidney transplantation (SPK) is the treatment of choice to improve glycemic control and prevent the development of secondary complication of diabetes. The management of pancreatic transplantation in the long term is limited by a lack of clinically relevant markers of graft dysfunction. Oral glucose tolerance test (OGTT) is performed to monitor pancreas graft function. The aim of our study was to focus on longitudinal assessment of insulin secretion and sensitivity data that can be derived from OGTT and to study their performance for the prediction of pancreas graft failure in the long term.
Patients and Methods: Between 2004 and 2013, patients with pancreas transplantation were retrospectively analyzed from the clinical database of the transplantation department, Edouard Herriot Hospital, Lyon, France. Clinical parameters, fasting blood glucose, HbA1c and OGTT were analysed at baseline, 3 months and every year after pancreas transplantation. Patients who lose their pancreas transplant within the first 3 months post transplantation were excluded from the analysis. Pancreas graft loss was defined as re-introduction of insulin therapy or anti-diabetic oral agents.
Results: 204 pancreatic graft recipients (6 PTA, 191 SPK, 7 PAK) were analyzed. Patient and pancreas graft survival at 10 years were 94.8% and 73.7% respectively. 28 patients lose their pancreas graft after a median of 31 months for vascular thrombosis (21.2%), pancreas necrosis (3%), biopsy proven acute rejection (24.2%), diabetes reccurrence (9.1%), lymphoma (3%) and unknown reason (24.2%). After 1 year post transplantation, 2 hour glucose from the OGTT and area under the curve for glucose were the most predictive for pancreas graft failure whereas Matsuda index, HOMA-IR and QUICKI, best predicted graft dysfunction after 2 years post transplantation. Fasting plasma glucose, HbA1c and OGTT at 3 months were not able to predict graft failure.
Discussion: Our study confirms the interest of the OGTT performed one year after pancreatic transplantation for the identification of patients at risk for pancreas graft loss. The relevance of complementary explorations (graft biopsy, search for rejection and recurrence of autoimmunity and other biomarkers) based on glucose metabolism parameters should be assessed in a prospective study.
Conclusion: In pancreas transplantation parameters derived from OGTT performed at one year yearpost transplantation may predict pancreas graft failure in the long term.

321.4

Pancreas transplantation results are not more progressing and require significant innovations

Diego Cantarovich[1], Cecile Guillen-Gueguin[1], Florent Le Borgne[1], Julien Branchereau[1], Jacques Dantal[1], Magali Giral[1], Gilles Blancho[1], Claire Garandeau[1], Aurélie Meurette[1], Maryvonne Hourmant[1], Georges Karam[1].
[1]Institut de Nephrologie, Urologie, Transplantation (ITUN), Nantes University Hospital, Nantes, France.

Background: We started our clinical pancreas transplant program in November 1987. Today (May 2015), 517 transplantations were done.
Aim: The goal of this analysis was to audit all procedures included in the database specifically created for this type of transplant and discuss possible changes in our practice to improve results.
Results: The selected period ranged from January 2000 to December 2013. During this period, 323 transplantations (Tx) were done (255 SPK and 68 PTA), with a minimum of 13 Tx in 2011 and maximum of 31 in 2004. According to each studied year, mean recipient age ranged from 37.1 to 43.6 years (20 to 65) and mean donor age from 31 to 39.8 (9 to 55), both without significant evolution over time. Mean BMI of recipients was quite similar each year, from 21.4 to 23.8 (17 to 33). Same thing for donor BMI (mean, 21.2 to 23.8; range, 16 to 33).Time on the waiting list was also relatively stable each year with a mean which never exceeded 1.4 years (maximum time observed was 6 years). In case of SPK, pre-emptive kidney Tx ranged from 9.1 to 68.4 % according to years, with a mean global value of 38%. Pancreas mean cold ischemia time (CIT) decreased from 13.2 hours in 2000 to 10.5 hours in 2013, with overall CIT of 12,7. Kidney DGF ranged from 6.2 to 29.4%, with a whole percentage of 16.6%. Percentage of patients under tacrolimus significantly increased from 43.8% in 2000 to 89.6% in 2013. All patients were under MMF in 2000. EC-MPA was initiated in 2005 and 26% of patients were under EC-MPA in 2013. Steroid-free patients represented 100% of the cohort in 2000; this percentage decreased progressively to 70% in 2013. Acute rejection (including kidney and pancreas ones) incidence, graft survival and death remain unvaried throughout this 14-year period time. Long-term pancreatic results (death-censored) were significantly impacted by donor BMI ≥25, recipient BMI ≥25 and recipient age >45 years (20% difference in survival in each 3 categories at 8 years). On the contrary, long-term kidney graft results were similar according to recipient BMI and donor BMI. We did not observe any influence of donor age or CIT on pancreas survival, although increased CIT was associated with more venous thrombosis.
Discussion: Measures to improve clinical pancreas transplantation should explore the mechanisms involved in pancreas (but not kidney) poor outcomes in relation to donor and recipient BMI. Complement and TNF-alpha blockade, hypothermic machine perfusion, medical optimized metabolic control, may be some suggestions to prevent pancreas Tx failures. Patients over 45 years may probably require a more frequent and extended evaluation before surgery. New trials should consider negative risk factors for pancreas success and test new tools to improve results. Alternatives such as kidney Tx alone, simultaneous kidney-islets or even new strategies of dialysis and medical diabetes care should also be considered in such future studies which might require multicenter collaboration.

321.5

Recipient and pancreas graft survival after kidney-pancreas transplantation in Australia and New Zealand: a cohort study 1984-2014 using ANZIPTR

Angela Webster[1,2], Alex Xi Peng[1], Henry Pleass[1], William Mulley[3], Helen Pilmore[4], Patrick J.Kelly[2].
[1]Centre for Transplant and Renal Research, Westmead hospital, Westmead, Australia; [2]Sydney School of Public Health, University of Sydney, Sydney, Australia; [3]Department of Nephrology, Monash Medical Centre, Clayton, Australia; [4]Department of Renal Medicine, Auckland City Hospital, Auckland , New Zealand.

Introduction: We evaluated survival for kidney-pancreas recipients (SPK) in Australia and New Zealand.
Methods: Data 1984-2014 from the Australia and New Zealand Islet and Pancreas Transplant Registry were used to analyse time to pancreas failure (first of; pancreatectomy, insulin-dependence, with and without death) or death (all-cause), using Kaplan-Meier survival curves, censoring at last follow-up. We used Cox models (Hazard ratios HR, with 95%CI) to identify prognostic factors.
Results: We included 627 recipients, with 5,370 years of observation, 119 (19%) deaths and 214 (34%) pancreas failures. Patient survival was 97% at 1 year, 93% 5 years, 81% 10 years, 69% 15 years and 64% 20 years (figure 1). After adjusting for other differences, risk of dying decreased by 48% for people receiving SPK in 2010-2014 compared to 1989-1994 (HR0.52; p<0.01). Recipient age increased risk of death 4% for every year older at transplantation (HR1.04; p=0.04). There was no evidence of increased risk with any other factors (p>0.05). Pancreas survival was 84% at 1 year, 76% 5 years, 64% 10 years, 56% 15 years and 50% 20 years (figure 2). Pancreas failure decreased 40% between 1989-1994 and 2010-2014 (HR0.60; p<0.02). After adjusting for other differences, risk of pancreas failure increased by 2% for every year of donor age (HR1.02; p=0.03). There was some suggestion that longer time on RRT associated with higher risk of pancreas failure (p=0.08).
Conclusion: There has been substantial improvement in patient survival and a substantial reduction in the risk of pancreas failure since SPK first began in ANZ.

Figure 1: Recipient survival by age at transplantation

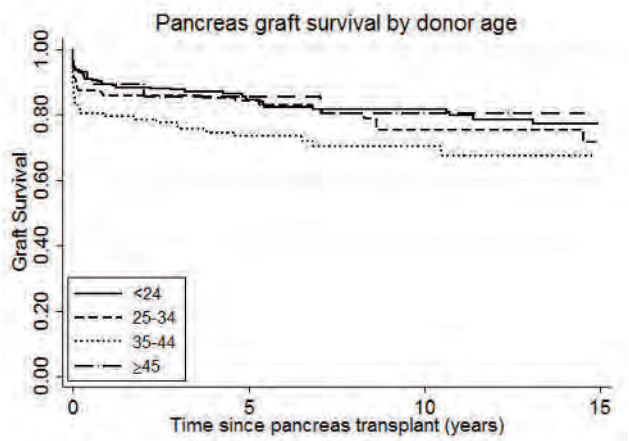
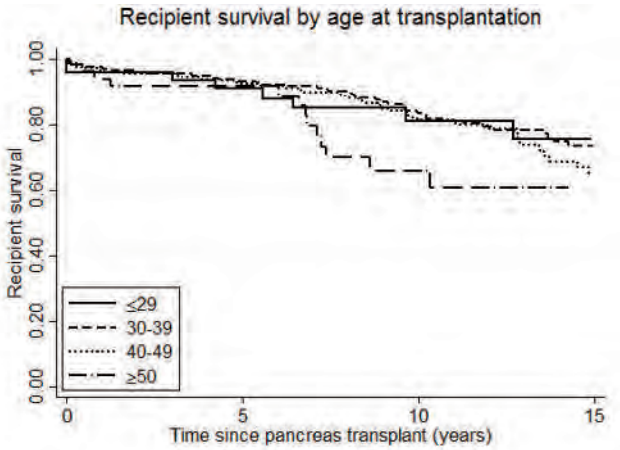


Figure 2: Pancreas transplant survival (censored for death) by donor age



ANZIPTR contributors

321.6

Pancreas-after-islet transplantation: A path for long term insulin independence

Steven Wisel[1], James M.Gardner[1], Garrett R.Roll[2], Jack Harbell[2], Dixon Kaufman[3], Andrew Posselt[2], Peter G.Stock[2].
[1]Department of Surgery, University of California, San Francisco, San Francisco, United States; [2]Division of Transplantation, University of California, San Francisco, San Francisco, United States; [3]Division of Transplantation, University of Wisconsin, Madison, United States.

Introduction: Islet transplantation has emerged as an attractive, minimally invasive approach for treating severe Type 1 diabetes with hypoglycemic unawareness. However, islet transplantation failure rates are high, and success rates following reinfusion of islets have been compromised by allosensitization from prior islet infusions as well as lack of funding for this procedure in the US. Here we describe our series of seven consecutive patients with history of failed islet infusions and subsequent solid organ pancreas transplantation, who have achieved long-term insulin independence and preservation of renal function up to 9 years (median 6.25 years) following pancreas transplantation.
Materials and Methods: Seven consecutive patients receiving care at our institution from 2007-2016 for pancreas-after-islet (PAI) transplantation were included in this study. All patients had a prior history of preuremic type 1 diabetes and at least one lifetime episode of hypoglycemic unawareness, with 1 to 3 previously failed islet infusions prior to PAI transplant. Six patients underwent PAI at our institution; one transplant was performed at an outside institution with subsequent follow-up care at our institution. Patients received anti-thymocyte globulin and methylprednisone for induction therapy, with a standard four-drug maintenance therapy including low dose tacrolimus (trough 5-7ng/ml), mycophenolic acid (180-720 mg/d), and prednisone 5 mg/d. Low-dose mTOR inhibition was added one month following transplant, with cessation of mTOR inhibition or MMF at one year following transplant. All patients underwent routine surveillance, including protocol biopsy between 3 and 6 months post-transplant.
Results: Despite highly variable panel-reactive antibody (PRA)-positivity prior to transplant (mean 36± 39%), all seven patients achieved stable and durable insulin independence with a mean follow-up of 75.2± 31.9 months. HgbA1C values improved significantly from post-islet, pre-pancreas levels (mean 8.1± 1.5%) to post-pancreas levels (mean 5.12± 0.30%, Figure 1a). Four patients experienced acute rejection episodes successfully managed with thymoglobulin and prednisone. Renal function was preserved in this preuremic cohort (Figure 1b), with 100% allograft function as defined by insulin independence (Table 1).
Conclusion: These results suggest that pancreas-after-islet (PAI) transplantation is a viable strategy to achieve long-term insulin independence following islet graft failure. Successful outcomes are dependent on a multidrug immunosuppression regimen which minimizes toxicities of any single agent, as well as routine surveillance biopsy to addres subclinical rejection.

Table 1. Patient Demographics

Patient	Number of Prior Islet Infusions	Panel Reactive Antibody (PRA)	Date of PAI	Follow-up (months)	Insulin Independence	Last HgbA1c (%)
1	3	91%	10/4/2006	114	YES	5.0%
2	2	76%	8/27/2007	103	YES	5.2%
3	3	0%	1/2/2008	99	YES	5.2%
4	1	10%	8/26/2010	66	YES	5.3%
5	1	10%	10/4/2010	65	YES	5.6%
6	2	62%	3/26/2011	59	YES	5.0%
7	2	2%	5/10/2014	22	YES	4.6%

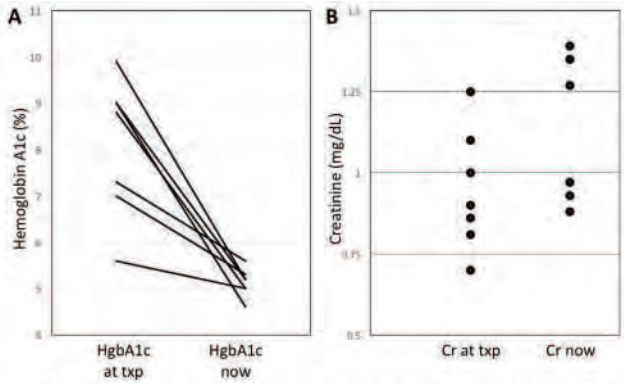


Figure 1. a) Serum Hemoglobin A1c, measured at time of PAI transplant and most recent laboratory panel; **b)** Serum creatinine, measured at time of PAI transplant and most recent laboratory panel.

321.7

Clinical pancreas transplantation from deceased donor in Japan

Takashi Kenmochi[1], Toshinori Ito[2], Ichiro Nakajima[3].
[1]Department of Organ Transplant Surgery, Fujita Health University, School of Medicine, Toyoake City, Aichi, Japan; [2]Department of Surgery, Osaka University School of Medicine, Osaka, Japan; [3]Department of Kidney Surgery, Tokyo Woman's Medical University School of Medicine, Tokyo, Japan.

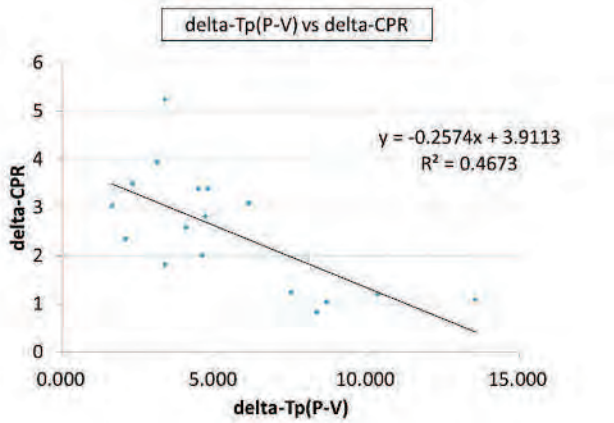
237 pancreas transplantations (PTx) [208 from DBD, 2 from DCD, 27 from live donors] were performed from 2000 to 2014 in Japan. We evaluated the effectiveness of PTx from deceased donors and 16 simultaneous pancreas and kidney transplantations from live donors (LDSPK) of my own experiences.
210 deceased donors PTXs in Japan: 132 donors (63%) were over 40 years, which was older as compared to the US and Europe. The Cause of death was cerebrovascular diseases in 117 donors (56%). 160 donors (76.2%) were marginal donors. Category of PTx were SPK; 167 (79.5%), PAK; 32 (15.2%) and PTA; 11 (5.2%). The duration of insulin therapy and hemodialysis of the recipients were 27.4 and 6.9 years. Induction therapy by @IL-2R or ATG was performed in 203 patients (97%) and immunosuppression was maintained by tacrolimus (98.1%) or cyclosporin (1.9%) combined with MMF and steroid. Enteric drainage was selected in 174 patients (83%) and bladder drainage in 36 patients (17%). 5-year recipient survival was 95.8%. Pancreas graft survivals were 84.3% at 1-year, 77.0% at 3-year and 70.4% at 5-year. While, kidney graft survivals were 92.5% at 1- and 3-year, and 89.2% at 5-year, respectively.
16 LDSPKs of my own experiences: I have introduced the first LDSPK in 2004 and performed 16 cases.
Recipients: Age and gender were 34.2+-5.7 years and 6 males / 10 females. All of them showed a negative serum C-peptide level (<0.03ng/ml).
Donors: Donors were 12 parents and 4 siblings. 6 donors were ABO-incompatible.
Operations and Immunosuppression: Donor operation was performed by right nephrectomy followed by distal pancreatectomy with open (8 donors) or laparoscopic procedure (8 donors). LDSPK was performed using pancreatico-cystostomy. Immunosuppression was achieved by a quadruple therapy with tacrolimus, MMF, predonisolone, and basiliximab. For ABO-incompatible cases, desensitization with rituximab, DFPP and PEX was performed.
Results: Although a pancreatic fistula was developed in one donor and a pancreatic cyst in one donor, 14 donors showed no complication including diabetes and renal dysfunction up to 11 years. One recipient developed primary nonfunction of the pancreas graft. Another patient developed venous thrombosis. Other 14 patients achieved insulin independency immediately after transplantation. These patients are maintaining insulin independency and showing the normal endocrine function. All six patients from ABO incompatible donors achieved insulin independency (100%) and withdrawal from hemodialysis (100%) without an episode of antibody mediated rejection.
Conclusion: Although majority of the deceased donors were marginal in our country, clinical outcome demonstrated that PTx was a potent and promising treatment for severe type 1 diabetic patients. Also, LDSPK can be recommended as a potent tool of treatment for type 1 diabetic patient with ESRD. Furthermore, our clinical data demonstrated that ABO-incompatible pancreatic graft survived as well as kidney graft.

321.8

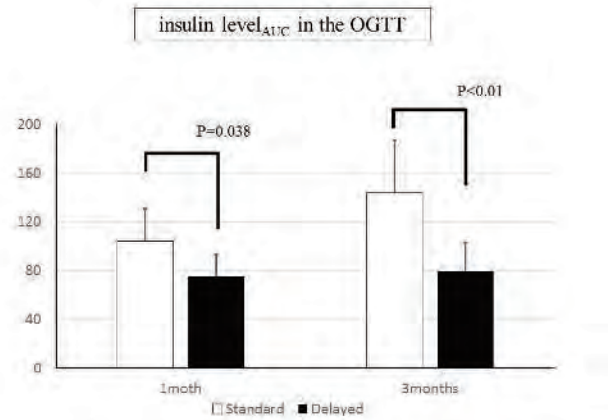
The prediction of the endocrine function of the graft using contrast-enhanced ultrasonography in clinical pancreas transplantation

Naohiro Aida[1], Takashi Kenmochi[1], Taihei Ito[1], Minoru Oshima[1], Toru Nishikawa[2], Akihiro Kawai[3], Mamoru Kusaka[3], Kiyotaka Hoshinaga[3], Hisahiro Matsubara[4].
[1]Department of Liver, Biliary Tract and Pancreas Diseases, Fujita Health University, School of Medicine, Toyoake, Japan; [2]Department of Transplantation and Regenerative Medicine, Fujita Health University, School of Medicine, Toyoake, Japan; [3]Department of Urology, Fujita Health University, School of Medicine, Toyoake, Japan; [4]Department of Frontier Surgery, Graduate School of Medicine, Chiba University, Chiba, Japan.

Introduction: In pancreas transplantation, maintaining blood circulation in the graft early after transplantation is important for organ protection. However, there is little study about the correlation between a blood flow and graft function. Contrast enhanced ultrasonography (CEUS) can be performed easily and safely, thus it is suited to the evaluation of a blood flow of the graft.
Objective: In the present study, we determined the early blood flow after transplantation by using CEUS and examined its relation with graft function.
Methods: Data from 17 cases of pancreas transplantation, performed between May 2012 and September 2015 in Fujita Health University Hospital, were retrospectively assessed. CEUS was performed within 24 hours after transplantation. The time-intensity curves of the parenchyma and vein were constructed, and each time to peak (Tp) were calculated. The time between the Tp of the parenchyma and that of the vein was measured to evaluate graft microcirculation and was defined as delta-Tp (parenchyma [P] – portal vein [V]).
Graft endocrine function was evaluated by oral glucose tolerance test [OGTT] (1month and 3 months after transplantation) and glucagon test (1 month after transplantation).
The relationship between delta-Tp(P-V) and graft endocrine function were analyzed.
Results: The delta-Tp(P-V) showed a significant negative correlation with the C-peptide increment in the glucagon test (R= -0.68).



The delta-Tp(P-V) also showed a significant negative correlation with insulin levelAUC in the OGTT at both 1 month and 3 months after transplantation (R= -0.42, R= -0.39). The patients were divided into the following two groups depending on the value of delta-Tp(P-V), ; Standard group(<7 seconds) and Delayed group (> 7 seconds). The Standard group had better insulin secretion than those in the Delayed group at both 1month and 3 months after transplantation (insulin levelAUC in the OGTT at 1month and 3 months after transplantation: 103.9 ± 26.9 vs. 75.0±18.2; P = 0.038, 143.8±43.2 vs. 79.4±23.5; P<0.01).



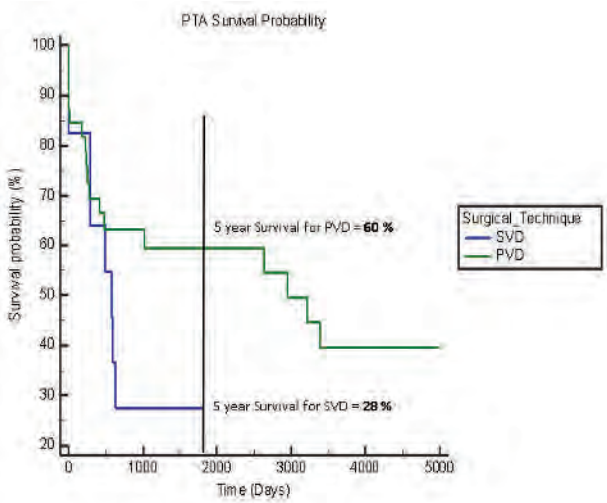
Conclusions: The present study demonstrated that delta-Tp (P-V) obtained in early period after transplantation was significantly correlated with the graft function at both 1 month and 3 months after transplantation. CEUS is able to evaluate a blood flow of the graft and useful for the prediction of the endocrine function of pancreas grafts.

321.9

Superior long-term outcome of pancreas transplantation alone with portal venous drainage vs. systemic venous drainage

Eric Siskind[1], Niket Todi[1], Lauren Pettinato[1], Amanda Bartosic[1], Blythe Fiscella[1], Eugene Schweitzer[1], Stephen Bartlett[1].
[1]Transplantation, University of Maryland Medical Center, Baltimore, MD, United States.

Introduction: There have been few studies analyzing portal venous drainage (PVD) versus systemic venous drainage in pancreas transplantation. The studies analyzing simultaneous kidney pancreas transplants have shown no benefit to PVD; however, these reports are confounded immunologically by systemic dr ainage of the co-transplanted kidney. Even the reports that show a benefit of PVD in pancreas transplants alone (PTA) are limited by short follow-up. We examined the difference in graft survival between portal venous drainage (PVD) and systemic venous drainage (SVD) in a long-term cohort of PTA cases.
Methods: From 1992 to present, 143 PTAs were performed at the University of Maryland. Patients were divided into two groups: PVD (n=95) or SVD (n=48).
Results: The mean graft survival of PTA is 2370 days from date of surgery calculated via a Kaplan Meier analysis. Patients with SVD had a mean graft survival of 580 days while those with PVD had a mean survival of 2680 days. (p=0.15). The five year graft survival was 28% for SVD and 60% for PVD (p=.004). There is a trend towards decreased episodes of rejection for PVD which approached significance.
Conclusion: Patients who undergo PTAs have significantly longer graft survival. We believe this is due to partial tolerance to antigen delivered into the portal system. The 60% 5-year PVD mean survival is superior to the national average of approximately 53% for PTAs[1].



References:
[1] Waki, K., Sugawara, Y., Kokudo, N., & Kadowaki, T. (2011). Long-term pancreas allograft survival in simultaneous pancreas-kidney transplantation by era. Clinical transplantation, 13-22. Chicago

321.10

En bloc dual kidney-pancreas transplantation into an adult recipient utilizing very small pediatric donor: No conflict with liver transplantation concerning anatomical division in respect to dividing arteries - all organs can be used

Johan Nordström, Carl Jorns, John Sandberg, Lars Wennberg.
Dept Transplantation Surgery, Division of Transplantation Surgery, CLINTEC, Karolinska Institute, Stockholm, Sweden.

Introduction: Simultaneous pancreas and kidney transplantation (SPK) is a treatment option for type 1 diabetes patients suffering from end-stage renal disease (ESRD). Upper age limit for donors is commonly 50-60 years but no lower age limit has been defined. Results are excellent using pediatric donors for adult recipients [2]. We present a combined pancreas and dual kidney en bloc transplantation from a 18 month-old, 12 kg donor. Surgical technique using donor aorta and cava as vascular pedicle not transecting neither pancreas nor kidneys is very feasible for SPK but raised concerns about possible problems for liver transplantation from same donor to child recipient.
Method: SPK from small pediatric donor using the en bloc technique to adult recipient. Only 1 vascular reconstruction is needed on backtable, namely an end-to-end anastomosis between the porta and upper vena cava. Several different surgical techniques has been described [5][3]. We used a previously described technique [4] with modification so that the proximal donor aorta was used for arterial anastomosis to the recipients a iliaca communis dexter. The urinary bladder was utilized aiming for bladder patch transplantation technique [1] for ureteral reconstruction but was finally not used due to excessively long ureters which raised concerns about risks for complications, i e ischemia i urethers leadine to urinary leakage and strictures as well as serving as an ileus mechanism. The 2 ureters was anastomosed separately to recipient urine bladder over 2 double-J-stents that was kept for 5 days post-op. Graft-duodenum was anastomosed to recipient jejunum approximately 40 cm distal to Ligamentum Treitz. No surgical complications was noted and organ function was immediate without need for dialysis or anti-hypertensive medicines and with excellent metabolic control. The liver was sent to another transplant center and pediatric whole liver transplantation was performed without problems. The fact that the donor had normal arterial anatomy made decisions easier, transecting liver and pancreas with sufficient vasculature for transplantation.
Result: No surgical complications. Organ function in SPK recipient was immediate. The liver was used for pediatric whole liver transplantation without problems. Our case shows that it is feasible to use very young donors for SPK en bloc without interfering with liver transplantation. Using the en bloc technique for SPK leads to short cold ischemia time (CIT). Outcome is excellent, one year follow-up: excellent metabolic control and GFR>60.
Discussion: The technique leads to short CIT which is greatly beneficial. The multiorgan procurement technique for en bloc in small pediatric donors is not complicated and does not take longer time compared to standard techniques. It can be done with no conflict with liver transplantation, at least in cases with normal arterial anatomy. The backtable is done with only one vascular reconstruction.

321.11

Long term results in pancreas re transplantation depending on the cause of primary graft failure

Lilia martinez de la Maza, Joana Ferrer, Maria Jose Ricart, Miguel Angel Lopez-Boado, Emiliano Astudillo, Ramon Rull, Santiago Sanchez-Cabus, Asma Salhi, Laureano Fernandez-Cruz, Juan Carlos Garcia-Valdecasas.
Hepato-Pacreatic-Biliary Surgery and Transplantation Department, Hospital Clinic de Barcelona, Barcelona, Spain.

Introduction: The late increase in the number of pancreas transplantations has lead to an increase in potential candidates for retrasplantation after graft failure due to technical complications or chronic immunological rejection.
Aim: To analyze the results in patients who where subject to a pancreatic retrasplantation in Hospital Clinic in Barcelona during the past 15 years weather the primary graft failure was due to immune causes or technical complications associated with surgery.
Material and Methods: We did a retrospective study to analyze the pancreas retrasplantations done from 2001 to 2015. The mean time between the first and second transplant was 57.3 ± 51.1 months. The back table end-to-end splenic with distal mesenteric artery anastomosis technique was used in 85.7% of the transplants. The donor pancreas was flushed either with UW (65.7%)) or Celsior (34.3%) solutions. The cold ischemia mean time was 11.8 ± 2.8 hours . Exocrine drainage was managed with a duodenojejunostomy in all cases. We used SPSS v20 for statistical analysis
Results: Between January 2001 and December 2015 a total of 312 pancreas transplantations were done in our institution, from which 35 were retransplantations. The group of retransplantation had mean age of 40±8 years old. Fifty one percent of these patients were men and 48.6% were women. Most of the primary graft failure was due to immune causes (57.1%), 40% was related to technical complications and in one patient the graft had to be removed because a lymphoma in the graft. In four cases (11.4%) a simultaneous pancreas-kidney retransplantation was done and in 31 patients (88.6%) a pancreas alone retransplantation was done with a functional kidney graft. The most common surgical related complication was vascular thrombosis without difference due to the primary graft failure. After a following of 82±47 months, the five-year survival was 94% in the group of immune cause of primary graft failure and 92% when primary graft failure was associated with surgical technical complications without significant difference. One year, 3 and 5 years graft survival was 79%, 73% and 61% respectively when primary graft failure was due to immune causes and 71%, 71% y 63% respectively when associated with technical complications as the cause of primary graft failure without significant difference.
Conclusions: Pancreas retransplantation can be considered as an effective and safe second option in patients with previous primary graft failure, reaching a graft survival equivalent to primary pancreas transplantation independently of the primary graft failure cause.

321.12

The optimal range of native kidney function before pancreas transplant alone minimizing the risk of end-stage renal disease

Sung Shin, Young Hoon Kim, Ji Yoon Choi, Hyun Wook Kwon, Joo Hee Jung, Duck Jong Han.
General Surgery, Asan Medical Center, Seoul, Korea.

Background: The optimal range of native kidney function minimizing the risk of end-stage renal disease (ESRD) after pancreas transplant alone (PTA) remains unclear.
Methods: A retrospective study was conducted on 84 recipients who underwent PTA at a single center from January, 1999 to August, 2015. Logistic regression was used to model the association between pre-transplant eGFR and the risk of ESRD after PTA.
Results: Nine patients (10.7%) had a pre-transplant estimated glomerular filtration rate (eGFR) <60, 10 patients (11.9%) 60 to 79.9, and 65 patients (77.4%) ≥80 ml/min/1.73m2. The cumulative probabilities of ESRD for eGFR<60, 60 to 79.9, and ≥80 ml/min/1.73m2 at 5 years were 36.5%, 0%, and 9.1% and at 8 years were 68.3, 0, and 18.2%, respectively. Pre-transplant eGFR < 60 ml/min/1.73m2 was found to be associated with a greater risk of ESRD (p = 0.04). Although there was no significant difference in the risk of ESRD between eGFR 60 to 79.9 and ≥80 ml/min/1.73m2, the post-transplant mean levels of eGFR in patients with pre-transplant eGFR 60 to 79.9 ml/min/1.73m2 were significantly lower than those with pre-transplant eGFR ≥ 80 ml/min/1.73m2 during the follow-up period (p = 0.042).
Conclusions: The ideal management of candidates for PTA with eGFR less than 80 ml/min/1.73m2 remains to be determined.

321.13

Hyperinsulinemia after pancreas transplantation: a risk for excessive weight gain, metabolic syndrome, and fatty liver disease

Richard Knight[1], Ana Islam[1], Samir Patel[2], Archana Sadhu[3], Larry Teeter[4], Edward Graviss[4], David Victor[3], Osama Gabor[1].
[1]Surgery, Houston Methodist Hospital, Houston, United States; [2]Pharmacy, Houston Methodist Hospital, Houston, United States; [3]Medicine, Houston Methodist Hospital, Houston, United States; [4]Pathology and Genomic Medicine, Houston Methodist Hospital, Houston, United States.

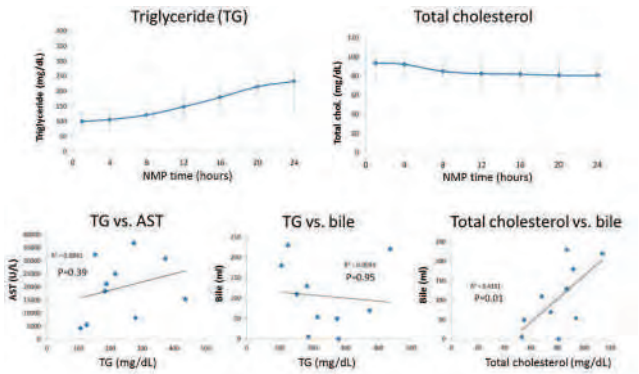
Aim: Recent studies have suggested that elevated insulin levels may be the cause rather that the result of obesity. We therefore hypothesized that hyperinsulinemia, due to portal-systemic drainage of the pancreas graft, may result in excessive weight gain (EWG) after transplantation, leading to metabolic syndrome and fatty liver disease. This study aimed to characterize EWG following pancreas transplantation and its association with hyperinsulinemia.
Methods: This is a single center review of 82 kidney-pancreas transplants for type 1 diabetes performed between 9/2007 and 4/2014. All transplants utilized portal-systemic venous drainage. Immunosuppression consisted of tacrolimus, mTOR inhibitor or mycophenolate mofetil, ± prednisone. EWG was defined as >18% weight increase at 1 -year post-transplant (upper limit of interquartile range of median percent weight gain at 1-year).
Results: Mean recipient age and body mass index at transplant were 41±9 years and 25±4 kg/m2, respectively. Mean weight gain at 1 year post-transplant was 11.2±13.4% of baseline. The incidence of EWG at 1 year was 29%. By multivariate analyses, independent pre-transplant risk factors for EWG included younger age (OR=0.92, p=0.01), HgbA1c >7% (OR=7.31, p=0.02), and C-peptide >0.2ng/mL (OR=3.59, p=0.05). No individual immunosuppression agent conferred a greater risk of EWG. Despite good pancreas function at 1 year in both groups (Hgb A1c 5.4±0.7% for EWG recipients vs. 5.3±0.6% for all others, p=0.40), EWG recipients had higher fasting insulin (26±15 vs. 19±11 mU/l, p=0.04), triglyceride (141±72 vs. 105±45 mg/dl, p=0.03), cholesterol (201±42 vs. 174±41 mg/dl, p=0.02), ALT (38±21 vs. 28±15 mg/dl, p=0.03), and AST (36±10 vs. 30±10 mg/dl, p=0.04) levels. Beyond 1-year the EWG group displayed continued weight gain and higher fasting insulin levels than the non-EWG cohort.
Conclusion: Almost one of 3 recipients experienced EWG within the 1st year post-transplant. Pre-transplant risk factors included younger age and poor glycemic control. Despite a well-functioning pancreas, EWG recipients had higher fasting insulin, lipids and transaminase levels. Beyond 1-year, EWG recipients continued to gain weight and remained hyperinsulinemic over time. These data suggest that hyperinsulinemia resulting from pancreas transplantation may cause EWG, potentially leading to metabolic syndrome and fatty liver disease.

322.1

Triglyceride exportation in the preservation of discarded steatotic human livers using 24 hours ex-vivo normothermic machine perfusion

Qiang Liu, Ahmed Nassar, Giuseppe Iuppa, Basem Soliman, Laura Buccini, Matthew Blum, Toshihiro Okamoto, Teresa Diago Uso, Ana Bennett, William Baldwin, Kareem Abu-Elmagd, John Fung, Charles Miller, Cristiano Quintini.
Cleveland Clinic, Cleveland, United States.

One primary reason to discard donor livers after procurement is severe steatosis. The impact of normothermic machine perfusion (NMP) on lipid metabolism and preservation of steatotic human livers has not been studied. We perfused 10 discarded human livers with oxygenated NMP for 24 hours (after cold ischemia of 4-6 hours). The perfusate consisted of packed RBCs and fresh-frozen plasma (2.5L total volume and 20-25% hematocrit). All livers had several degrees of steatosis. Perfusate analysis showed an increase in triglyceride (TG) levels in all livers starting from the 1st hour (median 127 mg/dL; interquartile range 95-149) to 24th hour (203;171-304)(p=0.004).



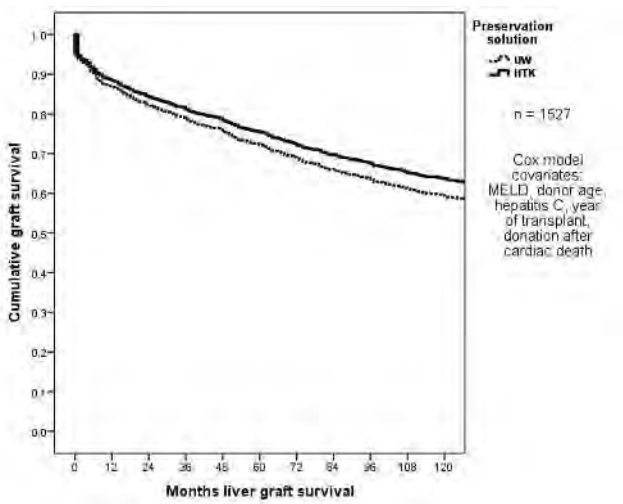
Total cholesterol decreased from 101(83-109) to 85(72-94) mg/dL (p=0.11). 50% of the livers produced a significant amount of bile (≥5 ml/hr). Bile production and aspartate transaminase (AST) release in the perfusate did not correlate to TG in perfusate at 24th hour NMP, while the total cholesterol had a significant positive correlation to bile production (r=0.66; p=0.01) but a non-significant correlation to AST(r=0.54; p=0.13). The semi-quantitative scoring of steatosis did not show a significant difference from start to end of NMP. There was no significant intra-individual difference in the degree of steatosis based on quantitative image analysis. This is the first study to determine the lipid profile of the perfusate using ex-vivo human liver perfusion in 24 hours NMP preservation. Our results indicate that active TG metabolism and exportation occur ex-vivo regardless of AST release and bile production (one of the most reliable marker of ex-vivo function). The long perfusion duration (24hrs) is not sufficient to decrease the amount of steatosis as measured by histological analysis. Pharmacological intervention may be needed to decrease the amount of steatosis in ex-vivo liver perfusion.

322.2

A comparison of liver transplant biliary complications for deceased donor drafts preserved with histidine-tryptophan-ketoglutarate and University of Wisconsin solutions

Richard Mangus, Jonathan A.Fridell, Chandrashekhar A.Kubal, Ray Chihara, Timothy Borup, William A.Marshall, A. J.Tector.
Surgery, Transplant, Indiana University School of Medicine, Indianapolis, IN, United States.

Introduction: Flushing of the biliary microvasculature at the time of whole organ liver graft procurement appears to be an important component in optimizing post-reperfusion blood flow to the biliary system. Two primary preservation solutions are most commonly employed in the United States, University of Wisconsin (UW) and Histidine-Tryptophan-Ketoglutarate (HTK) solutions. While HTK has a viscosity similar to that of water, UW is much more viscous. It has therefore been proposed that the less viscous HTK provides a better flush of the liver graft at the time of procurement. This then leads to the question of whether or not this improved flush results in a lower rate of biliary complications in HTK preserved livers, particularly donation after circulatory death (DCD) grafts. The present study evaluates biliary complications in 1527 consecutive liver transplant patients. The primary study variable is the choice of graft preservation solution, UW or HTK. Outcomes include post-transplant biliary complications, as well as graft and patient survival. Measured biliary complications include any need for bile duct imaging, intra- and extra-hepatic stricture formation, and bile duct leak. The secondary outcome was graft survival.
Methods: Data were extracted retrospectively from a large, single center database covering the period from 2001 to 2014. All primary imaging reports were reviewed. For this study, a patient was considered to have an anastomotic stricture if this is reported, and an intervention (balloon dilation or stent placement) is performed. Patients in whom sphincterotomy alone was performed were not considered to have a stricture. Any leakage of contrast from the biliary system was considered a biliary leak, though most were easily managed with temporary stenting of the duct.



Results: There were 1527 LTs reviewed, 1360 (89%) with duct-to-duct and 163 (11%) with Roux-Y choledochojejunostomy. Preservation included 1098 HTK (72%) and 429 UW (28%) grafts. One-year graft survival favored HTK preserved livers (88% vs 84%, p=0.02). Any biliary imaging was required for 50% of HTK and 59% of UW LTs (p=0.001). The risk of any leak was higher for UW (18%) versus HTK (7%) (p<0.001). The rate of any stricture was higher for HTK (45% vs 38%, p=0.01), but intrahepatic strictures were more common for UW

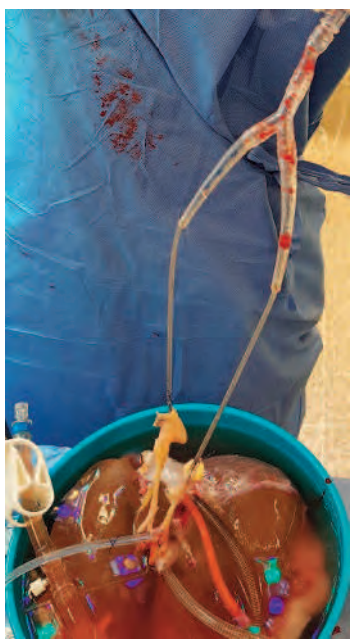
(5% vs 2%, p=0.001). Among donation after circulatory death (DCD) grafts, there was a higher risk of intrahepatic ischemic-type strictures in UW-preserved grafts (36% vs 8%, p=0.02). Multivariate Cox regression survival at 10-years favored HTK.
Conclusion: HTK liver graft preservation in deceased donors is associated with better graft survival, less imaging, less risk of biliary leak and intrahepatic strictures overall. HTK is also associated with a lower risk of intrahepatic strictures in DCD grafts.
References:
[1] Mangus RS. A comparison of histidine-tryptophan-ketoglutarate solution (HTK) and University of Wisconsin solution (UW) in extended criteria liver allografts. *Liver Transplantation.* 2008; 14:365-73.

322.3

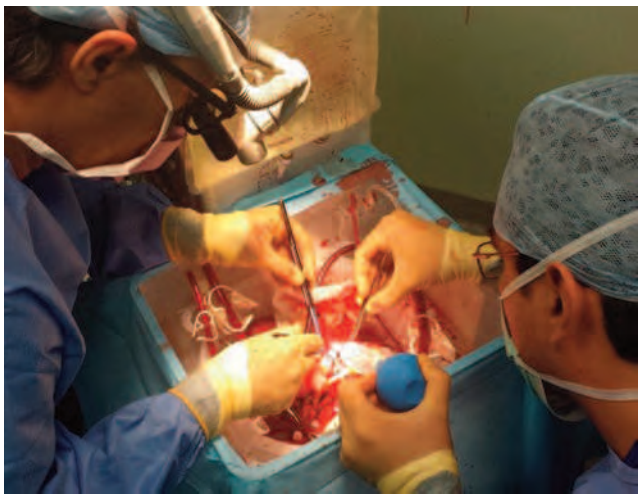
Surgical experience of normothermic machine perfusion in human liver transplantation

David Nasralla[1], Hynek Mergental[2], Thamara Perera[2], Paulo Muiesan[2], Annemarie Weissenbacher[1], Carlo Ceresa[1], Wayel Jassem[3], Constantin Coussios[4], Peter Friend[1].
[1]Nuffield Department of Surgical Sciences, University of Oxford, Oxford Transplant Centre, Oxford, United Kingdom; [2]Liver Transplant Surgery, Queen Elizabeth Hospital, Birmingham, United Kingdom; [3]Liver Transplant Surgery, King's College Hospital, London, United Kingdom; [4]Institute of Biomedical Engineering, University of Oxford, Oxford, United Kingdom.

Introduction: Normothermic machine perfusion (NMP) offers a potential solution to the organ shortage crisis affecting liver transplantation. It involves perfusing an organ with oxygenated blood, nutrients and medications at 37°C to preserve it in a functioning, physiological state. It requires the liver backtable to be performed at the donor hospital followed by cannulation of the vessels before commencing the perfusion. NMP continues during transport and storage until transplantation. We report the logistical impact and surgical expertise that has emerged during our first 100 transplanted NMP livers using the OrganOx *metra*.
Methods: 100 livers were successfully preserved using NMP until transplantation. For all livers the following details were recorded – cold ischaemic time (CIT; cross clamp to start NMP), backtable and cannulation time (liver explant to start NMP), cannulation technique, liver anatomy, ability to commence and complete NMP.
Results: Back-table preparation and cannulation has been identified as the critical step in achieving a successful liver NMP. DBD and DCD livers (71:29) were perfused. Average CIT was 2hrs 3min with no significant difference between DBD and DCD livers (1hr 59min vs 2hr 12min; p=0.09) and similar cannulation times in both groups (1hr 30min DBD vs 1hr 36min DCD; p=0.39). This did not seem to prolong the retrieval process unless abnormal anatomy was encountered (1hr 49min abnormal vs 1hr 24min normal; p=0.00002).
IVC cannulation problems were rare. The only challenge related to PV cannulation was the risk of twisting of the vein. This was identified by absent PV flow measured by the device and corrected by untwisting the vessel.



HA cannulation has posed the greatest challenge to NMP technique due to common aberrant anatomy. One liver was not perfused due to aberrant left hepatic artery (aLHA) arising directly from the aorta (not included in analysis). Aberrant arterial anatomy was encountered in 31/100 cases (10xaRHA, 16xaLHA, 5xaLHA+aRHA) requiring either arterial reconstruction to be performed at the time of retrieval (n=10), or for the aortic tube to be excised intact with coeliac and SMA, enabling cannulation and perfusion directly through the aorta (n=3). More recently a bifurcated cannula has been used to perform dual perfusion of aberrant vessels (n=2).
In 2 cases the distance between aortic patch and the origin of LHA was too short for cannulation, requiring the use of an extension graft. Reconstruction of an aberrant artery on to GDA or splenic artery has been performed during ex-vivo NMP on 5 occasions.



All livers were successfully transplanted.
Discussion: NMP can be employed with all types of commonly encountered aberrant liver anatomy. With normal liver anatomy NMP should not significantly prolong the retrieval process but abnormal anatomy can cause a delay before commencing NMP. A reasonable level of surgical expertise and meticulous cannulation technique are required for successful perfusion.
Les Russell; OrganOx Ltd, UK; Chris Morris; OrganOx Ltd, UK

322.4

Effect of machine perfusion and urokinase on the kidney transplants with glomerular thrombosis

Xiaopeng Yuan, Chuanbao Chen, Jian Zhou, Changxi Wang.
Third Division of Organ Transplant Center, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, People's Republic of China.
Background: Glomerular thrombosis of cadaveric kidneys usually occurred in donors with disseminated intravascular coagulation (DIC). DIC donors are frequently associated with open craniocerebral injury, massive blood transfusion and cardiopulmonary resuscitation. The transplant professionals may be unwilling to use these kidneys because of high rate of primary non-function. We studied the effect of machine perfusion with urokinase on kidneys with glomerular thrombosis.
Methods: Twelve kidneys from 6 DIC donors (5 cases of donation after brain death and 1 case of donation after cardiac death) were preserved by machine pulsatile perfusion with LifePort. Urokinase (2 × 105 units/L) was added into the KPS-1 solution. All kidneys showed extensive glomerular thrombosis on procurement biopsy. Wedge biopsies were repeated after perfusion.
Results: Causes of donor death included closed head trauma in 3, open head trauma in 2 and anoxia in 1. The terminal serum creatinine level was 409 ± 230 μmol/L (149 -816μmol/L) and the final 24-hour urine volume was 3284 ± 1460 mL(1200 - 5480mL). Twelve kidneys were transplanted and no case of hematoma occurred. The rate of glomerular thrombosis was 28.4% ± 7.1% (19.4% - 41.9%) on procurement biopsy, it was decreased to 12.8% ± 4.8% (6.1% - 20.0%) after machine perfusion and thrombolytic treatment. After a follow-up of 6 - 28 months, all renal graft survived. The estimated glomerular filtration rate (by the Modification of Diet in Renal Disease equation) of renal grafts at 3, 6 and 12 months were 33.9 ± 5.9, 37.7 ± 5.4 and 38.8 ± 6.9 ml/min/1.73m2, respectively.
Conclusions: Pre-treatment with machine perfusion and urokinase seems to be effective for kidneys with massive glomerular thrombosis. But the function of the renal grafts after thrombolytic treatment seems to be suboptimal.

322.5

A novel method of split liver ex vivo perfusion

Viola Huang[1], Negin Karimian[2], Sharon Geerts[2], Irene Beijert[3], Safak Mert[2], Korkut Uygun[2], James F.Markmann[1], Heidi Yeh[1].
[1]Transplant Surgery, Massachusetts General Hospital, Boston, United States; [2]Center of Engineering in Medicine/Surgical Services, Massachusetts General Hospital, Boston, United States; [3]Department of Hepatobiliary Surgery, University of Groningen, University Medical Center Groningen, Groningen, Netherlands.

Introduction: Each year in the United States, hundreds of procured deceased donor livers are deemed unsuitable for transplantation and discarded[1]. Ex-vivo machine perfusion (EVMP) is a promising method for improving organ preservation and viability post procurement, and is being used in clinical trials in North America and Europe[2],[3]. However, the heterogeneity of clinical deceased donor livers makes it difficult to compare the results of EVMP to standard cold storage, both in clinical and pre-clinical research using discarded human livers. We therefore developed a split liver ex-vivo machine perfusion technique to provide matched controls for each liver.
Materials and Methods: Our group established a subnormothermic (21°C) machine perfusion protocol that allows assessment of liver function, cellular injury, and recovery in real time[4]. Previously, we had only used this system on whole livers. We adapted our protocol to test whether two lobes of the same liver would be comparable to each other during perfusion, and behave similarly to whole livers. Six discarded livers were split anatomically into right and left lobes. Each lobe was perfused separately for 3 hours via its own inflow vessels, and bile output was measured. Flow, resistance, and a biochemical profile of each lobe were monitored at regular time intervals.
Results: As with whole liver perfusions, each lobe exhibited decreasing portal venous and arterial resistance, lactate production, and ALT release over time. Overall bile and ATP production was low in the split livers compared to whole liver perfusions. Within each liver, right and left lobe behavior during perfusion was comparable: portal venous and arterial resistance, ATP levels, and lactate clearance showed no significant differences. ALT release per gram of liver weight were slightly higher in left lobes compared to right lobes.

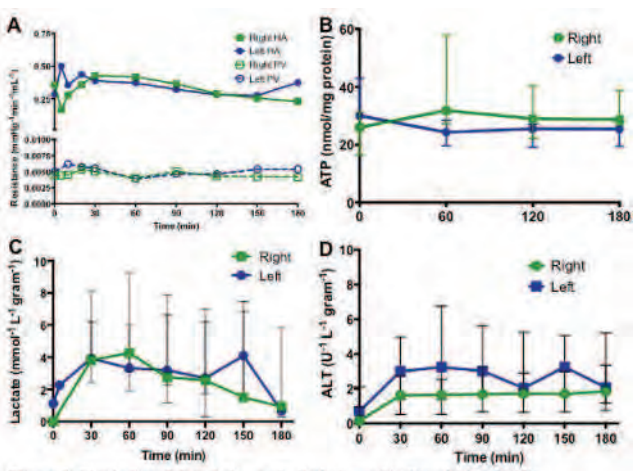


Figure 1: Single liver lobes behave similarly to each other during EVMP
Median portal venous and arterial resistance (A), cellular ATP content (B), arterial lactate (C), and ALT (D) measurements during ex-vivo machine perfusion of single liver lobes. Lactate and ALT are corrected for disparate lobe weights. Data presented as median and interquartile range.

Discussion: Single liver lobes behave similarly to whole livers during EVMP, and more importantly, right and left lobes behave similarly to each other, except for ALT production. More work needs to be done to understand why injury markers do not correspond between lobes as other physiologic and biochemical markers do.

Conclusion: Split liver perfusion is a novel approach that may allow direct comparison of different preservation techniques and treatments on the same liver, circumventing the need for extremely large numbers of experimental and control livers necessary with the wide variability of discarded human livers. Future experimental treatments could include de-fatting agents, anti-inflammatory factors, and immunosuppressants. Optimizing perfusion techniques using this system may eventually increase the supply of viable donor organs so that fewer need to be discarded.

References:

[1] Kim WR, Lake JR, Smith JM, Skeans MA, Schladt DP, Edwards EB, Harper AM, Wainright JL, Snyder JJ, Israni AK, Kasiske BL. OPTN/SRTR 2014 Annual Data Report: liver. *Am J Transplant*. 2016 Jan.
[2] Ravikumar R, Jassem W, Mergental H, Heaton N, Mirza D, Perera MT, Quaglia A, Holroyd D, Vogel T, Coussios CC, Friend PJ. Liver transplantation after ex vivo normothermic machine preservation: a Phase 1 (first-in-man) clinical trial. *Am J Transplant*. 2016 Jan 11.
[3] Guarrera JV, Henry SD, Samstein B, Reznik E, Musat C, Lukose TI, Ratner LE, Brown RS Jr, Kato T, Emond JC. Hypothermic machine preservation facilitates successful transplantation of "orphan" extended criteria donor livers. *Am J Transplant*. 2015 Jan;15(1):161-9.
[4] Bruinsma BG, Yeh H, Ozer S, Martins PN, Farmer A, Wu W, Saeidi N, Op den Dries S, Berendsen TA, Smith RN, Markmann JF, Porte RJ, Yarmush ML, Uygun K, Izamis ML. Subnormothermic machine perfusion for ex vivo preservation and recovery of the human liver for transplantation. *Am J Transplant*. 2014 Jun;14(6):1400-9.

322.6

Ex vivo perfusion of human livers induces leukocyte mobilisation

David Nasralla[1], William Critchley[2,3], Alexandra Ball[2,3], Rebecca Edge[2,3], Kavit Amin[2,3], Constantin Coussios[4], Peter Friend[1], James Fildes[2,3].
[1]Nuffield Department of Surgical Sciences, University of Oxford, Oxford, United Kingdom; [2]Manchester Collaborative Centre for Inflammation Research, University of Manchester, Manchester, United Kingdom; [3]The Transplant Centre, University Hospital of South Manchester, Manchester, United Kingdom; [4]Institute of Biomedical Engineering, University of Oxford, Oxford, United Kingdom.

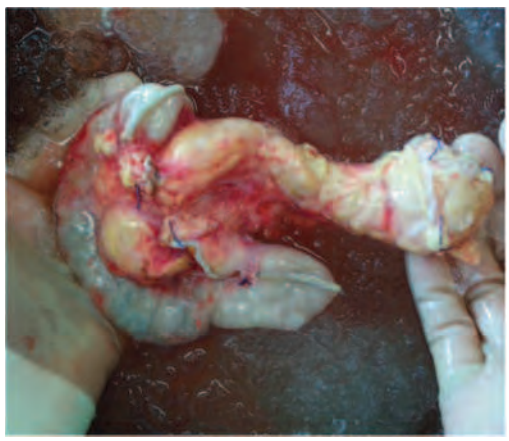
Introduction: Use of extended criteria donors has become widespread due to a lack of organs deemed acceptable for liver transplantation. However, the use of such organs is associated with graft dysfunction post-transplant. Ex vivo normothermic machine perfusion (NMP) is a preservation technique with the potential to enable evaluation and reconditioning of such marginal organs. We have previously demonstrated that ex vivo organ perfusion diminishes passenger leukocyte content; however, the effect on donor livers has not previously been assessed. We aimed to quantify major leukocyte populations within the perfusate at serial time points to determine whether ex-vivo NMP induces immune cell migration out of the organ. Leukocyte diapedesis was then correlated with clinical variables pre- and post-transplantation.
Methods: For each NMP, donor type (DBD/DCD), level of steatosis (n=14 none/mild vs n=7 moderate/severe), warm and cold ischaemic times (WIT/CIT), donor BMI and donor age were recorded. Peak AST values within 7 days post-transplant were also recorded. NMP was performed using the OrganOx metra device for up to 14 hours. Perfusate samples from n=21 procedures were obtained prior to organ attachment and at 30, 60 and 240 minutes. Leukocyte populations were phenotyped and quantified using flow cytometry.
Results: NMP induces the significant migration of leukocytes into the circuit within 30 minutes (B cells, T cells, NK cells, classical monocytes and non-classical monocytes, all $p<0.01$). Leukocyte number in the circuit is then diminished to marginally above baseline at 1 and 4 hours. Donor leukocyte diapedesis is not altered by donor type ($p=0.982$), WIT ($p=0.465$), CIT ($p=0.262$), donor BMI ($p=0.316$) or donor age ($p=0.285$) but is increased in association with steatosis severity ($p=0.016$). Additionally, post-transplant peak AST values are not affected by the extent of leukocyte migration ($p=0.726$). One liver experienced a second period of warm ischaemia during NMP, which induced a subsequent peak in immune migration into the circuit at the end of perfusion.
Discussion: Ex vivo NMP of the donor liver induces early diapedesis of passenger leukocytes into the circuit, an effect that is elevated in severely steatotic organs. The reduced immune cell count at 1 and 4 hours suggests that these leukocytes either adhere to the circuit or repopulate the organ. The additional immune migration in response to the second warm ischaemic injury suggests that immune return to the organ may occur. Our findings provide insight into the immunological processes that occur following liver transplantation, and specifically the relationship between steatosis severity and ischaemia reperfusion injury. Incorporating a leukocyte filter into the circuit may maximise the removal of passenger leukocytes and their transfer into the recipient, thereby modulating direct allorecognition.

322.7

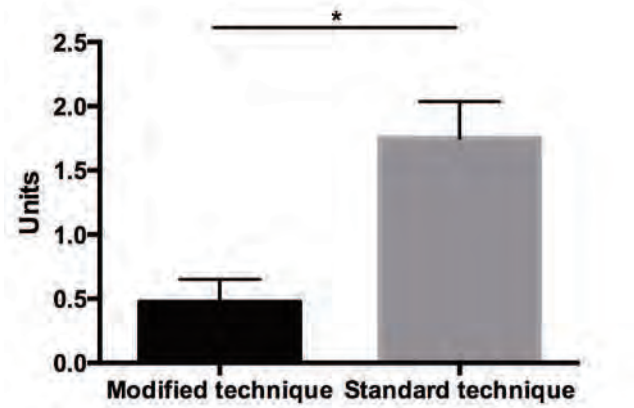
Harmonic scalpel in the rapid procurement of the pancreas for transplantation: Recipient outcomes

Ahmer Hameed[1,4], Teresa Yu[4], Lawrence Yuen[1,2], Vincent Lam[1,2], Brendan Ryan[1], Wayne Hawthorne[1,2], Richard Allen[1,2,3], Jerome Laurence[2,3], Henry Pleass[1,2,3].
[1]Department of Surgery, Westmead Hospital, Sydney, Australia; [2]Discipline of Surgery, University of Sydney, Sydney, Australia; [3]Department of Surgery, Royal Prince Alfred Hospital, Sydney, Australia; [4]Sydney Medical School, University of Sydney, Sydney, Australia.

Introduction: Dissection of the superior and inferior margins of the pancreas during the en bloc recovery of the pancreas is time consuming and is often associated with significant blood loss in recipients[1]. We have learnt from other surgical procedures that newer energy devices can be used to minimize dissection time and blood loss.
Methods: Here we describe a novel, two-phase technique for the procurement of the pancreas using ultrasonic shears (Harmonic Scalpel), both in situ and on the back-table, at a major tertiary centre. The Harmonic Scalpel is especially useful in fashioning a narrow SMA/SMV pedicle, overall allowing rapid skeletonization of the pancreas. It can further be employed on the back-table to divide the spleen and remove any excess/fatty tissue.



Recipient outcomes utilizing these donors were analyzed from 2011-2015, and included blood loss, packed red blood cell (PRBC) requirement, and primary graft thrombosis/loss rates. These were subsequently compared to a subset of pancreas recipients in whom this modified technique was not utilized (standard technique). Statistical analyses employed the Mann-Whitney and Fisher's exact tests for continuous and categorical variables, respectively (GraphPad Prism



Results: The mean PRBC requirement for all modified technique pancreas recipients (n = 21) was 0.5 units (95% CI 0.1-0.9), compared to 1.8 units (95% CI 1.2-2.3) in the standard technique group (n = 36; p < 0.01).
Mean blood loss in standard group was 928 ml (95% CI 533-1322), compared to 488 ml (95% CI 324-652 ml) in the modified technique patients (p = 0.14). During the whole study period, one of 20 (5%) simultaneous pancreas-kidney transplant patients in the modified technique group underwent graft pancreatectomy (day 9) due to primary graft vessel thrombosis compared to 6/102 (5.8%) in the standard technique group (p = 0.68). The mean time to graft loss in the standard group was 2.5 days.
Conclusions: Use of the Harmonic Scalpel in pancreas procurement simultaneously reduces blood product requirement in the recipient whilst allowing rapid organ procurement, without any increase in other adverse outcomes, including graft loss rates due to thrombosis.
Paul Robertson
References:
[1] Mizrahi SS, Jones JW, Bentley FR. Preparing for Pancreas Transplantation: Donor Selection, Retrieval Technique, Preservation, and Back-Table Preparation. *Transplantation Reviews*. 1996; 10: 1-12.

322.8

Hypothermic machine perfusion of en bloc kidneys from infant and neonatal donors

Abul Siddiky, Imeshi Wijetunga, Vivek Upasani, Muhammed Jameel, Lutz Hostert, Niaz Ahmad.
Department of Transplantation, St. James's University Hospital, Leeds, United Kingdom.

Introduction: Renal transplantation from donors less than two years of age have been rare in the United Kingdom (UK) at a rate of one transplant per year over the last two decades. Renal transplantation from a donor less than two-months of age was first performed in the UK in 2013. Until recently (2015), UK legislation did not permit the diagnosis of brain-stem death in infants under two-months of age and only donations after cardiac death (DCD) have been possible. In our experience, in situ hypothermic flush of the kidneys is often inadequate in these donors and the kidneys cannot be effectively flushed on the back-table after procurement. In this abstract we describe our experience with hypothermic machine perfusion (HMP) of infant and neonatal kidneys.
Methods: All renal transplants from donors less than two-months of age from the period March 2013 to January 2016 were included in this review. All kidneys were retrieved and transplanted en bloc with the abdominal aorta and inferior vena cava. Hypothermic machine perfusion was initially used for inadequate in situ flush and subsequently for all kidneys from such donors. The LifePort Kidney Transporter (Organ Recovery SystemTM) machine was used with Kidney Perfusion Solution (KPS-1) as the perfusion solution. Perfusion was started at a pressure of 30 mmHg which was gradually reduced to 20 mmHg over a period of 5 minutes, and maintained at this pressure up until transplantation.
Results: In the review period, nine transplants were performed from donors less than two-months of age; eight from DCD and one from donation after brain death (DBD). Two kidneys had static cold storage (SCS) and seven underwent HMP. All the kidneys in the HMP group had blood removed from the kidney once on the machine and had visibly improved flush. There was one primary non-function (SCS) and one delayed graft function (HMP). Seven kidneys had primary function (1 SCS, 6 HMP). A very high resistance and low flow rate were observed in the machine perfused kidneys with a mean resistance of 1.15 ± 0.48 and flow rate of 19.00 ml/min ± 7.05 (see Table 1).

Table 1. Summary of demographic data and machine parameters at time of transplantation

En bloc	Age (days)	Weight (Kg)	Flow (ml/min)	Resistance
1	23	3.2	21	1.02
2	30	4.2	10	1.98
3	6	3.4	10	1.64
4	58	4.2	22	0.95
5	0.5	1.3	20	0.90
6	238	7.2	20	0.95
7	2	3.0	30	0.60
Mean (SD)	51.07 (84.87)	3.79 (1.79)	19 (7.05)	1.15 (0.48)
Range	0.5-238	1.3-7.2	10-30	0.60-1.98

Discussion: This is the first reported series of hypothermic machine perfusion of kidneys from infant and neonatal donors. The current machines are not calibrated for paediatric, particularly infant and neonatal, kidneys. A high resistance and low flow rate has been observed in these kidneys and our evidence suggests that this is not an indicator for function or quality but normal for en bloc kidneys. Delayed graft function may have to be redefined in cases of infant and neonatal donor kidneys.

322.9

Perfusion characteristics during controlled oxygenated rewarming and subnormothermic machine perfusion of steatotic human livers

Dieter P. Hoyer[1], Anja Gallinat[1], Juergen W.Treckmann[1], Tamas Benkö[1], Ursula Rauen[2], Andreas Paul[1], Thomas Minor[1].
[1]General, Visceral and Transplantation Surgery, University Hospital Essen, Essen, Germany; [2]Institute of Physiological Chemistry, University Hospital Essen, Essen, Germany.

Introduction: Controlled oxygenated rewarming (COR) of cold stored livers by machine perfusion demonstrated convincing results in the first clinical pilot series[1] and was, hence, now applied in steatotic liver allografts. This study analyzes short-term available perfusion characteristics.
Methods: Between 03/2015 and 06/2015 eight distally procured livers from DBD-donors, which had been rejected by other transplant centers and had a histologically proven steatosis of ≥30% were subjected to the COR protocol before transplantation: Immediately after arrival in our transplant center hepatic artery and portal vein were cannulated. Gentle rewarming of the graft was effectuated thereafter by pressure controlled oxygenated machine perfusion (Organ Assist, Groningen, NL) while gradually increasing perfusate temperature up to 20°C. Real-time biochemical analysis of perfusate parameters was carried out in constant time intervals during the controlled rewarming.
Results: Median donor age was 60 (50-71) years, ischemic times were 7.6 (6.7-13.5) hours and the DRI was 2.1 (1.6-2.5). 2 out of 8 organs were not transplanted due to the macroscopic appearance and the overall risk profile. During the rewarming the median arterial flow increased constantly from 105 to 159.5 ml/min and the median portal flow from 165 ml/min to 255 ml/min. Weighing of the allografts showed similar values before and after perfusion (median weight ratio after/before perfusion= 1.01). Histological analysis did not reveal any negative perfusion associated effects, especially no injury to the endothelium or edema. Oxygen consumption increased steadily during the rewarming period, while acid base homeostasis and lactate levels remained stable. Perfusate analysis demonstrated rising glucose concentrations from median 133.5 to 256 mg/dl, triglyceride values from median 16.5 up to 33.5 mg/dl and potassium levels from 21.9 to 30 mmol/l. Enzyme leakage peaked after reaching the subnormothermic temperature range (Median LDH, AST and ALT: 7686 U/l, 3263 U/l and 2395 U/l, respectively). Mitochondrial release of GLDH showed increasing values from median 80.5 to 137.1 U/l during the rewarming period. The two discarded livers demonstrated a continuous release of aminotransferases, with significantly higher highest peak values in the perfusate (8542 U/l & 5186 U/l) compared to transplanted organs.
A significant correlation ($r2=0.8$; $p<0.05$) was observed for the AST release into the perfusate with the postoperative aminotransferase peak. After minimum follow-up of 6 months after transplantation, all recipients are alive with excellent graft function.
Conclusions: Short-term available perfusion parameters during rewarming to subnormothermic temperatures help to characterize steatotic allografts. Further validation of correlations with the outcome in clinical trials represents a possible new tool to assess eligibility for transplantation and might therefore reduce the rate of discarded organs.
References:
[1] Hoyer et al. Transplantation. 2015 Jan;100(1):147-52.

322.10

Peri-operative organ perfusion with ATG-fresenius results in improved graft function in clinical liver and kidney transplantation

Paul Ritschl[1], Julia Günther[2], Manuel Maglione[2], Lena Hofhansel[2], Susanne Ebner[2], Sascha Weiss[1], Nicole Bergmann[2], Annemarie Weissenbacher[2], Christian Margreiter[2], Benno Cardini[2], Matthias Biebl[1], Robert Sucher[1], Christian Denecke[1], Felix Aigner[1], Robert Öllinger[1], Stefan Schneeberger[2], Johann Pratschke[1], Katja Kotsch[1].
[1]Department of General, Visceral and Transplantation Surgery, Charité – Universitaetsmedizin Berlin, Berlin, Germany; [2]Center for Operative Medicine, Department of Visceral, Transplant and Thoracic Surgery, Medical University of Innsbruck, Innsbruck, Austria.

The gap between the number of patients awaiting an organ and the number of available organs is steadily increasing. Therefore the use of marginal donor organs with the consequences of higher risk of unfavorable transplantation (Tx) outcome has become reality. The most prominent characteristics impacting organ quality organs include age, cold ischemia, or donation after cardiac death (DCD). Consequently these organs are prone to a higher grade of ischemia-reperfusion injury (I/RI). In order to improve marginal allograft outcome, machine perfusion of solid organs has regained the highest attention. However, further research is necessary before it will become an established technique for the daily transplant routine. As antithymocyte globulin (ATG) is an established clinical therapeutic agent and has been further demonstrated to reduce I/RI in both clinical and experimental Tx, we hypothesized that pre-operative perfusion with ATG may be beneficial in reducing I/RI-related inflammation leading to improved graft function post Tx. We therefore performed a randomized controlled clinical trial involving 30 liver (LTx) and 50 kidney recipients (NTx) at the Department of Surgery, Innsbruck Medical University. Primary endpoints were defined as graft and patient survival after 7 days and one year; secondary endpoints were defined as initial graft function, presence of acute rejection, and clinical parameters. Prior to implantation organs were perfused and incubated for 5 min. with ATG-Fresenius (n=24 for kidney with 12,5mg ATG dissolved in 0.5l 0.9% NaCl; n=16 for liver with 25mg ATG dissolved 1l 0.9% NaCl) and subsequently flushed with NaCl. Control organs were perfused with saline only (n=26 kidney; n=14 liver). No significant differences were observed regarding donor and recipient age, gender, cold ischemia time, HLA mismatch, body mass index or hospital stay between ATG perfused (AP) and control perfused (CP) organ recipients. Mean age of all NTx recipients was 55.04 ± 13.64 yrs and of liver recipients 57.23 ± 9.65 years. During the early hospitalization phase, 16 out of 26 CP-NTx patients required dialysis during the first 7 days post Tx, whereas only 10 out of 24 AP-NTx patients received dialysis (p=n.s.). AP-NTx recipients illustrated clearly better graft function until day 15 post Tx reflected by lower creatinine and urea levels. This result was more evident in younger AP-NTx recipients (<55 yrs) compared with aged AP-NTx (≥55 years) patients (p=0.049 at day 7 post Tx) – an effect which was not observed for CP-NTx patients. This result was further confirmed for young AP-LTx compared to CP-LTx patients reflected by reduced gamma-GT levels at day 10 post Tx (p=0.034). Moreover, AP-perfused liver biopsies illustrate lower levels of CRP compared with CP livers post reperfusion (p=0.02). We present here the first clinical study on peri-operative organ perfusion with ATG suggesting an age-dependent effect independent from the organ transplanted.

322.11

What should be moved, graft or machine? Travel strategy in an expanded criteria donor program. organ procurement and transplant organizations (OPO) perspective

Francisco Javier Burgos[1], Victoria Gomez-Dos Santos[1], Victor Diez-Nicolas[1], Sara Alvarez[1], Vital Hevia[1], Adolfo Martinez[2], Laura Martinez[1], Ana Fernandez[3].
[1]Urology, Hospital Ramón y Cajal, Madrid, Spain; [2]Transplant Coordination , Hospital Ramón y Cajal, Madrid, Spain; [3]Nephrology, Hospital Ramón y Cajal, Madrid, Spain.

Introduction: The growing demand for organs has led to the expansion of donor source to include expanded criteria donors (ECD) and donors after cardiac death (DCD). The Machine Preservation Trial has showed that HMP decreases the incidence of PNF and DGF, and increases 1-year graft survival compared to standard static cold storage.
A critical issue for OPOs is to determine if HMP is necessary all along the cold isquemia time before transplantation or if only a period of "in-house" HMP after cold storage (hypothermic reconditioning) could be enough to improve kidney graft function. Several groups have proposed reconditioning of cold-stored kidneys even if clinical logistics only permit one hour of therapy. The aim of the study is to assess the impact of MP in local and extrahospitalary donors in terms of DGF. The study protocol was approved by the Hospital Ethics Committee in Clinical Research.
Material and Methods: Study data were prospectively collected in a cohort of consecutive ECD between February 2012 and September 2015. Ethical approval was obtained from Ethics Review Board. Pulsatile HMP was provided by LifePort® Kidney Transporter machine. Local kidneys were placed on LifePort in the donor operating room and delivered to the recipient on the pump. Kidneys imported from other organ procurement hospital were initially placed on ice until reaching our transplant center. No graft was discarded attending to perfusion parameters. DGF was defined as no fall in serum creatinine concentration of 10% or more within 48 hours.
Results: 112 ECD donors and 148 recipients were included in the analysis. 68.2% donors were of extrahospitalary origin. Local and extrahospitalary donors were different for brain death etiology, donor age, HTA and DM rates (Fig 1) with worse risk factors for local donors.

LOCAL AND EXTRAHOSPITALARY DONORS CHARACTERISTICS			
Sex (Male / Female)	Local Donor	59.6% / 40.4%	0.455
	Extrahospitalary	53.0% / 47.0%	
Brain Death Etiology (CVA / Trauma)	Local Donor	91.5% / 0%	**0,031**
	Extrahospitalary	72.3% / 13.9%	
Donor age (Me CI 95%)	Local Donor	65.5 (60.5; 70.4)	**0.000**
	Extrahospitalary	74.0 (71.8; 76.3)	
HTA	Local Donor	89.4%	**0,003**
	Extrahospitalary	66.0%	
DM	Local Donor	51.1%	**0,000**
	Extrahospitalary	18.6%	

As we expect total cold isquemia time and relative machine perfusion time were significantly different (Fig 2).

Preservation Characteristics and DGF rate			
Glomerulosclerosis (%) (Me; CI 95%)	Local Donor	8.1 (6.1-10.0)	0.553
	Extrahospitalary Donor	9.1 (7.6-10.7)	
Cold isquemia time (h)	Local Donor	16.2 (15.1 – 17.3)	**0,002**
	Extrahospitalary Donor	18.4 (17.4 – 19.3)	
Relative Machine Time	Local Donor	0.92 (0.89 – 0.95)	**0,000**
	Extrahospitalary Donor	0.44 (0.40 – 0.48)	
DGF	Local Donor	13.0% (6/46)	**0.032**
	Extrahospitalary Donor	29.5% (28/95)	

DGF rate were also significantly different for local and extrahospitalary donors respectively (13.0% vs 29.5%; p= 0.032). DGF rate was higher in the extrahospitalary donor group that are iniatilly preserved on ice and only connected to the pump when they reached our hospital. So pump lenght of time was lower. In multivariable risk analysis machine preservation time was a protective factor. Each hour in the pump diminishes DGF risk in a 20%.
Conclusions: Despite positive results of short periods of HMP reconditioning in the experimental setting, better results in terms of immediate graft function in grafts have been obtained from local donors placed on LifePort for the complete period of cold isquemia time. This conclusion could be translated in a machine travel policy recommendation for OPOs.

322.12

Hyperbranched polyglycerol-based organ preservation solution: Advantages for cold kidney perfusion

Caigan Du[1], Shadan Li[1], Iren Constantinescu[2], Qiunong Guan[1], Manu T.Kalathottukaren[2], Donald E.Brooks[2,3], Christopher Y.C.Nguan[1], Jayachandran N.Kizhakkedathu[2,3].
[1]Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada; [2]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada; [3]Department of Chemistry, University of British Columbia, Vancouver, BC, Canada.

Background: Both efficient and effective perfusion as well as effective cold storage of donor organs with a preservation solution are critical for the prevention of donor organ injury during procurement; however current preservation solutions including University of Wisconsin (UW) solution do not always yield the expected outcomes. Our preliminary study has demonstrated that hyperbranched polyglycerol (HPG)-based solution is a novel alternative to current solutions in cold storage of donor organs[1]. The current research was designed to evaluate the advantages of HPG-based solution for cold kidney perfusion.
Methods: Perfusion efficiency of preservation solutions (HPG versus UW) was tested in mouse kidneys, and their effectiveness of blood washout was evaluated using a semi-quantitative scoring system. Tissue damage was examined by histology. The interactions of preservation solutions with human red blood cells (RBCs) were examined using both sedimentation and aggregation tests.
Results: here, we showed that the lower viscosity of HPG-based solution was correlated with faster and more efficient perfusion through donor kidneys compared to UW solution. HPG-based solution was also more effective in removing RBCs from the kidney compared to UW solution, and was associated with less tissue damage in donor kidneys. The in vitro examination of the preservation solution – RBC interactions showed that unlike UW solution that significantly accelerated RBC sedimentation and induced RBC hyperaggregation, HPG solution had minimum impact on the RBC sedimentation and prevented RBC aggregation.
Conclusion: Our data suggest that HPG solution is more efficient and effective than UW solution in the removal of the blood from donor kidneys and caused less tissue damage, suggesting that HPG solution is a promising candidate to supplant standard UW solution for donor kidney perfusion in transplantation.
References:
[1] Gao S, Guan Q, Chafeeva I, et al. Hyperbranched polyglycerol as a colloid in cold organ preservation solutions. PLoS One 2015; 10 (2): e0116595.

322.13

Ex vivo normothermic perfusion reduces kidney immunogenicity prior to transplantation via removal of passenger leukocytes

John Stone[1,2], Alexandra L.Ball[1,2], William R.Critchley[1,2], Triin Major[1,2], Rebecca J.Edge[1,2], Kavit Amin[1,2], Marc Clancy[3], James E.Fildes[1,2].
[1]Manchester Collaborative Centre for Inflammation Research (MCCIR), University of Manchester, Manchester, United Kingdom; [2]The Transplant Centre, University Hospital of South Manchester, Manchester, United Kingdom; [3]The Transplant Unit, Western Infirmary Hospital, Glasgow, United Kingdom.

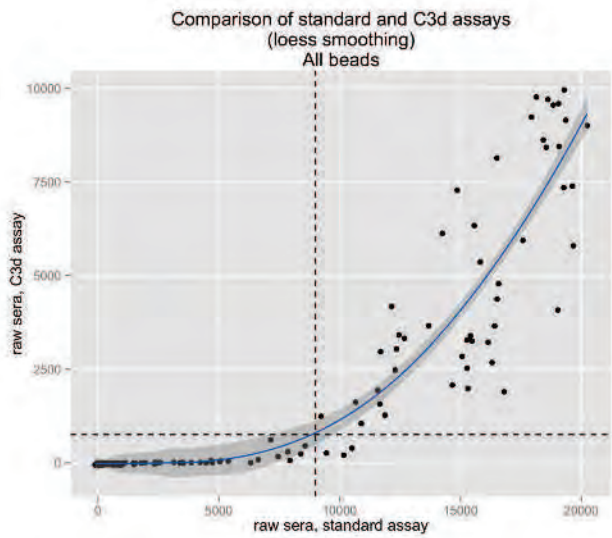
Introduction: Ex vivo normothermic perfusion (EVNP) can improve access to kidney transplantation by enabling the safe preservation and evaluation of extended criteria donor organs, following both circulatory and brain stem death. Aside from evaluation, EVNP can be used to characterise the inflammatory and immunological contribution of the donor kidney in isolation. Here we demonstrate that the kidney possesses a clinically relevant immune repertoire that can orchestrate allorecognition and drive pro-inflammatory responses. Furthermore, EVNP can be used as an immunomodulatory strategy to manipulate the donor kidney prior to transplantation.
Methods: Explanted porcine kidneys (n=5) underwent 2 hours of static cold storage followed by 6 hours of EVNP. Perfusate samples were collected at baseline and hourly intervals. Immune cells were characterised via flow cytometry and an inflammatory profile was generated via cytokine quantification. Cell free mitochondrial and genomic DNA was also determined as markers of cell death.
Results: All kidneys functioned within normal parameters and met the criteria for transplantation at the end of perfusion. Throughout perfusion there were continuous increases in pro-inflammatory cytokines including IFN-γ ($p=0.021$), IL-1α ($p=0.025$), IL-1β ($p=0.010$), IL-1RA ($p=0.025$), IL-2 ($p=0.017$), IL-6 ($p=0.007$), IL-8 ($p=0.007$), IL-12 ($p=0.007$) and IL-18 ($p=0.009$). IL-10 also increased ($p=0.007$), however GM-CSF, IL-4 and TNF-α did not change from baseline, suggesting EVNP drives a non-specific pro-inflammatory response. Increasing concentrations in cell free mitochondrial and genomic DNA were also observed ($p=0.008$ and $p<0.001$ respectively), suggestive of cell death. During perfusion, there was also a marked cellular diapedesis of T cells ($p=0.052$), B cells ($p=0.026$), NK cells ($p=0.015$) and monocytes ($p=0.040$) from the kidney into the circuit. Minor populations of neutrophils, eosinophils and macrophages were also detected.
Discussion: EVNP initiates a non-specific pro-inflammatory cytokine storm and a release of mitochondrial and genomic DNA. This is likely to be responsible for immune cell activation and mobilisation into the circuit prior to transplantation. Interestingly however, the pro-inflammatory EVNP environment did not impact upon renal function. This data therefore suggests that EVNP can be used to immunodeplete and saturate the pro-inflammatory capacity of the donor kidney prior to transplantation. In addition it could provide a platform for the development of novel immunomodulatory therapies.

323.1

Correlation Between C3d-fixing Donor Specific HLA Antibody and Luminex MFI

Paolo Malvezzi[1], Morgane Villemaire[1], Thomas Jouve[1], Anne Legall[2], Dominique Masson[2], Lionel Rostaing[1].
[1]Clinique de Néphrologie, CHU Grenoble, Grenoble, France; [2]Laboratoire de Histocompatibilité, EFS , Grenoble, France.

Introduction: Chronic antibody–mediated rejection is the main cause of late kidney graft loss. Finding in the serum donor-specific antibodies (DSA) is the main criteria for this diagnosis. Single-antigen (SA) Luminex assays provide DSA identification and a semi-quantitative estimate of the amount of antibody by mean fluorescence intensity measurement (MFI). Recent data have shown that patients whose DSA fix C3d have a worse clinical outcome, implying that C3d specific Luminex assays might provide useful prognostic data. In this work, we compared C3dDSA to standard MFI in a cohort of patients having developed de novo class 2 DSA and analyzed graft survival at one year following detection.
Methods: We included kidney graft recipients transplanted between 2005 and 2015 in our center, who developed de novo class 2 DSA. Serum was tested by standard SA Luminex technique and by C3d – fixing antibody detection system (IMMUCOR®) according to the manufacturer's instructions. Clinical data was analyzed regarding graft function and survival at DSA detection and one year later.
Results: 41/924 patients (4.4%) developed class 2 DSA. 65 serum samples were analyzed. Among them, 43 serum samples were negative for C3dDSA (66%). As shown in figure 1, an MFI threshold of 9000 in SA Luminex assay permitted to differentiate between the negative and the positive C3dDSA. This also holds true when all single bead results are taken into account.
From a clinical standpoint: 23% of patients doubled their creatinine level or lost their graft at one year. C3d positivity was not significantly associated to worse survival.
Conclusion: We show that C3d fixing antibody detection is highly correlated to SA Luminex MFI: in our cohort an MFI threshold of 9000 in SA Luminex predicts C3d positivity. Furthermore we do not find any short-term correlation between C3d positivity and graft survival. We conclude that when chronic antibody mediated rejection occurs the concentration of antibodies probably determine complement activation.



323.2

Pretransplant AT1Rabs are associated with acute rejection in 2-haplotypes kidney transplant recipients

Jose Manuel Arreola[1,2], Josefina Alberu[2], Mario Vilatoba[2], Alan Contreras[2], Julio Granados[2], Luis Morales-Buenrostro[3].
[1]Transplantation Department, Centenario Hospital Miguel Hidalgo, Aguascalientes, Mexico; [2]Transplantation Department, National Institute of Medical Sciences and Nutrition "Salvador Zubirán", Mexico DF, Mexico; [3]Nephrology and Mineral Metabolism, National Institute of Medical Sciences and Nutrition "Salvador Zubirán", Mexico DF, Mexico.

Kidney transplant recipients (KTR) who share 2-haplotypes (2-H) with their donors are privileged patients with a mean reported graft survival of 20 years. Nevertheless these patients are not free of acute rejection (AR) episodes and a high proportion of them reject the graft with discontinuation of immunosuppression. This immunological events are not explained by HLA antigens, then other non-HLA antigens could play a role in this AR episodes. Angiotensin II type 1 receptor antibodies (AT1Rabs) are non-HLA abs that has been associated with acute allograft rejection and graft loss even in 2-haplotypes KTR.
Methodology: This is a retrospective cohort study of 2-H living donor KTR between March/2009 and Sept/2013. All the patients have pre KT AT1Rabs measured by ELISA (CellTrend GMBH, One Lambda Canoga Park, CA, USA) and at least one protocol biopsy at 12 months of follow-up; all other biopsies were performed for graft dysfunction. Biopsy findings were classified according to BANFF 2013 criteria.
Results: Twenty-two KTR were included, with a mean age of 31.5 yr (min-max 18 – 51); M:F 12/10. The mean posttransplant follow-up was 2.8 yrs (min- max 0.9 – 5.8 yrs). During this period, 6/22 (27.2 %) had AR episodes (Cellular AR= 4, antibody mediated AR = 1, and mixed AR=1). Three of them had the AR episode during the first year of follow-up and the other three patients had the episode after that time. The median pre KT level of AT1Rabs was 11.7 U (IQR 7.19 – 18.93). Patients with AR had higher AT1Rabs level (19.7 IQR 14.1 – 89) than patients without AR (9.76, IQR 6.51 – 13.18) (p=0.055). AT1Rabs with cutoff value of 14 U was the best predictor of AR (Figure 1).

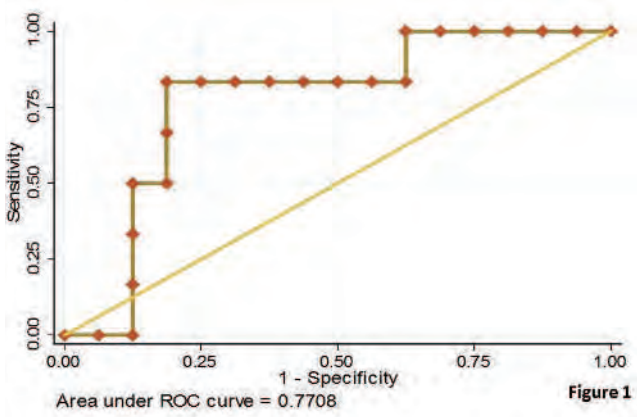


Figure 1

In the univariate analysis only AT1Rab > 14 U significantly predicted AR. (OR 21.6, 95% CI 1.81 – 260, p=0.015). Also, after the first 6 months of follow-up there was a significant difference in eGFR between KTR with and w/o AT1R>14 U (Figure 2).
Conclusions: AT1Rabs are related with AR episodes and worse graft function in 2-H KTR population. These observations add information to the role of non-HLA antigens in KTR.

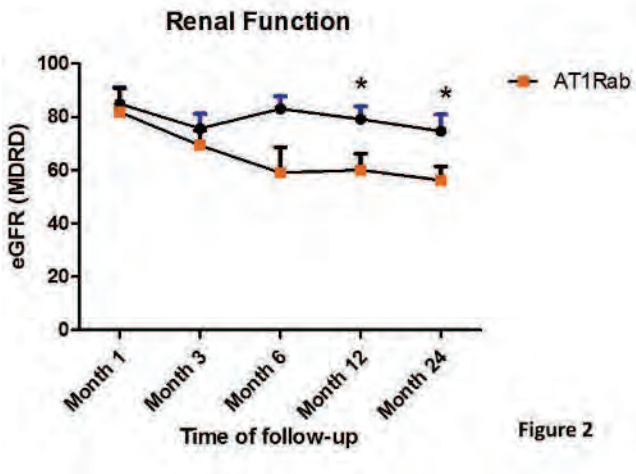


Figure 2

References:
[1] 1. Dragun D. Angitensin II type 1-receptor activating antibodies in renal-allograft rejection. N Engl J Med 2005; 352:558-69.
[2] Reinsmoen NL. Anti-angiotensin type 1 receptor antibodies associated with antibody mediated rejection in donor HLA antibody negative patients. Transplantation 2010; 90:1473-7
[3] Reinsmoen NL. Role of angiotensin II type 1 receptor-activating antibodies in solid organ transplantation. Hum Immunol 2013; 74:1474-7

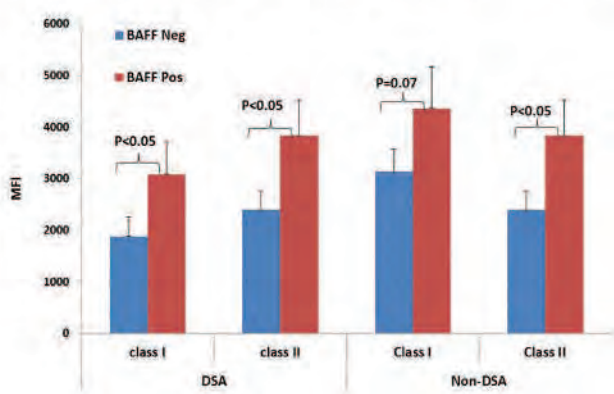
323.3

The levels of de novo HLA antibodies are determined by soluble B cell-activating factor belonging to the TNF family (BAFF) post transplantation

Junchao Cai[1], W. Huang[1], L.M. Rebellato[2], C.E. Haisch[3], KP Briley[2], P. Bolin[4], S.A. Kendrick[5], C. Morgan[2], P.I. Terasaki[1], M.J. Everly[1].
[1]Terasaki Foundation Laboratory, Los Angeles, CA, United States; [2]Department of Pathology, East Carolina University, Greenville, NC, United States; [3]Department of Surgery, East Carolina University, Greenville, NC, United States; [4]Department of Medicine, East Carolina University, Greenville, NC, United States; [5]Eastern Nephrology and Associates, Greenville, NC, United States.

It has been shown that excessive level of BAFF causes abnormally high antibody production which results in autoimmune diseases. Here we investigate the effect of BAFF on the production of HLA antibody after renal transplantation. A total of 171 transplant recipients who received renal transplants during 2004-2009 were enrolled into this study. The sera before transplantation and 3 to 6 month post-transplantation were tested for BAFF using Luminex technology. Sera from 20 normal healthy males were used as negative controls. The cutoff value to determine the positivity of soluble BAFF was set at 3500 MFI based on the average level of normal controls plus 3 standard deviations. . HLA class I and class II antibodies were detected using single antigen bead assay (One Lambda Inc, Canoga Park, CA). The level of BAFF post-transplant was significantly lower than that of pre-transplant sera (3100+/-1534 vs 3475+/-1289, p=0.0032). 63% of patients had a decreased level of BAFF while 37% of patients had an increased level of BAFF post-transplant. Interestingly, the peak levels of de novo HLA

Figure 1. The soluble BAFF is associated with high levels of both DSA and NDSA



In conclusion, the level of BAFF in peripheral blood was significantly reduced post-transplantation for the majority of renal transplant recipients, possibly due to immunosuppression. However, patients with higher levels of BAFF post-transplant developed de novo HLA antibodies. These data suggest that strategies to interfere with the production of BAFF may help inhibit the production of HLA antibodies.

323.4

Preformed C1q-binding DSA are more clearly associated with adverse outcomes after HLA-incompatible kidney transplantation than DSA strength

Jorge Malheiro[1], Ana Castro[1], Sandra Tafulo[2], Leonidio Dias[1], La Salete Martins[1], Idalina Beirão[1], António Castro-Henriques[1].
[1]Nephrology & Kidney Transplantation, Centro Hospitalar do Porto, Porto, Portugal; [2]Histocompatibility Lab, Centro do Sangue e Transplantação do Porto, Porto, Portugal.

Introduction: Transplantation in the presence of donor-specific antibodies (DSA) is challenging given the high risk of acute and/or chronic active antibody mediated rejection (AMR). We aimed to study the impact of preformed DSA characteristics (strength and C1q-binding ability) on posttransplant outcomes.
Materials and Methods: We retrospectively studied 60 kidney-transplanted patients with negative cytotoxic crossmatch but with preformed DSA detected by single antigen beads (SAB) assays (IgG and C1q kits).
Results: Cohort median DSA MFI was 6500. Thirteen patients had C1q-binding DSA. Eighteen patients experienced AMR.

Comparison of clinical and immunological characteristics according to C1q DSA status

	C1q- DSA N=47	C1q+ DSA N=13	P
At transplant			
Recipient age, mean	48	48	0.920
Male recipient, %	32	46	0.349
Retransplant, %	34	77	0.006
Cytotoxic PRA (%), median	4	35	0.036
HLA mismatch, mean	3.68	4.00	0.327
DSA MFI, median	5300	20000	0.00002
DSA number, median	2	4	0.001
ATG induction, %	57	92	0.023
Desensitization with Ivig+PP+Rtx, %	9	31	0.059
After transplant			
Delayed graft function, %	34	39	0.755
AMR, %	17	77	0.00003
eGFR at 1-year, mean	57	43	0.029
Proteinuria > 0.5g/g at 1-year, %	12	55	0.006
DSA detectable at 1-year, %	36	82	0.010

ROC curve analysis showed that the optimal DSA MFI cut-off for prediction of AMR was 10000.

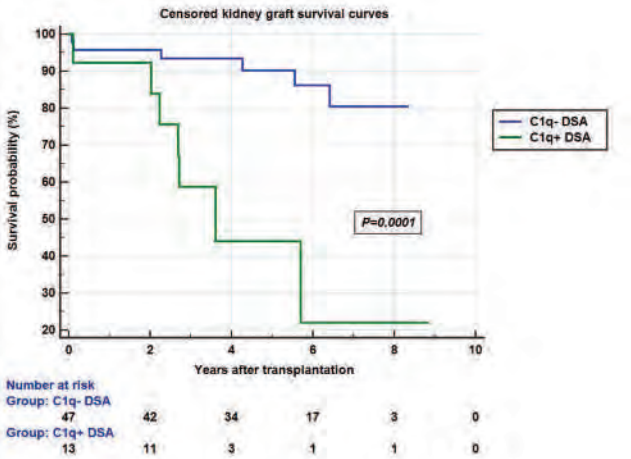
Analysis of DSA characteristics as predictors of AMR

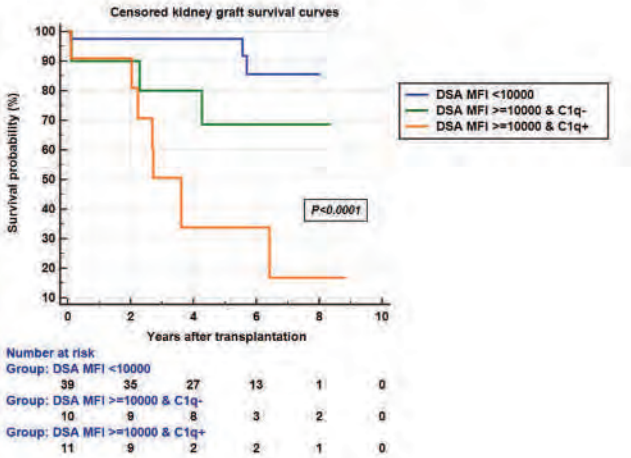
	Sensitivity (%)	Specificity (%)	Positive predictive value (PPV, %)	Negative predictive value (NPV, %)	Diagnostic odds ratio (DOR)
DSA MFI ≥10000 (n=21)	72	81	62	87	11.1
C1q+ DSA (n=13)	56	93	77	83	16.2

Comparison of clinical and immunological characteristics according to C1q DSA status in patients with a DSA MFI ≥10000

	C1q- DSA N=10	C1q+ DSA N=11	P
At transplant			
Retransplant, %	40	91	0.013
Cytotoxic PRA (%), median	50	52	0.832
HLA mismatch, mean	3.80	4.09	0.510
DSA MFI, median	18000	21000	0.313
ATG induction, %	70	100	0.050
Desensitization with Ivig+PP+Rtx, %	20	27	0.606
After transplant			
AMR, %	40	82	0.049
eGFR at 1-year, mean	47	39	0.314
Proteinuria > 0.5g/g at 1-year, %	22	56	0.147
DSA detectable at 1-year, %	56	100	0.023

Censored kidney graft survival at 6-years was 81.3% in patients with C1q- DSA and 44.1% in those with C1q+ DSA.





Censored kidney graft survival at 6-years was 85.6% in patients with DSA MFI <10000, 68.6% in those with C1q-/MFI ≥10000 DSA and 33.7% in those with C1q+/MFI ≥10000 DSA.

Conclusions: C1q-binding DSA was highly correlated with DSA strength. Nevertheless, AMR was better predicted by the presence of C1q+ DSA (higher DOR and PPV) than by using the optimal DSA MFI cut-off. Moreover, C1q+ DSA was associated with lower graft function, increased proteinuria and higher persistence of DSA at 1-year. C1q+ DSA patients had poorer graft survival than C1q- DSA ones, even within those with strong (MFI ≥10000) DSA.

323.5

ABO incompatibility might protect against chronic antibody mediated rejection caused by de novo DSA after renal transplantation

Manabu Okada[1,2], Kenta Futamura[1], Takayuki Yamamoto[1], Takahisa Hiramitsu[1], Makoto Tsujita[1], Norihiko Goto[1], Shunji Narumi[1], Yoshihiko Watarai[1], Kenta Iwasaki[2], Takaaki Kobayashi[2].
[1]Transplant and Endocrine Surgery, Japanese Red Cross Nagoya Daini Hospital, Nagoya, Japan; [2]Transplant Surgery, Aichi Medical University, Nagakute, Japan.

Introduction: There seems to be a quite difference in graft outcome between ABO-incompatible and HLA-incompatible renal transplantation (ABO-I and HLA-I), although anti-donor (anti-A/B and anti-HLA) antibodies could similarly be attached to the graft. Chronic antibody mediated rejection (AMR) which remains a major obstacle to long-term graft survival could be influenced by anti- HLA antibody, but not by anti-A/B antibodies. We have demonstrated that anti-A/B antibody binding to endothelial cells might induce "graft accommodation" (no injury even in the presence of anti-donor antibody) through up-regulation of CD55 and CD59, and down-regulation of HLA class I and II expression by in vitro experiments, unlike anti-HLA antibody. The aim of this study was to elucidate the potential benefit of ABO-incompatibility, regarding chronic AMR.
Methods: 521 living donor renal transplantation were performed at Nagoya Daini Red Cross Hospital between 2005 and 2011. Pretransplant DSA-positive recipients (n=34) were excluded. Patients (n=25) who could not be followed up from 2011 due to graft loss, death or changing hospital, were also excluded. Thus, a total of 448 (129 ABO-I and 319 ABO-Id/C) were included in this study. Annual HLA antibody screening and HLA specificity tests have detected de novo DSA, which encouraged patients to undergo indication biopsy even without renal dysfunction.
Results: 5-year graft survival rates of ABO-I, ABO-Id/C and HLA-I were 93%, 97% and 75%, respectively. Main cause of graft failure in ABO-I was death with functioning grafts such as infection, CVD and malignancy, which was considered to be related to desensitization.
Between 2011 and 2015, de novo DSA was detected in 11/129 (8.5%) of ABO-I, and in 46/319 (14.4%) of ABO-Id/C. Indication biopsy was conducted in 40 patients who had de novo DSA. Biopsy-proven CAMR were diagnosed in 18 patients. 22 patients had no CAMR pathologically. Biopsy-proven subclinical CAMR was detected in 2/8 (25%) of ABO-I, whereas it was observed in 16/32 (50%) of ABO-Id/C.
Conclusion: The possibility has been suggested that anti-A/B antibody binding might have a protective effect on chronic AMR caused by de novo DSA. Promising results might be expected in ABO-I, if the adverse effect of desensitization therapy could be minimized.

323.6

De novo donor specific antibodies in kidney transplantation - a systematic review and meta-analysis

Ankit Sharma[1,2], Joshua R.Lewis[1,2,3], Wai H.Lim[3,4], Suetonia Palmer[5], Giovanni Strippoli[1,2], Jeremy R.Chapman[6], Jonathan C.Craig[1,2], Germaine Wong[1,2].
[1]Centre for Kidney Research, Children's Hospital at Westmead, Westmead, Australia; [2]School of Public Health, The University of Sydney, Sydney, Australia; [3]School of Medicine and Pharmacology, University of Western Australia , Perth, Australia; [4]Department of Renal Medicine, Sir Charles Gairdner Hospital, Perth, Australia; [5]Department of Medicine, University of Otago, Christchurch, New Zealand; [6]Centre for Transplant and Renal Research, Westmead Hospital, Westmead, Australia.

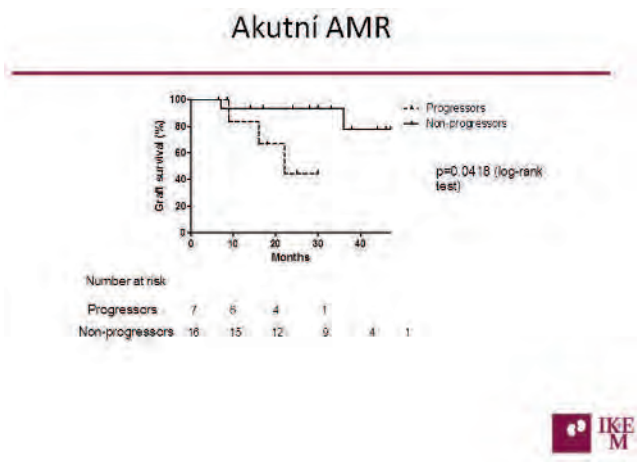
Pre-transplant donor specific anti-human leukocyte antigen antibodies (DSA) are associated with poorer graft outcomes after kidney transplantation. De novo DSA (dnDSA) are common but the implications of these antibodies for patient-relevant outcomes are uncertain. We undertook a systematic review and meta-analysis of prospective and retrospective cohort studies of adult and paediatric kidney or adult simultaneous kidney-pancreas transplant recipients to determine the association between dnDSA and transplant and patient outcomes. We searched MEDLINE and Embase for studies published by November, 2015. Thirty-seven studies were eligible, involving 9844 transplant recipients. Associations between dnDSA and outcomes were estimated by random effects meta-analysis with univariate meta-regression used to explore sources of between-study heterogeneity. Pre-specified outcomes were graft loss (23 studies, 636/5177), any acute rejection (13 studies, 352/2103), antibody mediated rejection (14 studies, 149/2395) and death (6 studies, 64/1517). The prevalence of recipients with dnDSA was 16.3%. Transplant recipients with dnDSA experienced an increased relative risk (RR) for any acute rejection (RR 3.12; 95% CI 2.04-4.79, antibody−mediated rejection (RR 12.98; 7.09-23.76), graft loss (RR 4.17; 3.31-5.24) and death from any cause (RR 2.32, 1.00-5.37). There was moderate to substantial heterogeneity in estimates between studies (I2 41-80%). Study-level factors explaining some heterogeneity included proportion of living donors, recipient age, and diagnostic assay for dnDSA. These findings suggest the presence of dnDSA is associated with an increased risk of acute rejection, particularly antibody mediated rejection and subsequent graft loss. Therefore studies evaluating the effects of interventions to lower dnDSA with graft and patient outcomes are needed.

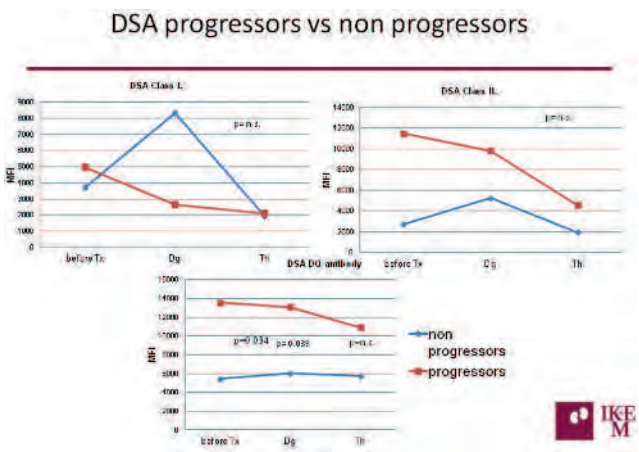
323.7

Do we know how to treat resistant antibody-mediated rejection effectively? A single centre experience

Janka Slatinska[1], Eva Honsova[2], Vladimir Hanzal[1], Petra Bohacova[3], Tomas Marada[4], Petra Hruba[5], Micheala Zelinova[1], Ondrej Viklicky[1,5].
[1]Department of Nephrology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [2]Department of Pathology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [3]Department of Immunology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [4]Department of Transplant Surgery, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [5]Transplant Laboratory, Institute for Clinical and Experimental Medicine, Prague, Czech Republic.

Introduction and Aims: Acute antibody-mediated rejection (AMR) remains one of the major barriers to successful long-term outcomes. Previously reported therapeutic superiority of combination of plasmapheresis (PP) and intravenous immunoglobulin (IVIG) may however fail in some resistant cases. Thus, the aim of this work was to analyze the efficacy and safety of administration of bortezomib (B) and rituximab (RTX)-based treatment of resistant AMR.
Methods: We retrospectively analyzed documentation of 772 patients who underwent renal transplantation between 1/2012-6/2015. Novel therapeutic approach to resistant acute AMR in kidney transplant recipients was applied in 23 patients (3%) based on administration of B [1 cycle of 4 doses of B (1.3 mg/m2)], small doses of i.v. corticosteroids, PP and a dose of RTX (375mg/m2). This protocol was administrated after conventional treatment had failed. Resistant AMR was defined as a persisting deterioration or non-function of renal allograft in patients with histological verification of AMR, positive C4d staining and detection of donor specific antibodies (DSA) receiving standard antirejection treatment with PP + IVIG. Patients (pts) were followed for 6-48 months.
Results: Therapy of resistant acute AMR was administered to 23 pts after kidney transplantation with median peak PRA 52%, actual PRA 36%, mean HLA mismatch in HLA-A 1.2 ± 0.4, HLA-B 1.7 ± 0.5, HLA-DR 1.3 ± 0, with median of 5.8 years on dialysis. 3 pts underwent 1st kidney transplantation, while 20 patients retransplantation (2nd Tx n=10, 3rd Tx n=6, 4th Tx n=4). Immunosuppressive protocol consisted of induction with antithymocyte globulin (n=22) or basiliximab (n=1). Diagnosis of resistant acute AMR was made on 14.th POD.Based on therapeutic effect, 15 pts received 1, 7 pts 2 cycles and 1 patient was treated with 3 cycles of B. We observed DGF in 26.1%.Using B regimen in treating resistant acute AMR led to decrease in DSA quantity in HLA especially in class I (p=0.005), class II (p = 0.015), but not in DQ (p= 0.2). No significant improvement of renal function was observed during the follow-up.The pts with the levels of serum creatinine increased more than 25% of baseline level in 6 months after administration of protocol with B, are progressors (n=7). The progressors graft survival was 57% in 20 months. The quantity of MFI DQ antibodies was significantly higher than in pts with stable renal function (non progressors). The side-effects observed were UTI (35%), colitis (13%), polyneuropathy (26%), hepatotopathy (13%), fluid retention (22%), thrombocytopenia (78%), leucopenia (56.5%), sepsis (30.4%).
Conclusions: Traditional AMR therapeutic strategies have focused on antibody removal and B-cell depletion while not directly focusing on plasma cell depletion. Bortezomib was effective against HLA I and II class antibodies, the problem with DQ antibodies is still unsolved. Bortezomib-related toxicities were all transient.



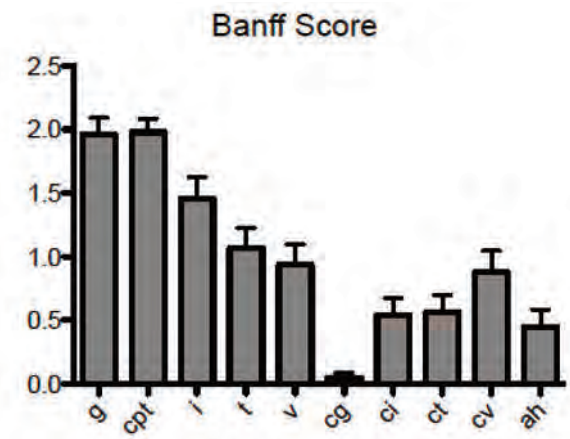


323.8

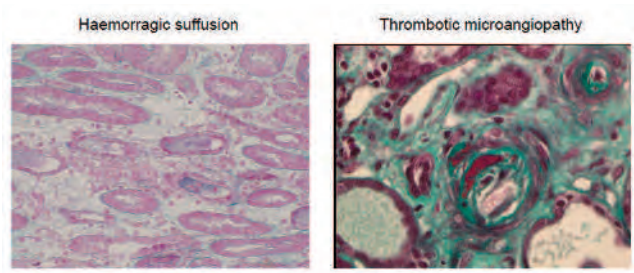
Early acute humoral rejection in the absence of anti-HLA antibodies: clinico-pathological description from a French nation-wide study

Marianne Delville[1], Marion Rabant[22], Jean-Paul Duong[22], Sylvain Pagie[20], Philippe Gatault[9], Magali Giral[10], Olivier Thaunat[13], Antoine Sicard[13], Nadia Arzouk[3], Marc Hazzan[11], Alexandre Hertig[2], Marie Matignon[4], Christophe Mariat[15], Sophie Caillard[6], Nassim Kamar[12], Johnny Sayeg[18], Pierre-François Westeel[19], Didier Ducloux[8], Cyril Garrouste[7], Marc Ladriere[14], Vincent Vuiblet[24], Joseph Rivalan[17], Dominique Bertrand[5], Andre Le Moine[21], Anne Cesbron[23], Stéphanie Castagnet[23], Christophe Legendre[1], Beatrice Charreau[20], Dany Anglicheau[1].
[1]Transplantation rénale , Hopital Necker, APHP, Paris, France; [2]Transplantation rénale , Hopital Tenon, APHP, Paris, Finland; [3]Transplantation Rénale, Hopital La Pitie Salpetriere, APHP, Paris, France; [4]Transplantation Rénale, Hopital Mondor, APHP, Paris, France; [5]Transplantation rénale , CHU, Rouen, France; [6]Transplantation rénale , CHU, Strasbourg, France; [7]Transplantation rénale , CHU, Clermont-Ferrand, France; [8]Transplantation rénale , CHU, Besançon, France; [9]Transplantation rénale , CHU, Tours, France; [10]Transplantation rénale , CHU, Nantes, France; [11]Transplantation rénale , CHU, Lille, France; [12]Transplantation rénale , CHU, Toulouse, France; [13]Transplantation rénale , CHU, Lyon, France; [14]Transplantation rénale , CHU, Nancy, France; [15]Transplantation rénale , CHU, St Etienne, France; [16]Transplantation rénale , CHU, Bordeaux, France; [17]Transplantation rénale , CHU, Rennes, France; [18]Transplantation rénale , CHU, Angers, France; [19]Transplantation rénale , CHU, Amiens, France; [20]ITUN, CHU, Nantes, France; [21]Transplantation Rénale, Hopital Erasme, Bruxelles, Belgium; [22]Anatomie et Cytologie Pathologiques , Hopital Necker, APHP, Paris, France; [23]Laboratoire d'histocompatibilité et d'immunogénétique, CHU , Nantes, France; [24]Transplantation rénale , CHU, Reims, France.

Background: Antibody mediated rejection (ABMR) is associated with poor transplant outcome. Pathogenic alloantibodies are usually directed against HLA antigens. However evidence of ABMR in the absence of anti-HLA antibodies strongly suggests the implication of non-HLA antibodies, usually identified under the generic term of anti-endothelial cell antibodies (AECA). Despite the severity of non-HLA ABMR, the available data remain elusive.
Materials and Methods: We implemented a retrospective nation-wide study to improve undertanding of non-HLA ABMR. Inclusion criteria were: first or re-transplantion, deceased or living-donor; acute allograft dysfunction during the first three months; allograft biopsy showing a total score of glomerulitis and peritubular capillaritis g+ptc≥3 according to the Banff classification; absence of identified anti-HLA DSAs using Luminex® .
Results: Demographical and clinical parameters were collected for 47 cases. Recipient age was 46±2 yrs. Male/female ratio was 3/1. Mean time of dialysis was 3.7±0.6 yrs. Donor age was 51±2 yrs. Cold ischemia time was 15.8±1.5 hrs. All patients but one received induction therapy (85% basiliximab, 13% thymoglobuline), a CNI (72% tacrolimus, 28% cyclosporine), MPA and steroids.
Rejection was diagnosed at day 15±4 in the absence of graft function recovery in 47% of patients. The remaining 25 patients defined a poor serum creatinine nadir of 228±28 µmol/L. Acute graft dysfunction (318±42 µmol/L) led to the diagnosis of acute rejection at day 25±6. No anti-HLA DSA were observed. Mean g+ptc score was 3,9 [3; 6]. In addition, intimal arteritis and interstitial inflammation (i≥1) were observed in 48% and 72% of cases, respectively.



Additional lesions included haemorragic suffusion and thrombotic microangiopathy in 21% and 23% of cases, respectively.



Therapeutic strategies included steroids pulses (89%), thymoglobulines (23%), rituximab (30%), IVIG (45%) and plasmapheresis (60%). Primary non function was observed in 2 cases and one patient was back to dialysis at 2 years. At last follow up (3±1 yrs) serum creatinine was 182±15 µmol/l.
Conclusion: This study provides a close clinico-pathological description of non-HLA ABMR. Non-HLA ABMR is a severe condition associated with graft lost. Additional histological and mechannistic analyses are upcoming. Therapeutic issue is challenging and will need further studies.

323.9

Post-transplant monitoring of donor-specific anti-HLA antibodies and their characteristics improves risk stratification of kidney allograft loss

Denis Viglietti[1], Alexandre Loupy[2], Carol Bentlejewski[3], Christophe Legendre[2], Denis Glotz[1], Adriana Zeevi[3], Carmen Lefaucheur[1].
[1]Saint-Louis Hospital, Paris, France; [2]Necker Hospital, Paris, France; [3]University of Pittsburgh Medical Center, Pittsburgh, PA, United States.

Circulating donor-specific anti-HLA antibodies (DSA) are associated with allograft failure in solid-organ transplantation. We investigated the pathogenic characteristics of DSA that may improve risk prediction of allograft loss in a population-based study.
We enrolled consecutive patients who received kidney allografts at two Paris centers between 2008 and 2011. Patients were screened for the presence of circulating DSA at the time of transplantation (day-0), at one year and two years after transplantation or during an episode of acute rejection in the first two years after transplantation. We assessed DSA characteristics, including specificity, HLA class, mean fluorescence intensity, C1q-binding capacity, and IgG subclasses at day-0 and at the time of first post-transplant detection.
Of the 858 patients included in the study 88 (10.3%) patients had day-0 DSA and 184 (21.6%) patients were identified with post-transplant DSA. When we considered all immunologic parameters at the time of transplantation, the detection of day-0 DSA was the strongest independent immunologic risk factor of allograft loss (HR=2.7, 95% CI: 1.6-4.6; p<0.001). The post-transplant independent immunologic determinants of allograft loss were the detection of C1q-binding DSA (HR=4.4, 95% CI: 2.2-8.8, p<0.001) and the detection of IgG3-positive DSA (HR=3.5, 95% CI: 1.6-7.4, p=0.001).We built a multivariate model for allograft loss at the time of transplantation integrating donor age, donor type, cold ischemia time and day-0 DSA. This baseline model showed a moderate discrimination capacity (C-statistic of 0.66). The addition of post-transplant DSA increased significantly the performance of the baseline model (C-statistic of 0.71). The detection of C1q-binding DSA and IgG3-positive DSA further improved the risk prediction of allograft loss: C-statistic, 0.75 (1000 bootstrap mean difference: 0.029, 95% CI: 0.028-0.030) and 0.74 (1000 bootstrap mean difference: 0.022, 95% CI: 0.021-0.023), respectively, and integrated discrimination improvement, 0.07 (p<0.001) and 0.06 (p<0.001), respectively.
Post-transplant serial monitoring of DSA improves risk stratification of kidney allograft loss. The characterization of DSA by complement-binding capacity and IgG3 subclass further improves the performance to predict allograft loss beyond their simple detection.

323.10

Complement-binding donor-specific anti-HLA antibodies are associated with severe kidney allograft arteriosclerosis

Alexandre Loupy[1], Denis Viglietti[2], Jean-Paul Duong Van Huyen[1], Denis Glotz[2], Christophe Legendre[1], Adriana Zeevi[3], Carmen Lefaucheur[2].
[1]Necker Hospital, Paris, France; [2]Saint-Louis Hospital, Paris, France; [3]University of Pittsburgh Medical Center, Pittsburgh, PA, United States.

The role of circulating donor-specific anti-HLA antibodies (DSA) in the development of accelerated arteriosclerosis have been recently reported in kidney transplant recipients. This study investigated the characteristics of DSA that are associated with the severity of allograft arteriosclerosis.
We enrolled 744 consecutive kidney transplantation performed between January 1, 2004 and January 1, 2010 at Necker Hospital (Paris, France), with systematic assessment of injury phenotype and arteriosclerotic lesions using the vascular fibrous intimal thickening (cv) Banff score on allograft biopsies performed at one year after transplantation. We assessed circulating DSA and their characteristics (specificity, HLA class, mean fluorescence intensity [MFI] and C1q-binding) at six months after transplantation.
We identified 281 patients with cv0 score, 213 patients with cv1 score, 189 patients with cv2 score and 61 patients with cv3 score. The distribution of DSA according to cv score was the following: 47/281 (17%) in cv0 patients, 39/213 (18%) in cv1 patients, 63/189 (33%) in cv2 patients and 28/61 (46%) in cv3 patients. Immunodominant DSA (iDSA) MFI level was positively correlated with the severity of arteriosclerosis (Spearman's rho=0.23, p=0.002), with a mean MFI of 3204.0±3725.2 in cv0 patients, 3760±3598 in cv1 patients, 4892±4676 in cv2 patients and 5541±3892 in cv3 patients. C1q-binding DSA prevalence increased with the severity of allograft arteriosclerosis: 8/281 (3%) in cv0 patients, 6/213 (3%) in cv1 patients, 25/189 (13%) in cv2 patients and 9/61 (15%) in cv3 patients (p<0.001). Patients with C1q-binding iDSA had a higher cv score compared with patients with non-C1q-binding DSA (1.7±1.0 versus 1.3±1.1, respectively, p=0.01). The C1q-binding capacity of DSA was associated with increased microvascular inflammation (p<0.001) and C4d deposition in peritubular capillaries or arteries (p<0.001).
This study shows a biological gradient between DSA MFI level and the severity of allograft arteriosclerosis. The complement-binding capacity of DSA is associated with an increased severity of arteriosclerosis and complement deposition in allograft.

323.11

The natural history of donor specific antibodies in kidney transplant recipients and associated clinical outcomes

Susan Wan[1,2], Kate Wyburn[1,2], Jian Lin Yin[1,2], Narelle Watson[3], John Saunders[1], Josette Eris[1,2].
[1]Department of Renal Medicine, Royal Prince Alfred Hospital, Sydney, Australia; [2]Sydney Medical School, University of Sydney, Sydney, Australia; [3]NSW Transplantation and Immunogenetics Laboratory, Clinical Services and Research Division, Australian Red Cross Blood Service, Sydney, Australia.

Introduction: Kidney transplantation is the treatment of choice for end stage kidney disease. While there have been significant advances in early post-transplant graft survival, there has been little change in long-term outcomes. The causes of graft loss are multifactorial, and the previously under-recognised role of donor specific antibody (DSA) is now considered to contribute significantly. DSA have been implicated in the development of rejection and graft dysfunction. However, the natural history of DSA remains poorly characterized.
Aim: To characterize the development of DSA and the associations with clinical outcomes in kidney transplant recipients (KTx).
Materials and Methods: Serum samples from adult KTx performed at a single unit between July 2010 and May 2015 were prospectively collected pre-transplant and at Days 7, 28 and Months 3, 6, 9, 12 post-transplant and tested for Class I and II DSA and C1q binding on a luminex platform. Clinical outcomes including acute rejection (AR) and graft function were recorded and all episodes of AR were biopsy proven.
Results: 166 recipients had pre- and post-transplant DSA testing. Mean follow-up was 35 ± 17 months. Ninety-two (55%) patients had DSA; 62 (37%) had pre-existing DSA only, 14 (8%) had denovo (dn)DSA only, and 16 (10%) had pre-existing and dnDSA. Of patients with dnDSA; 9 (30%) had Class I antibodies only, 16 (53%) had Class II antibodies only and 5 (17%) had Class I & II antibodies. In 3 patients (11%) the dnDSA was C1q-binding. Twenty-five (32%) patients with pre-existing DSA and 17 (57%) patients with dnDSA had AR. Patients with dnDSA vs those without were more likely to develop AR (OR 3.63; 95%CI 1.61-8.22; P=0.002), however there was no increased risk in patients with pre-existing DSA only (OR 0.71; 95%CI 0.36-1.42; P=0.337). Median time to rejection was 1.9 months (IQR 0.2-4.9). Mean creatinine at 3, 6 and 12 months post-transplant was higher in patients with dnDSA compared to those without dnDSA (Mean 12-month creatinine: 172 ± 67 vs 128 ± 38 μmol/L; t62=-3.22; P=0.002).
Conclusion: While there was no association between the presence of pre-existing DSA and rejection, the development of dnDSA, which occurred in 18% of patients, was associated with a higher risk of AR and worse graft function. Monitoring the emergence of dnDSA may assist with determining and mitigating the risk of rejection.

323.12

Non-validation of pre-transplant sensitization against angiotensin II type1 receptor as risk factor of graft and patients outcomes in kidney transplantation

Clément Deltombe[1], Florence Florence[2,3], Dany Anglicheau[3,4,6], Emmanuel Morelon[4,7], Katy Trébern-Launay[1,4], Florent Le Borgne[2], Marie Rimbert[8], Pierrick Guérif[1], Stéphanie Malard-Castagnet[3,5], Yohann Foucher[2], Magali Giral[1,2,3,4].
[1]Institute for Transplantation, Urology and Nephrology (ITUN), Nantes University Hospital, Nantes, France; [2]SPHERE-EA4275, Nantes University, Nantes, France; [3]LabEx Transplantex, Founds, Nantes, France; [4]Thematic Research and Care Network RTRS, Centaure, Nantes, France; [5]HLA Laboratory, Etablissement Français du Sang, Nantes, France; [6]Department of Kidney Transplantation, Necker University Hospital, AP-HP, Paris, France; [7]Department of Transplantation and Clinical Immunology, Hospices Civils de Lyon, Hopital Edouard Herriot, Lyon, France; [8]CIMNA platform, Nantes University Hospital, Nantes, France.

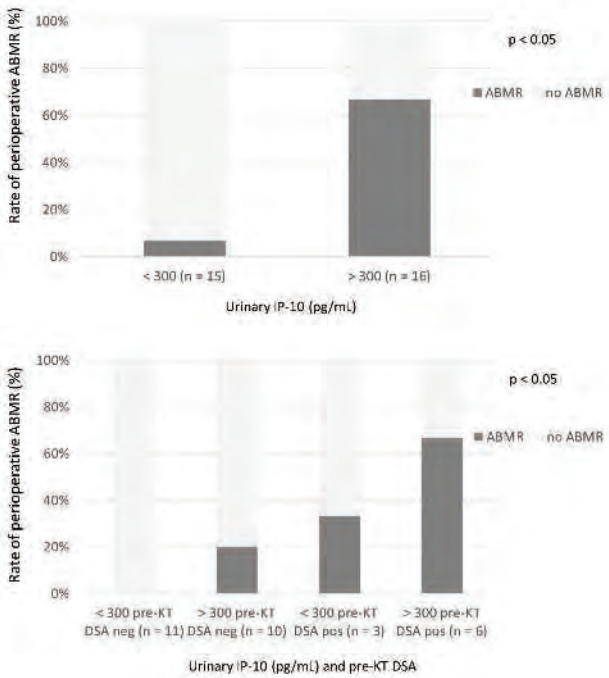
Context: Several studies have suggested that anti-angiotensin II type 1 receptor antibodies before transplantation constitute a risk factor of acute rejection episode and graft failure. We aimed to propose an external validation of such an etiologic role.
Methods: We studied 940 kidney transplant recipients from 3 French centers of the DIVAT cohort. Multivariable Cox models were performed to estimate the association between pre-transplant anti-angiotensin II type 1 receptor antibodies and time to acute rejection episode or time to graft failure.
Results: There were 387 patients (41.2%) with a pre-transplant anti-angiotensin II type 1 receptor antibodies higher than 10U/mL, i.e. the level above which we previously reported higher risks of acute episode rejection and graft failure. However, the results of the present study indicated that a pre-transplant anti-angiotensin II type 1 receptor antibodies higher than 10U/mL was not significantly associated with higher risks of acute rejection episode (HR=1.04, 95%CI from 0.80 to 1.35), nor with risk of return to dialysis (HR=0.86, 95%CI from 0.56 to 1.33).
Conclusion: Our study did not confirm the association between the pre-transplant anti-angiotensin II type 1 receptor antibodies and the kidney transplant outcomes.

323.13

Urine IP-10 as the biomarker for ABMR in Kidney transplant recipient with DGF

Natavudh Townamchai[1], Asada Leelahavanichkul[2], Jade Supapol[3], Yingyos Avihingsanon[1], Kearkiat Praditpornsilpa[1].
[1]Department of Medicine, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand; [2]Department of Microbiology, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand; [3]Department of Surgery, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand.

Background: In the setting of DGF in kidney transplantation (KT), early acute ABMR, which needs early treatment, has to be differentiated from simple ischemic acute tubular necrosis (ATN). Concerning the higher risk of bleeding complication during this period, the clinician is reluctant to perform kidney biopsy. The biomarkers are needed for acute ABMR prediction during this vulnerable period.
Methods: There were 115 incident recipients during September 2012 to December 2014, which all of them were negative for CDC-AHG crossmatch. The total number of recipients with DGF were enrolled (n=31). None of them underwent desensitization prior transplantation. The urine IP-10 (also called CXCL-10) at day 7 were measured by ELISA method in all DGF recipients. The allograft biopsy was performed at day 7 for detection of ABMR. Recipients were stratified in to four groups according to pre-KT DSA status and urine IP-10 level.
Results: None of our patients was anuria at day 7 after transplantation. Seven of 31 DGF recipients had ABMR (22.6%). The median urine IP-10 level of 31 recipients was 326.6 pg/mL (range from 0 to 6999 pg/mL). Based on ROC analysis, the most optimal cut point for low vs high IP-10 level was at 300 pg/mL with 85.7% sensitivity and 58.3% specificity. The recipients with high IP-10 level had higher rate of ABMR compared to low IP-10 recipients, 37.5% vs 6.7%, p<0.05 (figure 1).



The rates of ABMR among negative pre-KT DSA/low IP-10, negative DSA/high IP-10, positive DSA/low IP-10, and positive DSA / high IP-10 recipients were 0, 20.0, 33.5 and 66.7%, respectively (p<0.05, figure1).

Conclusions: In DGF, the perioperative ABMR can be predicted by urine IP-10 at day 7. Together with pre-KT DSA, urine IP-10 provides additional predictive value for ABMR prediction.

324.1

Combined en-bloc heart-liver transplantation for patients with congenital heart disease complicated by cirrhosis

C. Andrew Bonham[1], Jackie Lee[1], Thomas Pham[1], Waldo Concepcion[1], Sarah Conlon[1], Katsuhide Maeda[1], Seth Hollander[2], Carlos Esquivel[1].
[1]Surgery, Stanford University Medical School, Palo Alto, CA, United States; [2]Pediatrics, Stanford University Medical School, Palo Alto, CA, United States.

There are over 1 million adolescent and young adults with congenital heart disease (CHD), with estimates the number will increase by 5% per year. Hepatic complications correlate with elevated hepatic venous pressures and low cardiac index, and are related to duration of follow-up, with odds ratios of 4.4 and 9 at 11-15 and 16-20 years post-Fontan[1]. Hepatic complications including variceal bleeding, protein losing enteropathy, hepatopulmonary syndrome, ascites, hepatic encephalopathy and hepatocellular carcinoma contribute to significant morbidity and mortality. Cardiac transplantation for congestive heart failure in the setting of cirrhosis carries a high risk of liver failure. Combined heart-liver transplantation (CHLT) corrects both defects. Guidelines as to which patients would benefit from this procedure remain poorly defined. Historically, the most common indication for CHLT was amyloidosis. CHD patients with cirrhosis are considerably more challenging, with multiple sternotomies, portal hypertension and coagulopathy. We report our experience of CHLT using a novel en-bloc technique with excellent results.
Ten patients received CHLT between August 2006 and May 2015. Indications for CHLT included end stage heart disease complicated by cirrhosis identified on imaging or biopsy with evidence of portal hypertension (varices, splenomegaly, ascites). Seven patients had CHD, with failed Fontan the most common diagnosis. All patients had complications secondary to cirrhosis. Two patients were sensitized. Prospective crossmatch at time of organ offer was negative in all patients. Immunosuppression included anti-thymocyte globulin induction, tacrolimus, mycophenolate, and steroids.
There were no deaths, with mean follow up of 53.3 months. Over 100 endomyocardial biopsies were performed after transplant, with no evidence of rejection in any patient. The en bloc procedure shortens ischemic time and provides excellent results in these challenging patients. Inclusion of the liver appears to be immunoprotective for the heart.

References:

[1] 1. Late hepatic complications after Fontan operation; non-invasive markers of hepatic fibrosis and risk factors. Baek JS, Bae EJ, Ko JS, Kim GB, Kwon BS, Lee SY, Noh CI, Park EA, Lee W. Heart. 2010 Nov;96(21):1750-5

324.2

Effects of acute and chronic amiodarone use in patients before heart transplantation on post-transplant outcomes

Rasmus Rivinius[1], Matthias Helmschrott[1], Arjang Ruhparwar[2], Bastian Schmack[2], Christian Erbel[1], Christian A.Gleissner[1], Mohammadreza Akhavanpoor[1], Lutz Frankenstein[1], Fabrice F.Darche[1], Patrick A.Schweizer[1], Dierk Thomas[1], Philipp Ehlermann[1], Tom Bruckner[3], Hugo A.Katus[1], Andreas O.Doesch[1].
[1]Department of Cardiology, Angiology and Pneumology, Heidelberg University Hospital, Heidelberg, Germany; [2]Department of Cardiac Surgery, Heidelberg University Hospital, Heidelberg, Germany; [3]Institute for Medical Biometry and Informatics, University of Heidelberg, Heidelberg, Germany.

Introduction: Major concerns about the safety of pre-transplant amiodarone use in patients with end-stage heart failure have been recently raised. Due to its long half-life, the newly transplanted heart is exposed to amiodarone posing potential risks such as increased mortality after heart transplantation (HTX). The aim of this study was to compare the effects of acute and chronic amiodarone use in patients before HTX on post-transplant outcomes.
Methods: This retrospective single-center study included 530 adult patients who received HTX between 06/1989 and 12/2012. Patients were stratified by their amiodarone therapy before HTX: No continuous amiodarone use (≤ 90 days before HTX), acute continuous amiodarone use (≤ 90 days before HTX), and chronic continuous amiodarone use (> 90 days before HTX). Analysis comprised recipient data, donor data, principal diagnosis for HTX, transplant sex mismatch, perioperative data, and initial post-transplant medication. The occurrence of early post-transplant atrial fibrillation (AF) ≤ 30 days after HTX and mortality after HTX were chosen as primary outcomes. Kaplan–Meier estimator using log-rank tests was applied for freedom from early post-transplant AF and survival after HTX.
Results: 412 patients (77.7%) were appointed to the "no use of amiodarone before HTX" group, 23 patients (4.4%) to the "acute use of amiodarone before HTX" group, and 95 patients (17.9%) to the "chronic use of amiodarone before HTX" group.
Indications for acute amiodarone use were AF in 1 patient (4.3%), ventricular tachycardia in 20 patients (87.0%), and ventricular fibrillation in 2 patients (8.7%). Indications for chronic amiodarone use included AF in 13 patients (13.7%), Wolff–Parkinson–White syndrome in 1 patient (1.1%), ventricular tachycardia in 73 patients (76.8%), and ventricular fibrillation in 8 patients (8.4%).
We found no statistically significant difference between the three groups in 30-day (P = 0.8656), 1-year (P = 1.0000), 2-year (P = 0.8763), 5-year (P = 0.5174), or overall follow-up mortality after HTX (P = 0.1936). Moreover, Kaplan–Meier survival analysis showed no statistically significant difference in 30-day (P = 0.8610) or overall survival (P = 0.2924).
Patients with chronic amiodarone use before HTX had the lowest rate of early post-transplant AF (no amiodarone before HTX [13.6%], acute use of amiodarone before HTX [13.0%], and chronic use of amiodarone before HTX [2.1%], P = 0.0065). Additionally, this group had a significantly lower rate of AF in the Kaplan–Meier analysis of freedom from early post-transplant (P = 0.0081).
Conclusion: In contrast to former publications, we found the administration of acute or chronic amiodarone in patients with end-stage heart failure and arrhythmias before heart transplantation not to be associated with an increased mortality after HTX. Importantly, chronic amiodarone use reduces the occurrence of early post-transplant AF whereas acute amiodarone therapy shows no such effect.

324.3

Terlipressin increases blood pressure and facilitates weaning from cardiopulmonary bypass in heart transplant recipients with refractory hypotension

Nukhet Akovali[1], Aynur Camkiran Firat[1], Duygu Taskin[1], Pinar Zeyneloglu[1], Bahadir Gultekin[2], Atilla Sezgin[2], Arash Pirat[1].
[1]Anesthesiology, Baskent University Hospital, Ankara, Turkey; [2]Cardivascular Surgery, Baskent University Hospital, Ankara, Turkey.

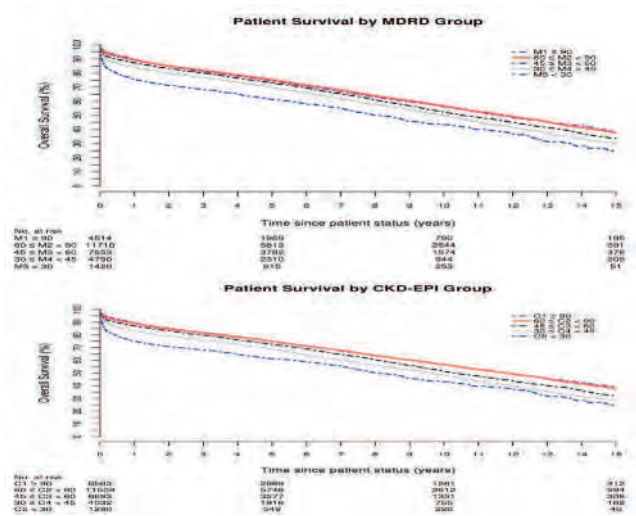
Introduction: Refractory hypotension may occur during the weaning period from cardiopulmonary bypass (CPB) in orthotopic heart transplantation (OHT) and can be multifactorial. Vasopressin plays major role in circulatory homeostasis, but vasopressin is currently not available in our country. So, we usually use terlipressin that is a synthetic analog of vasopressin and a prodrug.
Materials and Methods: We retrospectively collected data from the records of consecutive heart transplantations from June 2013 to December 2015. Eighteen patients underwent orthotopic heart transplantation (OHT) for end-stage heart disease at our institution and five patients were used terlipressin. All of them were retrospectively evaluated from anesthetic and surgical charts. Patients' characteristics potential to have impact on anesthestic management as well as postoperative outcome were recorded. Anesthesic technique included induction with midazolam and fentanyl followed by maintenance with desflurane, air/O2 and fentanyl infusion. Beside standard invasive monitoring, pulse contour cardiac output measurement device was used and pulmonary artery monitorization was performed in all of the patients.
Predefined strict hemodynamic criteria were used to assess the success of weaning at our instutions. If weaning was not successful, CPB was reinstituted and terlipressin bolus (0.01-0.02 mg/kg) was used. If the second weaning attempt failed, terlipressin infusion (3 mg/24 hours) was started. The data collected for each case were demographic features, duration of mechanical ventilation, early (<30 days) and late postoperative mortality.
Results: Of the 5 patients considered, one patient was younger than 16 years. Average age was 27.6±18.6 years and average body weight was 54.3±25.5 kg. The cross clamp time was 128.4±14.4 minutes, cardiopulmonary bypass time was 237±66.8 minutes, and total ischemia time was 240.0±44.3 minutes. Ultrafiltration was performed in 4 patients with an average amount of 1037±415 mL. Postoperative length of mechanical ventilation was 44.6±53.9 hours, lactate levels at the end of surgery was 8.9±4.0 mmol/L, 2 (40%) patients required renal replacement therapy and 1 (%20) patient had revisions due to bleeding. In-hospital mortality rate after the OHT was 20% (one patient), and there were no operative mortality.
Discussion: In this report we describe five patients undergoing OHT. Despite inotropic therapy (dopamine, dobutamine and adrenaline) the hemodynamic situation deteriorated, necessitating the administration terlipressin.
Conclusion: The case series have demonstrated an important beneficial effect of terlipressin when the weaning from CPB attempt failed, especially as a rescue treatment in patients with long total ischemia time or impaired donor left ventricular function undergoing heart transplantation. It may be beneficial for its postoperative hemodynamic profile, may reduce the doses of requirements of catecholamines and contribute to prevention of the postcardiotomy vasoplegic shock.
References:
[1] 1. Eiki Tayama.Interact CardioVasc Thorac Surg 2007; 6(6):715-719.
[2] 2. Michael Argenziano. J Heart Lung Transplant 1999;18:814–817.
[3] 4. Mars Licker. Annals of Cardiac Anaesthesia 2012;15(3):206-223

324.4

Pre-orthotopic heart transplant (OHT) estimated glomerular filtration rate (eGFR) predicts post-OHT mortality and renal outcomes

Hani M. Wadei[1], David Hodges[2], Parag C.Patel[1], Philip J.Habib[1].
[1]Transplant, Mayo Clinic, Jacksonville, FL, United States; [2]Health Sciences Research, Mayo Clinic, Jacksonville, FL, United States.

Background: There are limited information on the relationship between pre-OHT estimated glomerular filtration rate (eGFR) and adjusted short and long term survival and renal outcomes post-OHT.
Methods: Using the United Network of Organ Sharing (UNOS) database we estimated pre-OHT eGFR using the Modification of Diet in Renal Disease (MDRD) and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations in patients aged ≥18 years who received OHT between 1988 and 2013 in the US. Cox proportional hazards models were used to investigate the relationship between eGFR and post-OHT mortality. The following variables were adjusted for: age, sex, ethnicity, BMI, cold ischemia time, diabetes, inotrope use, era of transplant, listing status and ventricular assist device (VAD).The primary outcome was to determine if pre-OHT eGFR independently predicted post-OHT mortality.
Results: 30,090 patients were included, of these 46.1% and 39.9% had an eGFR <60 mL/min/1.73 m2 by MDRD and CKD-EPI, respectively. The 1, 3, 5 and 10-year patient mortality was progressively lower with each drop in GFR category using either equation.



Compared with eGFR ≥90 mL/min/1.73 m2, the adjusted hazard ratio of mortality was 1.09 (95% CI: 1.02-1.26) for eGFR 45-59 mL/min/1.73 m2, 1.22 (95% CI-1.23-1.31) for eGFR 30-44 mL/min/1.73 m2 and 1.55 (95% CI 1.41-1.70) for eGFR <30 mL/min/1.73 m2 by MDRD. Using CKD-EPI, 4,955 (16.5%) patients were reclassified upward, 40 (0.1%) downward and the remaining 25,092 (83.4%) remained in agreement with the MDRD category.There was no advantage of CKD-EPI over MDRD in determining post-OHT mortality. Pre-OHT eGFR by either equation was predictive of post-OHT end stage renal disease (ESRD) and the need for kidney transplantation with the highest risk in those with pre-OHT eGFR <30 mL/min/1.73 m2 by either equation.
Conclusions: Pre-OHT eGFR was independently associated with mortality, ESRD and kidney transplantation following OHT. There was no advantage of CKD-EPI over MDRD in determining post-OHT mortality or renal outcomes.
Grant support for biostatistics was awarded through the Mayo Clinic

References:
[1] 1. Singh TP, Givertz MM, Gauvreau K: Risk stratification for in-hospital mortality after heart transplantation using the modification of diet in renal disease and the chronic kidney disease epidemiology collaboration equations for estimated glomerular filtration rate. Transplantation 2014;98:1000-6.
[2] 2. Hong KN, Iribarne A, Worku B, et al.: Who Is the High-Risk Recipient? Predicting Mortality After Heart Transplant Using Pretransplant Donor and Recipient Risk Factors. The Annals of Thoracic Surgery 2011;92:520-7.

324.5

Relationship between brachial artery flow-mediated dilation and clinical characteristics in heart transplant recipients

Takuya Watanabe[1,2], Norihide Fukushima[1], Yoshihiro Miyamoto[2], Osamu Seguchi[1], Masanobu Yanase[1], Tomoyuki Fujita[1,3], Kunihiro Nishimura[2], Takuma Sato[1], Haruki Sunami[1], Seiko Nakajima[1], Eriko Hisamatsu[1], Kensuke Kuroda[1], Norihiro Okada[1], Hiroki Hata[1,3], Junjiro Kobayashi[3], Takeshi Nakatani[1].
[1]Transplantation, National Cerebral and Cardiovascular Center, Osaka, Japan; [2]Preventive Cardiology, National Cerebral and Cardiovascular Center, Osaka, Japan; [3]Cardiovascular Surgery, National Cerebral and Cardiovascular Center, Osaka, Japan.

Introduction: Peripheral endothelial (PE) dysfunction assessed by brachial artery flow-mediated dilation (BA-FMD) has a potential prognostic value in patients with various cardiovascular diseases. However, clinical implication of BA-FMD in heart transplant (HTx) recipients remains unclear and associated factors with them are not fully elucidated.
We sought to clarify the relationship between BA-FMD and clinical characteristics in HTx recipients.
Materials and Methods: We measured BA-FMD in HTx recipients who survived at least 1 year post-HTx. Forty-three HTx recipients underwent BA-FMD measurement at a mean 5.3 years after HTx. BA-FMD was first treated as a continuous variable. Subsequently, BA-FMD value was treated categorical variables by defining the first quartile value as the cut-off point in PE dysfunction. A cut-off point of 5.2% in BA-FMD was chosen.
Results: The indication for HTx was non-ischemic heart disease in 41 recipients (95.3%). Mean BA-FMD was 8.0±3.4% (range 2.1-14.4%). PE dysfunction was observed in 11 recipients (25.6%). Recipients with past smoking history exhibited lower BA-FMD (6.0±2.6% vs 9.0±3.3%, p=0.005) and higher prevalence of PE dysfunction (50.0% vs 13.8%, p=0.011) compared with never smokers. Follow-up duration from HTx to BA-FMD measurement was negatively associated with prevalence of PE dysfunction in logistic regression analysis with adjustment of recipient sex and age (Odds ratio [OR]=0.75, 95% confidence interval [CI]=0.56-0.99, p=0.043). Ten recipients who survived more than 10 years post-HTx did not exhibit a PE dysfunction. In multivariable logistic model including recipient sex, age, smoking history, follow-up duration from HTx, previous hypertension, diabetes mellitus and hyperlipidemia, smoking history was independently associated with the high prevalence of PE dysfunction (OR=9.33, 95%CI=1.31-66.48, p=0.026).
Discussions: BA-FMD in our recipients may be higher than that in previous studies, which suggests the association of atherosclerotic conditions prior to HTx with BA-FMD value after HTx, because majority of our recipients were diagnosed with non-ischemic cardiomyopathy. Regarding the association between PE function and follow-up duration from HTx, the present study may add a concept that BA-FMD may be improved even after 1 year post-HTx as time passed after HTx. Since presence of ischemic heart disease prior to HTx and smoking history have been reported to be associated with poor prognosis after HTx, this independent association between impaired BA-FMD and these factors may confirm the potential relationship between PE dysfunction and clinical prognosis in HTx recipients.
Conclusion: These results suggest that BA-FMD in maintenance periods of HTx was associated with potential risk factors for poor outcome in HTx recipients, therefore, BA-FMD measurement after HTx may be useful for predicting clinical outcome in HTx recipients.
References:
[1] Kubo SH. Effects of cardiac transplantation on endothelium-dependent dilation of the peripheral vasculature in congestive heart failure. *Am J Cardiol* . 1993; 1: 88-93.

[2] Patel AR. Heart failure etiology affects peripheral vascular endothelial function after cardiac transplantation. *J Am Coll Cardiol*. 2001; 37: 195-200
[3] Roig E. Assessment of peripheral endothelial-dependent vasodilatation within the first year after heart transplantation. *J Heart Lung Transplant* . 2009; 38: 299-304.
[4] Lund LH. The registry of the International Society for Heart and Lung Transplantation. *J Heart Lung Transplant* . 2015; 34: 1244-1254.

324.6

Effects of exercise training on cardiac reserve and autonomic activity in post heart transplant patients and patients receiving left ventricular assist devices support by assessment of heart rate profile

Katherine Fan[1], Jenny Ng[3], Cyril Ko[1], Kandra Chan[1], Kathy Cheng[1], Cally Ho[2], Timmy Au[2].
[1]Cardiac Medical Unit, Grantham Hospital, Aberdeen, Hong Kong; [2]Department of Cardiothoracic Surgery, Queen Mary Hospital, Pok Fu Lam, Hong Kong; [3]Physiotherapy Department, Grantham Hospital, Aberdeen, Hong Kong.

Purpose: Despite improved survival after heart transplant (HT) and left ventricular assist devices (LVAD) implantation, some patients (pts) continue to experience exercise intolerance and functional limitations which might have been due to extended hospital stay and significant frailty. Chronotropic incompetance and abnormal heart rate recovery (HRR) immediately after exercise reflecting profound abnormalities of autonomic nervous system control during exercise have been reported in pts with chronic heart failure. These abnormalities are valid prognostic markers and strongly associated with increased mortality. We studied the effects of exercise training in pats with LVAD and post HT by assessing the heart rate profile [peak heart rate, chronotropic reserve (CR) and heart rate recovery (HRR)]**.**
Methods: A total of 42 pts: 26 pts with HT (58% men; mean age 49 yrs) and 16 pts with LVAD (81% men; mean age 50 yrs) underwent a 12 weeks' supervised exercise program starting 3 months after surgery. Symptom-limited cardiopulmonary exercise test were performed at baseline and at 3 months during the end of the program. Chronotropic response to exercise was evaluated by the percentage of CR= (peak HR – resting HR/220− age− resting HR) x 100%]. HRR was calculated as the difference between peak HR and HR 1 minute later (HRR1, bpm) during recovery.
Results: The results are shown in Table 1. Exercise training resulted in significant improvement in exercise capacity in both groups of pts at the end of program (METS and CR). There were significantly improved HRR1 in LVAD pts. but remained unchanged in post HT group however.
Heart rate profile at baseline and 3 months post training:

	Heart Transplant group			LVAD group		
	Baseline	3 months	P value	Baseline	3 months	P value
METS level	5.3± 1.5	8.0±2.3	0.0001	2.1±0.4	3.7±1.3	0.00013
Resting HR (bpm)	92.3±4.7	97.6±13.6	0.03	86±11.4	83±16	0.5
Peak HR (bpm)	114.3±16.7	126.3±16.8	0.0003	102.3±12.8	107.8±20	0.2
HRR1	2.5±4	2.6±4.6	0.9	6.3±6.7	10.1±8	0.0015
CR (%)	40±18	57.7±23	0.00015	26.7±8.5	40±22	0.03

Conclusions: Despite different pathophysiologic processes from different treatment strategies for end-stage heart failure in HT and LVAD pts, exercise capacity and CR were improved in both groups, suggesting an accentuated improvement in fitness due to the intervention. Exercise training also improved autonomic activities with in LVAD pts but not post HT pts, consistent with physiology of denervated heart.
Heart rate profile can potentially represent a simple, non-invasive tool to assess outcomes and possibility of becoming a therapeutic target during cardiac exercise rehabilitation.

324.7

Physical function and self-reported health status in pre-transplant patients with left ventricular assisted device - local experience of out-patient cardiac rehabilitation program in Hong Kong

Jenny Ng[1], Katherine Fan[2], Jacky Li[1], Karanda Chan[2], Ka Yan Cheng[2], Mun Yee Cheung[2], Derek Chan[1], Siu Lai Lam[3], Rita Li[3], Cally Ho[3], Timmy Au[3].
[1]Physiotherapy Department, Grantham Hospital, Aberdeen, Hong Kong; [2]Cardiac Medical Unit , Grantham Hospital, Aberdeen, Hong Kong; [3]Cardiothoracic Surgical Department , Queen Mary Hospital, Pokfulam, Hong Kong.

Introduction: Left ventricular assisted device (LVAD) has been accepted as durable mechanical circulatory support for patient with advanced systolic heart failure (HF) as bridge-to-transplant in Hong Kong. Many remained frail and severely deconditioned even after LVAD implantation while waiting for heart transplantation. Cardiac rehabilitation (CR) and exercise training has been shown to be safe and has an impact on physical capacity and quality of life.
Objective: To evaluate the effects of exercise training on physical function and quality-of-life in patients with advanced HF after LVAD implantation while waiting for heart transplantation in an out-patient CR program in Hong Kong.
Method: A total of 16 patients (13 men, 81%, mean age 50±11, BMI 25±5) joined the program at mean 4.7 months after LVAD implantation (15 HeartMate II & 1 HeartWare) and completed a 12 months' program of CR. Progressive low intensity aerobic treadmill walking exercise and light resistance training for the large muscle groups of lower limbs (<7 lb.) were chosen as predominant exercise. Exercise intensity is titrated to rating of perceived exertion (RPE 0-10) ≤3 and to an asymptomatic level. Data of Six-Minute Walk Test (6MWT), Hand Grips (HG) strength, and Short-Form 36 (SF-36) were obtained and compared with baseline, 6 months and 12 months (end of CR program). Changes from baseline to follow-up were analyzed with a paired t-test.
Results and Discussion: The functional class improved significantly in NYHA. Mean exercise training session was 17±8 visits per patient. Treadmill time and intensity improved significantly from mean duration 13±5 mins to mean 20±5 mins ($p<0.001$) and from mean 2±0.4 METs to mean 3.7±1.3 METs ($p<0.001$) at the end of program. There were statistically significant improvements in both 6MWT and HG strength. Physical domains of SF-36 showed significant improvement in both Physical Functioning and Role-Physical (role limitation due to physical problems). Social Functioning domain showed insignificant improvement at mid-term but gradually increased significantly at the end of the program. However, Mental Health domain decreased significantly at end of CR (See Table 1). There was no adverse event in >250 training sessions. Three of them were successfully bridged to heart transplantation.
Conclusion: Indicators of physical function and self-reported health status improved after patients with LVAD who attended CR. Cultural difference may affect LVAD patients' own perception of well being. There is a need for continuous psychological and emotional support in LVAD patients waiting for their heart transplant surgery, therefore a comprehensive CR is recommended. Whether such improvements translate into improved clinical outcomes remain to be determined.

324.8

Is perfect long-term result of continuous-flow LVAD for bridge to transplantation possible?

Minoru Ono[1], Kan Nawata[1], Haruo Yamauchi[1], Osamu Kinoshita[1], Mitsutoshi Kimura[1], Daisuke Nitta[2], Teruhiko Imamura[2], Eisuke Amiya[2], Masaru Hatano[2].
[1]Department of Cardiac Surgery, The University of Tokyo, Tokyo, Japan; [2]Department of Cardiovascular Medicine, The University of Tokyo, Tokyo, Japan.

Introduction: There are many recent reports stating improved long-term results of continuous-flow LVAD for bridge to transplantation (BTT) and destination therapy. However, significant adverse events were not infrequent, some of which may lead to death even in the era of improved device mechanism and patient management. We analyzed LVAD implants for BTT indication at our institute, and compared long-term results between early and recent cases.
Material and Methods: Since November 2007, 100 continuous-flow LVAD implantations for BTT indication were performed. There were 26 females with an average age of 39.5 years (11 – 64). Primary heart diseases were non-ischemic dilated cardiomyopathy in 71, ischemic cardiomyopathy in 9, dilated-phase hypertrophic cardiomyopathy in 8 and others in 12. We examined outcomes after LVAD implantation focusing on survival and transplant (Tx) eligibility, and compared long-term results between early implant group (n = 46: November 2007 to September 2013) and recent implant group (n = 54: October 2013 to the present time).
Results: Five devices were used such as 34 HeartMate II, 24 EVAHEART, 22 Jarvik 2000, 19 DuraHeart and 1 HVAD. Average support duration is 555 days (9 – 1405). Twenty-eight patients underwent heart Tx after 833-day support, 3 were weaned from LVAD due to functional recovery after 260-day support, 5 experienced device change without 1 death due to device infection after 728-day support, 7 died after 302-day support and 57 in ongoing support. All Tx patients were alive but 1 who died from pneumonia 25 months after Tx. There was no patient who is ineligible for heart Tx among those of ongoing support at the time of evaluation. Actuarial survivals of patients in early and recent implant groups were 89.1%, 87.0% and 81.9% at 1, 2 and 3 years, respectively, and 100% and 100% at 1 and 2 years, respectively ($p < 0.03$ by log-rank test).
Discussion: Long-term results of LVAD implantation for BTT indication in recent years were satisfactory in many centers and perfect in our institute. We have revised and refined implant techniques and patient management aiming at prevention of surgical bleeding and less transfusion, migration of pump and cannulae and prevention of driveline infection. These refined strategies seemed to have a strong and favorable impact on recent improvement of long-term results.
Conclusions: Long-term results of continuous-flow LVAD for BTT indication greatly progressed thanks to refined implant techniques and patient management. This management strategies may have positive impact on better heart Tx results.

324.9

Medical utilization and costs of herpes zoster hospitalizations following heart transplantation in Taiwan

Kwai-Fong Lee[1,2], Yi-Chang Lin[3], Yi-Ting Tsai[3], Chih-Yuan Lin [3], Chung-Yi Lee[3], Hung-Yen Ke [3], Feng-Yen Lin[7], Wei-Hwa Lee[4,5], Chien-Sung Tsai[2,3,6].
[1]Biobank Management Center, Tri-Service General Hospital, Taipei, Taiwan; [2]Graduate Institute of Life Sciences, National Defense Medical Center, Taipei, Taiwan; [3]Division of Cardiovascular Surgery, Tri-Service General Hospital, Taipei, Taiwan; [4]Pathology, Taipei Medical University Shuang Ho Hospital, Taipei, Taiwan; [5]Graduate Institute of Injury Prevention and Control, Taipei Medical University, Taipei, Taiwan; [6]Division of Cardiovascular Surgery, Taoyuan Armed Forces General Hospital, Taoyuan, Taiwan; [7]Internal Medicine, Taipei Medical University, Taipei, Taiwan.

Background: Herpes zoster is a significant complication in solid organ transplant recipients. The data of incidence, medical utilization and costs of herpes zoster hospitalizations following heart transplantation in Taiwan is limited.
Methods: Heart transplantation patients with more than 90-day follow up between 2001 and 2012 were identified by Taiwan National Health Insurance research database. We used Student's t test to exam the difference and Kaplan Meier method to calculate the probability of developing herpes zoster hospitalizations following heart transplant.
Results: After exclusion of prior heart transplant and follow up less than 90 days, a total of 702 heart recipients were included with a median follow up of 42 months. 38 patients (5.4%) developed herpes zoster hospitalizations; the median time to onset was 14.2 months and overall incidence was 13.4 cases per 1000 person-years. There was no gender difference of incidence but older recipients (age ≥ 55 years old while heart transplantation) had high occurrence of herpes zoster hospitalizations ($p<0.01$). Of these 38 recipients with 50 herpes zoster hospitalizations, the average of total medical cost was $118,994 NTD (3,606 USD) and mean length of stay was 13.4 day. Fees of drug and room accounted for 54 % of total medical cost (34.3% and 20.0%, respectively). Twenty-seven of the herpes zoster hospitalization episodes (54.0%) were treated with acyclovir. Older recipients (age ≥ 55 years old while heart transplantation) had longer stay in hospital ($p=0.03$) but not total medical cost.
Conclusion: Older recipients have higher incidence and need more medical utilization for herpes zoster hospitalizations. Further strategies against herpes zoster infection after heart transplantations are crucial.

324.10

Metabolic profile of donation after circulatory death hearts during reconditioning

Hong Chee Chew[1], Ling Gao[1], Jeanette Villaneuva[1], Mark Hicks[1], Kumud Dhital[1,2], Peter MacDonald[1,2].
[1]Transplant Laboratory, Victor Chang Cardiac Research Institute, Sydney, Australia; [2]Heart Lung Clinic, St Vincent's Hospital, Sydney, Australia.

Aim: Donation after Circulatory Death (DCD) hearts have been shown to be viable after appropriate period of resuscitation on ex-vivo reperfusion circuits. These hearts were subsequently transplanted, and short term outcomes appear to be equivalent to brain dead (BD) heart transplants. Ischaemic events in the peri-mortem period cause considerable metabolic derangements which possibly impact cardiac reconditioning. Hence we aim to understand cardiac metabolic demands during early ex-vivo perfusion in order to limit ischaemic reperfusion injury (IRI) and improve functional recovery in DCD hearts.
Method: Landrace pigs (n=12) were anaesthetised and asphyxiated until circulatory arrest, followed by a stand-off period of 5mins. Blood collection and preservation flush occurred prior to explantation and installation on the ex-vivo device. Blood samples at baseline, following circuit priming, and every hour thereafter were taken. Arterial pH, haematocrit (Hct) and electrolyte profile (K+, Ca2+, and HCO3-) were measured using an I-Stat analyser.
Results: All results are presented in table below.

	Baseline	Blood + Priming Solution	Installation of Heart	180 mins post reconditioning
pH	7.36 ± 0.08	7.53± 0.06	7.27 ± 0.05	7.31 ± 0.06
Hct	23 ± 5.5%	15 ± 6.0%	13 ± 3.1%	
Ca^{2+} (mM)	1.3 ± 0.15	0.64 ± 0.32	0.53 ± 0.36	
K^{+} (mM)	3.89 ± 0.19	5.59 ± 1.78	4.66 ± 1.98	
HCO_3^- (mM)	33.78 ± 2.93	23.92 ± 3.15	20.42 ± 2.67	
Lactate	2.14 ± 2.33		2.64 ± 0.56	1.75 ± 0.63

Warm ischaemic time (WIT) and back-table time (BTT) was 21 +/- 5 and 29 +/- 6 min for the heart. The average blood volume collected was 1.6L: 1:1 dilution with Krebs resulted in significant hemodilution (from 23 +/- 5% to 15 +/- 6%) and hypocalcemia (1.30 +/- 0.15 to 0.64 +/- 0.32mM). This resulted in sub-optimal cardiac contractile recovery despite a favourable lactate profile. Changes in [Ca], [HCO} worsens overtime due to consumption, unless externally replaced. In contrast, a rise in [K] with circuit priming is partially corrected following heart installation. There is considerable alkalosis when introducing blood to the priming solution (500mls Krebs solution + Gelofusine) but this is overcompensated with acidosis when the heart is initially installed on the ex-vivo circuit. However, this acidosis is corrected overtime.
Conclusion: In porcine model, significant biochemical derangement occurs from the time of withdrawal. In addition to the dilutional effect of the priming solution, there is consumptive trend overtime unless externally replaced. Correction by the organ is limited and requires stringent replacement to ensure adequate recovery.
University of New South Wales

324.11

Low molecular weight heparine as alternative anticoagulation therapy in patients with left ventricular assist devices

Ozgur Ersoy[1], Bahadır Gültekin[1], Arbër Kutllovci[2], Ilknur Akkaya[1], H. Tankut Akay[1], Atilla Sezgin[1].
[1]Department of Cardiovascular Surgery, Baskent University, Ankara, Turkey; [2]Department of Cardiac Surgery, University of Prishtina Hasan Prishtina Faculty of Medicine, University Clinic Centre of Kosova, Prishtina, Kosovo.

Introduction: Anti-coagulation therapy is necessary in patients with left ventricular assist devices (LVAD). Low molecular weight heparine (LMWH) in the early post-operative period and oral anticoagulation (Warfarin sodium) in the late post-operative period are preferred for most of the patients with LVAD implantations. The aim of this study was to discuss the use of LMWH in patients with LVAD implantations who can not use oral Warfarin sodium.
Material and Methods: Thirty seven patients with LVAD implantations from January 2012 to February 2016 have been evaluated. Average age of patients is 36,0±24,62 years. We have to use LMWH for six of the patients. We used LMWH from the early post operative period for two of them, we started to use LMWH for four of them after discontinuing Warfarin sodium because of cerebrovascular accidents or while waiting for emergency heart transplantion list. LMWH acitvity was monitored by measuring of anti-factor Xa levels in plasma per week.
Results: Mean effective anti-Xa activity was 0,73±0.19 IU/mL which was within the defined range of efficacy 0,6 to 1 IU/mL. Mean period of anti-coagulation time with LMWH was 124 days±63 days (maximum 184 days, minimum days. There was no observed pump thrombus and thromboembolic complications. There was only one patient with gastrointestinal bleeding required an intervention.
Conclusion: Our results show that use of LMWH has constant biological efficacy and can be an alternative of anticoagulation therapy for long time¸ but more studies are needed to be assure.

324.12

Hospital readmissions after left ventricular assist device implantation

Christian Heim, Nattipong Mekkhala, Markus Kondruweit, Michael Weyand, Rene Tandler.
Cardiac Surgery, University of Erlangen, Erlangen, Germany.

Objectives: The number of patients provided with a continuous flow left ventricular assist device (LVAD) is steadily increasing. So far, little is known about health care resource utilization in the long term. Therefore the aim of this study was to analyse unplanned hospital readmissions of patients after LVAD implantation.
Methods: We analysed the clinical course of 62 patients who underwent continuous flow LVAD implantation (HeartWare HVAD or BerlinHeart Incor) between January 2010 and December 2014 and were followed up in our outpatient care unit. The cause of readmission, timing after LVAD implantation, and length of hospital stay were evaluated.
Results: 41 of 62 patients were readmitted 78 times (1.9 times/patient) until the end of follow up (cut off 08/31/2015). The median follow up after LVAD implantation was 17.5 months. The main causes of unplanned readmissions included neurological events (10% stroke and 8% ICB), assist device associated infections (12%), haemorrhage and gastrointestinal bleeding (13%) and device thrombosis (6%). Other causes included non-device related infections (11%). Analysis of device specific cause of hospital readmission revealed a trend of gastrointestinal bleeding and device thrombosis in HeartWare patients and stroke and haemorrhage in BerlinHeart Incor patients. Interestingly, readmissions did not significantly influence long-term survival in a negative manner (6.8% mortality [pts. without readmission] vs. 13.2% mortality [pts. with readmission]; p=.65).
Conclusion: Main causes of readmission after LVAD implantation included neurological events and bleeding. Interestingly, readmissions did not significantly influence long-term survival in a negative manner. Multiple readmissions of a single patient with the same clinical pattern might bias the analysis. Further investigations appear attractive to analyse health care resource utilization after LVAD implantation.

324.13

Prior clotting events as predictor of post LVAD morbidity and mortality

Justin Johannesen[2], Jabar Whittier[1], Matthew Carazo[1], Scott Feitell[1], Theresa Rowe[1], Wade Fischer[3], Shelley Hankins[1], Howard J.Eisen[1].
[1]Cardiology, Drexel University College of Medicine, Philadelphia, PA, United States; [2]Internal Medicine, Drexel University College of Medicine, Philadelphia, PA, United States; [3]Cardiothoracic Surgery, Drexel University College of Medicine, Philadelphia, PA, United States.

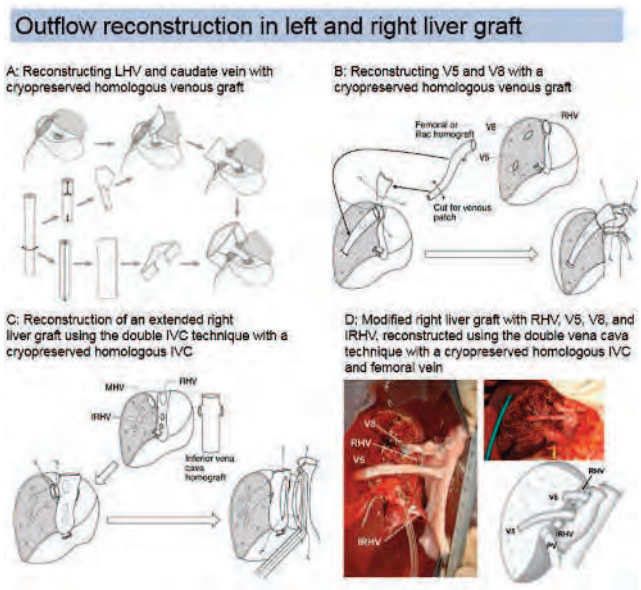
Introduction: Bleeding, pump thrombosis, and other thromboembolic events remain among the most feared post implantation complications for patients receiving LVADs. Despite these concerns, a patient's coagulopathy history is rarely discussed when patients are presented as potential candidates for device implantation. This project aims to determine if patients with previous histories of clotting are more prone to these post LVAD complications.
Methods: A retrospective chart review of 46 patients who received Heartmate II LVADs from 2008-2013 in a single university transplant center was performed. These patients were sorted into those who had no history of clotting events and those who did. Clotting events included PE, DVT, LV/LA thrombus, thrombophlebitis and CVA. The two groups were then examined to determine which group suffered more post LVAD complications including hemorrhage, pump thrombosis, death within 6 months, stroke and MI.
Results: Of a total of 46 patients, 12 had a history of previous clot while 34 did not. Seventeen patients had an adverse event post LVAD implantation. Of those 17 patients, nine had a history of prior clot. In our analysis we found a significant relationship between history of clot and adverse LVAD outcome ($p = .004$, OR 9.75, 95% CI 2.11-44.9).
Conclusion: Despite a limited number of patients, our results showed a statistically significant difference in post LVAD complications between those patients with prior clotting events and those without. While future larger studies are warranted, these results would argue for different coagulation parameters in patients with histories of clotting.

325.1

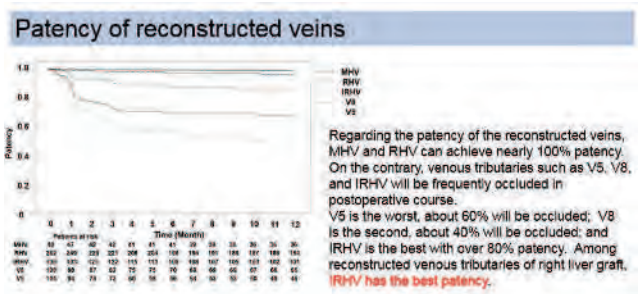
Outflow reconstruction using the homologous venous grafts in living donor liver transplantation: Experience at the University of Tokyo Hospital

Nobuhisa Akamatsu[1,2], Sumihito Tamura[1,2], Yoshihiro Sakamoto[2], Kiyoshi Hasegawa[2], Norihiro Kokudo[2].
[1]Tissue Bank, The University of Tokyo Hospital, Tokyo, Japan; [2]Artificial Organ and Transplantation Surgery Division, Department of Surgery, University of Tokyo, Tokyo, Japan.

Back ground: It is well accepted that not only the actual graft volume but also the uncongested volume of it is a key issue for the successful LDLT. Therefore, congested area should be minimized to the possible extent. Reconstructing the MHV tributaries, IRHV or caudate vein are important with this aspect, however, direct anastomosis between these vessels to IVC requires the technical difficulty and prolongs warm ischemic time. To overcome these problems, we have reported several innovations in the reconstruction of these veins at the bench surgery, utilizing the cryopreserved homologous veins (Figure 1).



Method: We have performed 462 adult LDLT. Of these 55% of the donors underwent right liver resection, 39% with left liver resection, and 7% with posterior liver resection. We routinely use the cryopreserved vein grafts for the outflow reconstruction, and herein present our way of venous reconstruction in LDLT.
Results: Left liver graft and right liver graft is equal in terms of short- and long-term outcome, provided the proper graft selection algorithm. 90 days mortality rate was 5%. The incidence of severe complications (Clavien Grade IIIb and more) was 38%. The incidence of outflow obstruction requiring interventional or surgical treatment was 1% in the left (2/179) and 2% in the right liver (6/257), respectively. Regarding the patency of the reconstructed veins, LHV MHV and RHV can achieve nearly 100% patency. On the contrary, venous tributaries such as V5, V8, and IRHV will be frequently occluded in postoperative course. V5 is the worst, about 60% will be occluded; V8 is the second, about 40% will be occluded; and IRHV is the best with over 80% patency. Among reconstructed venous tributaries of right liver graft, IRHV has the best patency (Figure 2).



Conclusion: In conclusions, outflow reconstruction is a key for the successful results. Cryopreserved vein graft is useful for the reconstruction.

325.2

Outcome of living donor liver transplantation using right liver allografts with multiple arterial supply

Kyo Won Lee[1], Min Jung Kim[2], Sanghoon Lee[1], Nuri Lee[1], Chan Woo Cho[1], Kyeong Sik Kim[1], Seounghyun Kim[1], Ji Soo Lee[1], Jong Man Kim[1], Gyuseong Choi[1], Jae-Won Joh[1].
[1]Department of Surgery, Samsung Medical Center , Seoul, Korea; [2]Department of Surgery , Jeju National University Hospital , Jeju, Korea.

Introduction: Partial liver graft with multiple hepatic arterial (HA) stumps can be faced in 10% of living donor liver transplantation (LDLT). In these cases, a smaller HA may elicit hepatic artery thrombosis. Therefore, whether to reconstruct all HA stumps on a graft is an ongoing debate. This study was designed to validate the safety of partial reconstruction of multiple HAs in LDLT using right lobe graft.
Method: From January 2000 to June 2014, 1149 patients underwent LDLT procedures at the Samsung Medical Center. Among them, 30 cases of LDLT using right lobe graft with multiple HA stumps and 149 cases of LDLT using right lobe graft with single HA sampled from database were enrolled. These cases were divided into three groups: single HA group (Group 1, n = 149), multiple HAs with total reconstruction (Group 2, n = 19), and multiple HAs with partial reconstruction (Group 3, n = 11). Biliary complication rate, HA complication rate, patient survival, and graft survival were compared among groups. Partial reconstruction was performed only when there was pulsatile back-bleeding after larger HA reconstruction and sufficient intrahepatic arterial flow was confirmed by Doppler ultrasound (DUS).
Result: In Group 2, the median size of donor HAs was smaller ($P < 0.001$) and the duration of HA reconstruction was longer ($P < 0.001$). However, there was no significant difference in biliary complication rate, artery complication rate, patient survival, and graft survival. Risk factor analysis revealed that multiple HAs itself and partial reconstruction of HAs were not related to biliary anastomosis stenosis, biliary leakage, hepatic artery stenosis, graft survival and patient survival.
Conclusion: In conclusion, partial reconstruction of HAs in LDLT using right lobe graft is feasible when intrahepatic arterial communication is confirmed by pulsatile back-bleeding from the smaller artery and DUS.
References:
[1] Sugawara, Y., et al. Surgery 149(6): 841-845.
[2] Kim, S. J., et al. Clin Transplant 25(6): 929-938.
[3] Uchiyama, H., et al. Surgery 147(6): 878-886.
[4] Suehiro, T., et al. Liver Transpl 8(5): 495-499.
[5] Marcos, A., et al. Liver Transpl 7(8): 673-679.

325.3

The effect of anhepatic phase extended by temporary portocaval shunt on anesthetic sensitivity to desflurane administered at a constant age-adjusted minimum alveolar concentration and postoperative cognitive function in patients undergoing living donor liver transplantation

Jong Hae Kim[1], Jin Yong Jung[1], Dong Lak Choi[2], Taeha Ryu[1], Sung Hye Byun[1], Joo Dong Kim[2].
[1]Department of Anesthesiology and Pain Medicine, School of Medicine, Catholic University of Daegu, Daegu, Korea; [2]Division of Hepatobiliary Pancreas Surgery and Abdominal Organ Transplantation,Department of Surgery, School of Medicine, Catholic University of Daegu, Daegu, Korea.

Introduction: Anesthetic requirements have been found to be lowest during anhepatic phase among the three phases of living donor liver transplantation (LDLT). However, the effect of duration of the anhepatic phase on anesthetic sensitivity and postoperative cognitive function has not been investigated when a constant minimum alveolar concentration (MAC) of inhalational anesthetic agent is administered. We assessed the anesthetic sensitivity to desflurane administered at a constant MAC and perioperative cognitive function in patients undergoing LDLT where the duration of the anhepatic phase was extended by temporary portocaval shunt (TPCS) to test the hypothesis that a prolonged anhepatic phase increases the anesthetic sensitivity, in turn resulting in postoperative cognitive decline.
Materials and Methods: This study was conducted in 67 consecutive patients undergoing LDLT from February 2014 to January 2016. Anesthesia was maintained at an end-tidal desflurane concentration, which corresponds to 0.6 age-adjusted MAC, between the peritoneal incision and closure. A TPCS was created in select cases, such as severe adhesions or massive bleeding. Throughout the surgery, bispectral index (BIS) was maintained below 60 and was averaged at 1-minute intervals. Mini-mental state examination in the Korean version of the consortium to establish a registry for Alzheimer's disease assessment packet (MMSE-KC) was performed 1 day before and 7 days after the LDLT. The averaged BIS values of each phase and preoperative and postoperative MMSE-KC scores were compared between the patients receiving TPCS (TPCS group) and those who did not (non-TPCS group).
Results: TPCS was performed in 16 patients (24%). The TPCS prolonged the duration of the anhepatic phase (125.9 ± 29.4 versus 54.9 ± 20.5 minutes [mean ± standard deviation], $P < 0.0001$) shortening that of the preanhepatic phase (80.1 ± 38.4 versus 137.2 ± 27.9 minutes, $P < 0.0001$). The averaged BIS values during the three phases were comparable between the two groups. No significant interval changes in the averaged BIS value were observed during the three consecutive phases. Likewise, there were no significant differences in MMSE-KC score assessed 1 day before and 7 days after the LDLT between the two groups. The preoperative MMSE-KC scores were unchanged postoperatively in the two groups.
Discussion: The extension of the anhepatic phase does not seem to affect the anesthetic sensitivity to desflurane and postoperative cognitive function according to the insignificant differences in BIS values during the three phases and perioperative MMSE-KC scores between the two groups as well as the insignificant interval changes in BIS values throughout the surgery and cognitive function between 1 day before and 7 days after the surgery.
Conclusion: The prolonged duration of anhepatic phase caused by TPCS does not influence anesthetic sensitivity to desflurane and postoperative cognitive function.
References:
[1] Hasanin AS, Mahmoud FM, Yassen KA. Entropy-guided end-tidal desflurane concentration during living donor liver transplantation. *Saudi J Anaesth.* 2013; 7: 399-403

325.4

Domino liver transplantation: Technique and outcome

Neslihan Celik, Frederick Vyas, Armando Ganoza, Geoffrey J.Bond, Kyle A.Soltys, Rakesh Sindhi, George V.Mazariegos.
Hillman Center for Pediatric Transplantation, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA, United States.

Aim: Domino liver transplantation (DomLT) using allografts with metabolic disorders enhances organ utilization but is not well described in children. Since the documentation of short and long term course of these patients are critical to decision making about the safety of this procedure, we reviewed the outcomes of DomLT at a single center.
Methods: All patients receiving DomLT were analyzed retrospectively with minimum one year follow-up period for patient and donor characteristics, early and late postoperative complications and patient and graft survivals. We also reviewed the MSUD (Maple Syrup Urine Disease) donors of these patients who received deceased donor liver transplantation at the same time in terms of age, weight, cold ischemia time, postoperative leucine levels and peak ALT (Alanine Aminotransferase) levels during postoperative 48 hours.
Results: Between 2007 and 2014, 10 patients underwent liver transplantation with domino live-donor allografts from MSUD patients. The indications were progressive familial intrahepatic cholestasis, cystic fibrosis, congenital hepatic fibrosis, embryonal sarcoma, primary sclerosing cholangitis, alpha-1-antitrypsin deficiency and chronic rejection after liver transplantation. All patients and grafts survived. Median recipient and donor ages were 19.4 and 16.6 respectively. There was no vascular complication at early postoperative period, one patient had portal vein thrombosis 3 years after transplantation and meso-Rex bypass was performed. Small for size syndrome occurred in reduced left lobe DomLT recipient and managed successfully. Biliary stricture developed in 2 patients and solved by stenting.
The data and comparison were listed in the Table below

Recipient Demographics and Functional Metrics	DomLT Recipient	MSUD Recipient	p-value
Mean age at transplantation (years)	22.4	15.9	0.28
Mean weight (kg)	56.1	55.1	0.93
Posttransplant mean leucine level (umol/dL)	12.3	18.1	0.000
Posttransplant mean cold ischemia time (minute)	302.4	349.0	0.26
Posttransplant peak ALT in 48 hours (IU/L)	288.1	984.8	0.007

Conclusion: Patient and graft survival in DomLT from MSUD donors has been excellent at long term follow-up. Metabolic function has been normal in all recipients on normal unrestricted protein intake. Ischemia preservation injury based on peak ALT has been significantly decreased in DomLT recipients. Domino transplant from pediatric and adult recipients with selected metabolic disease should be increasingly considered.

325.5

Outcome of partial reconstruction of multiple hepatic arteries in pediatric living donor liver transplantation using left lateral section grafts

Kyo Won Lee[1], Min Jung Kim[2], Jong Man Kim[1], Gyuseoung Choi[1], Jae-Won Joh[1], Nuri Lee[1], Kyeong sik Kim[1], Ji Soo Lee[1], Seounghyun Kim[1], Jonghwan Lee[1].
[1]Department of Surgery , Samsung Medical Center , Seoul, Korea; [2]Department of Surgery, Jeju National University Hospital , Jeju, Korea.

Introduction: In pediatric end-stage liver disease patients, the use of partial liver grafts from adult living donors has been increasing for liver transplantation due to a shortage of size-matched liver allografts. Partial liver grafts commonly involve multiple hepatic artery (HA) stumps. In these cases, a smaller HA may elicit hepatic artery thrombosis (HAT). Whether to reconstruct all HA stumps on a graft is an ongoing debate.
Methods: This study was designed to validate the safety of partial reconstruction of multiple HAs in pediatric living donor liver transplantation (LDLT) cases. From January 2000 to June 2014, 159 pediatric patients underwent LDLT. Among these, ABOi cases, re-transplantation cases, and right lobe cases were excluded. The remaining 136 patients were categorized into three groups: single HA group (group 1, n = 74), multiple HAs with total anastomosis group (group 2, n = 23), and multiple HAs with partial reconstruction group (group 3, n = 39). Biliary complication rate, HA complication rate, patient survival, and graft survival were compared between groups. Partial reconstruction was performed only when there was pulsatile back-bleeding after larger HA reconstruction and sufficient intrahepatic arterial flow was confirmed by Doppler ultrasound (DUS).
Results: In group 2, the median size of donor HAs was smaller ($P < 0.001$) and the duration of HA reconstruction was longer ($P < 0.001$). However, there was no significant difference in biliary complication rate, artery complication rate, patient survival, and graft survival. Risk factor analysis revealed that male recipients, bile duct diameter < 5mm, and total duration of operation were risk factors for biliary anastomosis stricture (BAS). Multiple HAs itself and partial reconstruction of multiple HAs were not risk factors of BAS.
Conclusion: In pediatric LDLT, partial reconstruction of HAs in a left liver graft with multiple HA stumps does not increase the risk of BAS or affect graft survival when intrahepatic arterial communication is confirmed by pulsatile back-bleeding from the smaller artery and DUS.
References:
[1] Julka, K. D., et al. Pediatr Surg Int 30(1): 39-46.
[2] Lee, S., et al. Transpl Int 28(7): 835-840.
[3] Suehiro, T., et al. Liver Transpl 8(5): 495-499.
[4] Uchiyama, H., et al. Surgery 147(6): 878-886.
[5] Takatsuki, M., et al. Surgery 140(5): 824-828; discussion 829.

325.6

The novel use biodegradable SX-ELLA biliary stent placed by percutaneous transhepatic aproach for the treatment of biliary stenosis after liver transplantation

Cristina Dopazo[1], Anna Curell[1], Iratxe Diez[3], Carla Gonzalez-Junyent[3], Mireia Caralt[1], David Hernandez[3], Jose Luis Lazaro[1], Javier Juamperez[4], Lluis Castells[2], Jesus Quintero[4], Mercedes Perez[3], Itxarone Bilbao[1], Ramon Charco[1].
[1]Department of HPB Surgery and Transplants, Hospital Universitario Vall D'Hebron, Barcelona, Spain; [2]Department of Internal Medicine, Liver Unit, Hospital Universitario Vall D'Hebron, Barcelona, Spain; [3]Department of Interventional Radiology, Hospital Universitario Vall D'Hebron, Barcelona, Spain; [4]Pediatric Liver Transplant Unit, Hospital Universitario Vall D'Hebron, Barcelona, Spain.

Introduction: The uncovered self-expandable metal stents placed by percutaenous transhepatic approach (PTA) was used routinely in our center for the treatment of biliary stenosis after liver transplantation (LT) when balloon dilatation was failed. However the rate of complications thereafter achieved more than 60%. Biodegradable SX-ELLA biliary stents are available in our center since August 2014 and no results in LT were published previously in the literature.
Objectives: To evaluate feasibility, safety and outcome of patients treated with biodegradable SX-ELLA biliary stents placed by percutaneous transhepatic approach for the treatment of biliary stenosis after LT in adult and pediatric setting.
Methods: Observational retrospective single-centre study including adult and pediatric patients underwent a first LT who developed biliary stenosis (anastomotic or non anastomotic stenosis) treated with Biodegradable SX-ELLA biliary stents. Once biliary stenosis is diagnosed by Magnetic Resonance Cholangio-pancreatography the percutaneous transhepatic balloon dilatation of biliary stenosis is the first approach in our center, and stenting when the stenosis is not resolved after two or more procedures. The stent size depends on balloon size and length of stenosis. The median patient follow-up period after stent was placed was 14 months (6-19 months).
Results: Between August 2014 and August 2015 ten patients required a biodegradable SX-ELLA biliary stent by PTA. Six patients were adults with a median age of 60 (49-68) years-old, the main indication for LT was HCC (67%) and all of them have received whole graft from brain-dead donors and the biliary reconstruction was end-to-end in all cases. Four patients were children with a median age of 1 (1-9) years-old, the cause of transplant was Wilson disease, hepatoblastoma, biliary atresia and cholestatic familiar disease and two patients received whole graft from brain-dead donors and two cases partial graft (split liver and living donor) being the hepatoyeyunostomy performed in 3 of them. The biliary complications after LT appeared in a median time of 15 (1-194) months.
After a median of unsuccessful balloon dilatation of 1.5 (1-4), biodegradable SX-ELLA biliary stents were placed due to anastomosis stenosis in 9 cases (with patent artery in 6 patients) and one case was due to non-anastomotic stenosis. Stents implantations were always feasible and no immediate major complications occurred in any case. Three adult patients with arterial thrombosis (30%) presented the following complications after procedure related to progression of ischemic cholangiopathy: one cholangitis at 30th day and two stent obstructions at 63rd and 85th day respectively. None of them required further surgical treatment.
Conclusions: Biodegradable biliary stents represents a new feasible option for treatment of biliary stenosis after LT either in adult or children LT patients with a low early morbidity.

325.7

V5/V8 hepatic vein thrombus after left lobe donation surgery

Kaori Kuramitsu, Takumi Fukumoto, Hisoka Kinoshita, Motofumi Tanaka, Masahiro Kido, Sachio Terai, Taku Matsumoto, Tadahiro Goto, Sadaki Asari, Hirochika Toyama, Tetsuo Ajiki, Yonson Ku.
Surgery, Division of Hepato-Biliary and Pancreatic Surgery, Kobe University, Kobe, Japan.

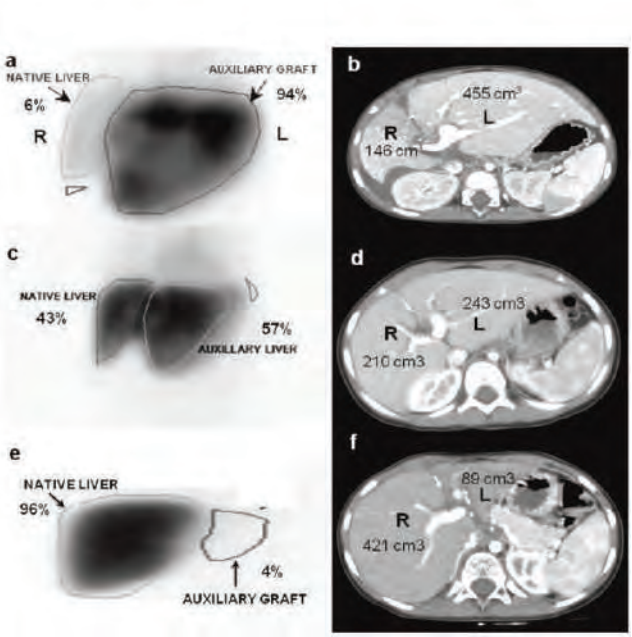
Background: Left lobe liver graft has been widely accepted as a favorable grafts for living donors. However up to date, detailed analysis is not well performed for potential risks for left lobe donors. We analyzed the effect of V5/V8 hepatic vein thrombus on left lobe donors after left lobe donation surgery.
Methods: From February 2004 to November 205, 40 cases of left lobe donation surgery were performed at Kobe University Hospital. Among them 35 cases (87.5%) were eligible for the study with CT images. Preoperative factors (donor age, liver/spleen ratio, % of remnant liver volume/whole liver volume, middle hepatic vein (MHV) dominancy), intraoperative factors (bleeding volume, operation time, intraoperative transfusion), and postoperative factors (AST/ALT/T-bil levels at peak and day 7, length of hospital stay, remnant liver regeneration at 1 week and 1 month) were analyzed as a potential risk factor for the formation of V5/V8 thrombus.
Results: V5/V8 thrombus was detected in 21 cases (60%). Among preoperative factors, MHV dominancy (p=0.01) was identified as a risk factor for the formation of V5/V8 thrombus. Among intraoperative factors, bleeding volume (p=0.02), operation time (p=0.03), and intraoperative transfusion (p=0.01) were identified as risk factors. Among postoperative factors, level of day7 ALT was identified as a risk factor. Postoperative remnant liver regeneration at 1 week and 1 month did not differ with the existence of V5/V8 thrombus with statistical difference.
Conclusion: V5/V8 thrombus was identified in 60% of cases after left lobe donation surgery. Although the existence did not affect postoperative donor courses, it is now proved that the formation of V5/V8 thrombus associated with surgical difficulties. To maintain zero donor mortalities, cautions should be taken for the selection of left lobe grafts especially for MHV dominant cases.

325.8

Auxiliary partial orthotopic liver transplantation (APOLT): A safe & effective alternative to orthotopic liver transplantation for patients with acute liver failure and non-cirrhotic metabolic liver disease

Mettu Reddy, Naresh Shanmugham, Joy Varghese, Ilankumaran Kaliamoorthy, Rajesh Rajalingam, Venugopal Kota, Gomathy Narasimhan, Mohamed Rela.
Institute of Liver Disease & Transplantation, Global Health City, Chennai, India.

Background: In Auxiliary Partial Orthotopic Liver Transplantation (APOLT), a part of the recipient's native liver is resected and a healthy partial graft is implanted orthotopically in its place. It has been used in selected cases of acute liver failure (ALF) and non-cirrhotic metabolic liver disease (MLD). In ALF, it acts as a bridge to native liver regeneration, while in MLD it supplements the native liver with the missing functional protein thus correcting the symptoms. It is not universally accepted because of concerns of technical complications. We present our series of APOLT procedures and discuss the indications, safety, and outcomes.
Methods: Data of all APOLT procedures performed in our institute between Sep 2009 and Feb 2016 was retrieved. Indications for APOLT, operative details, complications, short term and long term outcomes are reported.
Results: 14 APOLT procedures were performed during the study period, forming 2% of our overall transplant numbers. Follow-up ranged from 2 weeks to 75 months.
6 APOLT procedures were performed for ALF. There were 3 children & 3 adults (Age range 9 months to 31 years). All underwent LDLT-APOLT (Graft type: left lateral: 2, left lobe:1, right lobe:3) after native hepatectomy (left hepatectomy:3, right hepatectomy: 3). There were no early or late technical complications. One patient transplanted for yellow phosphorus poisoning died 4 weeks after transplant with multiple organ failure. Of the 5 survivors, two children had good native liver regeneration and were completely weaned off immunosuppression while 3 others were stable on immunosuppression.
8 APOLT procedures were performed for MLD (6 children, 2 adult). Indications were Criggler Najar syndrome type 1 (n=3), propionic acidemia (n=4) and citrullinemia (n=1). 5 patients underwent LDLT-APOLT while 2 underwent DDLTsplit- APOLT. All patients underwent left hepatectomy and received left lateral grafts (n=6) or left lobe grafts (n=2). All patients needed intra-operative portal flow modulation by graded portal banding to prevent portal steal. One child with propionic acidemia developed early HAT, which was managed successfully with surgical revascularisation. Another child with propionic acidemia developed recurrence of symptoms secondary to portal steal after 12 months. This was effectively managed by segmental native portal vein embolization. At followup all 8 patients were in good health with complete resolution of their original symptoms.
Discussion: Our data shows that APOLT is a safe alternative to orthotopic liver transplantation for selected patients with ALF. In our ALF cohort, two patients (33%) had become completely immunosuppression free. APOLT in MLD ensures adequate backup native liver function in case of graft related complications thus improving recipient safety. It also improves living donor safety as a smaller sized graft, (such as left lateral graft) is sufficient to treat the metabolic defect even in adult recipients. To our knowledge, this is the largest reported Asian series outside Japan.
Conclusion: APOLT is a viable alternative to OLT for selected patients with ALF and noncirrhotic MLD.



Serial HIDA and CT scans in a 2 year old child who underwent left sided APOLT for fulminant liver failure due to Hepatitis A infection. Figures a,b at 1 month, c,d at 24 months and e,f at 30 months. Serial scans show a gradual increase in size of native liver remnant (right lobe), while the left graft shrinks. This child was completely taken off immunosuppression 24 months after transplant.

325.9

Reducing non-anastomotic biliary strictures after donation after circulatory death liver transplantation, a matter of time?

Nicholas Gilbo, Ina Jochmans, Mauricio Sainz, Jacques Pirenne, Diethard Monbaliu.
Abdominal Transplantation Surgery and Coordination, University Hospitals of Leuven, Leuven, Belgium.

Liver Transplantation (LT) from donation after circulatory death (DCD) is associated with increased non-anastomotic biliary strictures (NAS) and graft loss. Donor and recipient demographics, transplant and outcome data were compared between recipients with NAS (NAS+) and those without (NAS-) in a cohort of 61 consecutive DCD-LT (01/2003-12/2013). Risk factors for NAS occurring <1y after DCD-LT were identified in multivariate regression. Median (IQR) is given.
13/61 developed NAS. Incidence of NAS decreased over time (30% in 2003-2010, 15% in 2011-2013) in parallel with a decrease in cold ischemia time (CIT) [6.8h (5.5-8) in 2003-2010, 5.4h (4.75-6.4) in 2011-2013, p=0.002]. Donor and recipient age and gender, warm ischemia time [NAS+ 20min (15-29) vs NAS- 22min (16-28), p=ns], donor peak AST/ALT did not differ. NAS+ had a higher DRI [3.01 (2.86-3.49) vs 2.67 (2.37-3.05), p=0.031], longer CIT [7.3h (5.95-8.52) vs 5.6h (4.97-6.75), p=0.004] and anastomotic time [55min (46.5-60.5) vs 46min (42-52.5), p=0.038]. NAS occurred more frequently in grafts exposed to CIT>5h vs CIT<5h (26.5% vs 0%, p=0.054). Peak ALT post-LT was higher in NAS+ vs NAS- [1114IU/L (745-1566) vs 645IU/L (318-1087), p=0.019]. No difference in early allograft dysfunction (NAS+ 23.1% vs NAS- 20.8%, p=ns) or acute kidney injury was observed. The need for re-LT and endoscopic biliary intervention was higher in NAS+ vs NAS- (7.7% vs 0% and 84.6% vs 10.4% respectively, p<0.0001). One year censored graft and patient survival were similar between NAS+ and NAS- (84.6% vs 89.6%, 92.3% vs 91.7% respectively, p=ns). NAS did not influence the risk of death (HR:1, 95%CI:0.28-3.6) or graft loss (HR:1.62, 95%CI:0.57-4.6). CIT was the only independent risk factor of NAS (HR:1.42, 95%CI:1.06-1.92). Even with overall short CIT (5.78h, 5.13-7.13), the risk for NAS development in the 1st year post-transplant increases 1,42 times by every additional hour of CIT. In the absence of interventions that might directly prevent NAS, active efforts to maximally reduce CIT in DCD-LT are essential.

325.10

Liver stiffness measurement using acoustic radiation force impulse (ARFI) in living and deceased donor orthotopic liver transplantation patients

Murat Haberal[1], Hale Turnaoglu[1], Adnan Ozdemir[1], Nihal Uslu[1], Nihan Haberal Reyhan[2], Gokhan Moray[3], Mehmet Haberal[3].
[1]Radiology, Baskent University, Ankara, Turkey; [2]Pathology, Baskent University, Ankara, Turkey; [3]General Surgery, Baskent University, Ankara, Turkey.

Background: Acoustic Radiation Force Impulse (ARFI) elastography is a new technology that provides numerical measurements (wave-velocity values) of tissue stiffness. It has been shown that ARFI is a reliable technique for diagnosing and excluding liver cirrhosis. However, the value of the ARFI technique is not yet well established in the clinical management of liver transplantation patients. The aim of this study was to evaluate the diagnostic efficiency of the ARFI technology of the assessment of fibrosis in orthotopic liver transplantation patients.
Materials and Methods: We enrolled 28 orthotopic liver transplantation patients (living and deceased donor), whose biopsy decision has been given clinically due to posttransplantation dysfunction, prospectively. ARFI elastographic measurements were applied before the biopsy or within three days after the biopsy, by two radiologists. Ten measurements in segment III-IV on the left lobe graft and segment VII-VIII on the right lobe graft could be obtained, avoiding large vessels or bile ducts. The measurements were performed about 3 cm in depth from the surface of the liver.
After the core needle liver biopsy, specimens of the patients were analyzed by an experienced pathologist, according to the modified Ishak scoring system. The measurements of ARFI sonoelastography and the results of pathology specimens were compared with the nonparametric Kruskal Wallis test (with Conover-Dunn multiple comparison test). The statistical significance was set at $p<0.05$.
Results: Orthotopic liver transplantation were deceased-donor in 5 patients (17.9%), and from living donor in 23 patients (82.1%). A total of 28 biopsies, 4 of fibrosis score were evaluated as F0 (14.3%), 16 of fibrosis score were evaluated as F1 (57.1%), 4 of fibrosis score were evaluated as F2 (14.3%) and 4 of fibrosis score were evaluated as F3 (14.3%). There were no specimens which fibrosis score evaluated as F4, F5 or F6. The ARFI measurements mean was calculated 1.4±0.07 in F0, 1.74±0.57 in F1, 2.19±0.7 in F2 and 2.18±0.35 in F3. There were no significant correlation of mean ARFI values between the F0 vs F1 and F0 vs F2 stages ($p= 0.956$, $p=0.234$, respectively). A statistically significant correlation of mean ARFI values was found between the F0 and F3 fibrosis stages ($p=0.046$).

Conclusions: ARFI imaging is a promising screening test for detecting significant liver fibrosis (≥F3 in modified Ishak scoring) in living or deceased donor orthotopic liver transplantation patients.

325.11

Efficacy of acoustic radiation force impulse (ARFI) elastography for predicting post-transplant clinical outcomes in living donor liver transplantation

Koji Natsuda, Akihiko Soyama, Mitsuhisa Takatsuki, Masaaki Hidaka, Takanobu Hara, Tota Kugiyama, Hajime Imamura, Zhassulan Baimakhanov, Satomi Okada, Shinichiro Ono, Amane Kitasato, Tamotsu Kuroki, Susumu Eguchi.
Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan.

Introduction: Although time-zero biopsy has been reported to predict adverse clinical outcomes after liver transplantation, the results will be known after transplantation. Recently, acoustic radiation force impulse (ARFI) elastography is widely used for evaluating liver fibrosis. The aim of this study was to evaluate the efficacy of preoperative ARFI elastography for evaluating a quality of the graft and clinical outcomes in living donor liver transplantation (LDLT).
Patients and Methods: Seventy-four LDLT donor candidates s in Nagasaki University Hospital between August 2010 and August 2015 who preoperatively underwent ARFI elastography were enrolled in this study. Velocity of shear wave (Vs) value of the right lobe was measured by ARFI elastography, and we conducted following analyses: 1, correlation between Vs and the age of donors, 2, correlation between Vs and fibrosis staging of time-zero biopsy, 3, correlation between Vs and the regeneration rate of remnant liver (liver volume 1 month after LDLT/ expected remnant liver volume), 4, correlation between Vs and 1-year survival rate.
Results: The median of Vs was 1.05, and range was 0.73-1.93. 1. There was no significant correlation between Vs and the age of donors. 2. The number of F0/F1/F2 in time-zero biopsy were 59/14/1, respectively. The Vs of F0 and F1/2 were 1.05 and 1.08 (N.S.). 3. We used two Vs cut-off values those were previously reported (Sporea et al. Eur J Radiol. 2012): 1.19 (equal to F1) and 1.33 (equal to F2). And all patients were separated by graft types, right lobe graft (RLG, n=29) and extended left lobe graft (ELLG, n=45). In RLG, the median Vs lower or higher than 1.19 (n=25, 4) and 1.33 (n=27, 2) were 2.27 and 1.60 ($P <0.01$), 2.22 and 1.77 ($P =0.13$), respectively. And in ELLG, the median Vs lower or higher than 1.19 (n=34, 11) and 1.33 (n=40, 5) were 1.30 and 1.27 ($P =0.35$), 1.31 and 1.18 ($P <0.05$), respectively. 4. One year survival rate of the recipients in the same category in section 3 were compared by log-rank test. One year survival rate lower or higher than 1.19 and 1.33 were 84.2% and 65.2% ($P =0.09$), 82.7% and 57.1% ($P <0.05$), respectively.
Discussion: Despite significant correlation between Vs and clinical outcomes, Vs was not correlated with donor age and the zero-biopsy. Hence, Vs was considered an independent predictor of post-transplant clinical outcomes.
Conclusion: The grafts with preoperative high Vs value resulted in poorer graft regeneration and worse short-term survival of recipient despite no differences in time-zero biopsy. Preoperative ARFI elastography might be an useful tool for predicting post-transplant clinical outcomes. ARFI elastography was considered an effective examination for preoperative evaluation in LDLT.

325.12

Early enteral feeding after living donor liver transplantation prevents infectious complications: A prospective pilot study

Jong Man Kim[1], Kyung-Sik Kim[1], Nuri Lee[1], Chan-Woo Cho[1], Hyun Jung Kim[1], Sung-Hye Kim[2], Miyong Rha[2], Gyu-Seong Choi[1], Choon Hyuck D.Kwon[1], Jae-Won Joh[1], Suk-Koo Lee[1].
[1]Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; [2]Department of Dietetics, Samsung Medical Center, Seoul, Korea.

Background: Infectious complications, including bacteria, virus, and fungus, often occur after liver transplantation and are the most frequent cause of in-hospital mortality. The present study prospectively analyze the effect of early enteral feeding in patients after living donor liver transplantation (LDLT)**.**
Methods: Between January 2013 and August 2013, 36 patients underwent LDLT. These patients were randomly assigned to receive enteral formula via nasointestinal feeding tubes (EN group, n=17) or maintenance on intravenous fluid until oral diets were initiated (control group, n=19). All patients completed the study.
Results: The pretransplant and perioperative characteristics of patients did not differ between the two groups. The incidence of bacterial infection was significantly lower in the EN group (29.4%) than in the control group (63.2%) (P=0.043). Additionally, the incidence of bile duct complications in the EN group was lower than in the control group (5.9% vs. 31.6%, P=0.041). Multivariate analysis showed that early enteral feeding was closely associated with bacterial infections (odds ratio, 0.178; P=0.041). There was no statistically significant difference in nutritional status between the two groups. There were no cases of in-hospital mortality.
Conclusion: Early enteral feeding after LDLT prevents post-transplant bacterial infection, suggesting the possibility of a reduction of in-hospital mortality as a result of decreased infectious complications.

325.13

Auxiliary partial orthotopic liver transplantation versus heterotopic auxiliary partial liver transplantation – single center experience

Kohei Miura, Takashi Kobayashi, Zhengkun Zhang, Daiki Soma, Yuki Hirose, Hirosuke Ishikawa, Tomohiro Katada, Taku Ohashi, Kazuyasu Takizawa, Kabuto Takano, Jun Sakata, Toshifumi Wakai.
Division of Digestive and General Surgery, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan.

Introduction: Small-for-size is a major obstacle in adult living donor liver transplantation (LDLT). Renal dysfunction is also a direct complication which affect to prognosis after LDLT. We have successfully performed auxiliary partial living donor liver transplantation for recipients with end stage liver disease. The aim of this study was to evaluate this procedure by comparing auxiliary partial orthotopic liver transplantation (APOLT) with heterotopic auxiliary partial liver transplantation (HAPLT).
Methods: The clinical records of eight patients who underwent auxiliary partial living donor liver transplantation from March 2000 to May 2009 were analyzed. In these patients, four underwent APOLT and the other four underwent HAPLT. We compared the clinical outcomes including characteristics of recipients and donors, intraoperative findings, and postoperative findings of these two different procedures.
Results: The primary disease of the patients were fulminant hepatitis 3, alcoholic liver cirrhosis 1 in APOLT, and fulminant hepatitis 3, primary biliary cirrhosis 1 in HAPLT. Mean age and MELD score of the patients were 35 years vs 57 years, and 27 vs 27 respectively (APOLT vs HAPLT). Child-Pugh grade was C in all 8 cases. Blood type was identical 3, compatible 1 in APOLT and identical 2, compatible 2 in HAPLT. Partial liver graft was right lobe 2, left lobe 2 in APOLT, and left lobe in all cases in HAPLT. Mean graft-to-recipient weight ratio (GRWR), operation time, and blood loss were 0.79% vs 0.73%, 917 min vs 825 min, and 39764 ml vs 18943 ml respectively. In APOLT, 2 of 4 (50%) patients were alive (96, 144 months) and 1 case achieved free of immunosuppression after sufficient recovery of the native liver. One was lost at 6 years after operation because of intraabdominal infection, and the other was lost at 10 months after surgery because of sepsis. In HAPLT, only 1 of 4 (25%) patient was alive (168 months). One was lost at post-operative day (POD) 3 because of cytomegalovirus pneumonia and the other 2 were lost because of hypoxic encephalopathy at POD 28, aspiration pneumoniae at 15 months after operation.
Conclusions: In APOLT, the age of recipient tended to be younger and the amount of bleeding tended to be larger than in HAPLT. Although auxiliary partial living donor liver transplantation might be a choice as an aid for severe liver failure cases such as small-for-size or renal dysfunction, further study was required to find appropriate indication for treating them.

326.1

What keeps TTS members up at night? Preliminary results of an international ethics survey

Dominique Martin[1], Kristof Van Assche[2], Katrina Bramstedt[3], Rudolf Garcia-Gallont[4], Jacob Lavee[5], Riadh Fadhil[6], Richard Trompeter[7], Beatriz Dominguez-Gil[8].
[1]Deakin University, Geelong, Australia; [2]University of Antwerp, Antwerp, Belgium; [3]Bond University, Robina, Australia; [4]Hospital General San Juan de Dios, Guatemala City, Guatemala; [5]Tel Aviv University, Tel Aviv, Israel; [6]Hamad Medical Corporation, Doha, Qatar; [7]UCL Centre for Nephrology, London, United Kingdom; [8]Organización Nacional de Trasplantes, Madrid, Spain.

Introduction: Professionals working in the field of transplantation may confront a number of ethical issues in their daily practice. Previous studies have explored professional attitudes concerning specific issues, but not the prevalence of such issues. We report here selected findings from a cohort of Transplantation Society members who were early participants in an international survey of transplant professionals investigating their experiences of ethical issues.
Methods: An invitation to participate in an online survey (hosted by SurveyMonkey) was emailed to all members of The Transplantation Society (TTS). Available in English and Spanish, the questionnaire included items assessing members' experience of ethical concerns or dilemmas in seven categories (living donation; deceased donation; organ allocation and transplantation; organ trading and transplant tourism; healthcare funding; research and publications; professional interactions).
Results: During the first five days of data collection, 163 participants representing 40 countries were recruited who identified as a member or possible member of TTS. The majority were physicians (41%) or surgeons (35%), male (70%), and from North America (38%; 22% Europe; 16% Latin America; 9% Oceania; 7% Asia; 4% Middle East; 4% Africa). 78% of participants reported experience of ethical dilemmas in the field of living organ donation (LD); of those reporting concerns about specific categories of LD (n=123), 68% were concerned about young adults, 42% about non-residents who travelled to donate, and 40% about donors who lack insurance or have limited access to healthcare. 61% of participants reported experience of dilemmas in deceased donation; of whom 46% identified extended criteria donors and those with potentially transmissible diseases as raising ethical concerns. Participants reported experience with ethical issues concerning waiting lists, selection and organ allocation(93%); organ trading, transplant tourism or financial incentives for donation(37%); healthcare funding (44%); research or publications(33%); and interactions with colleagues (56%). 47% reported experiencing an ethical dilemma in the last year (46% had not; 7% preferred not to answer). See **Table 1** for a list of issues commonly experienced within each category. Respondents identified follow up care of living donors(39%), equity in access to transplantation in developing countries(36%), and financial incentives for living donation(35%) as the most important issues for TTS to address. Priority activities were development of guidelines for management of specific ethical issues(63%); educational and training resources in clinical ethics(60%); position statements on key ethical issues in transplantation(56%).
Conclusions: These preliminary findings suggest that TTS members commonly experience a range of ethical concerns in their professional practice, and identify potential areas of need for professional guidance in transplant ethics.

Table 1: Three most commonly selected practices raising ethical concerns for professionals in specific areas of practice

Categories of practice/areas of practice	Three most commonly selected practices raising ethical concerns	Proportion of question respondents selecting these items (n)
Living donation – 78% (127) had experienced ethical issues		
Donor recruitment	Encouraging potential related donors without undue pressure	55% (64)
	Public solicitation of donors	52% (60)
	Managing those wishing to donate for money	47% (55)
Donor evaluation	Medical evaluation	61%(72)
	Psychosocial evaluation	59%(70
	Donor-recipient relationship evaluation AND detecting coercion of potential donors by family members	(equal third) 57% (68)
Other areas of concern	Follow-up care of living donors	79% (81)
	Financial costs for living donors	50%(51)
	Financial incentives for living donors	43% (44)
Deceased donation – 62% (101) had experienced ethical issues		
Process	Referral of potential donors prior to death determination (DDt)	49% (31)
	Evaluating medical suitability prior to DDt	49% (31)
	protocols for ante-mortem interventions in donation after circulatory determination of death	44% (28)
Consent/authorization	Cultural or religious objections to deceased donation	51% (39)
	Managing family disagreement	54% (41)
	Family disagreement in the context of registered donors	47% (36)
Waitlisting, candidate selection, or allocation – 93% (151) had experienced ethical issues		
Waitlisting/ candidate selection	Medical criteria governing eligibility for waiting list	62% (61)
	Listing for combined organ transplants	34% (34)
	Non medical criteria governing waiting list eligibility AND listing of foreign patients who travel for transplantation	(equal third) 32% (32)
Allocation of deceased donor organs	Organ allocation policies	56%(43)
	Inequities in organ sharing at the regional level	39%(30)
	Allocation to patients with acute illness due to self-harm or drug or alcohol abuse	26%(20)
Aspects of allocation policies	Conflicts between equality of opportunity and maximising utility	79%(73)
	Age matching	38% (35)
	Prioritization of pediatric candidates	25% (23)
Organ trading, transplant tourism or incentives for donation – 38% (61) had experienced ethical issues		
Trading, tourism or incentives	Managing patients who return after suspected transplant tourism	75% (45)
	Managing patients considering transplant tourism	67% (40)
	Managing living donor-recipient pairs where commercial relationship is suspected	50% (30)
Healthcare funding – 44% (71) had experienced ethical issues		
Funding	Patients unable to afford immunosuppression	63%(44)
	Patients unable to afford transplantation	60%(42)
	Patients unable to afford living donation AND living donors unable to health insurance or follow up care	(Equal third) 43%(30)
Research and Publications – 33% (53) had experienced ethical issues		
Research	Obtaining funding for research	50%(22)
	Excessive regulation of research	41%(18)
	Researcher conflicts of interest	34%(15)
Publication	Including colleagues as authors who don't meet journal criteria	
	Pressure not to publish problematic findings AND determining authorship AND reviewing journal articles	(Equal second) 33% (12)
Interactions with professional colleagues - 56% (91) had experienced ethical issues		
Professional interactions	Disagreement regarding suitability of transplant candidates	67%(61)
	Disagreement regarding suitability of potential living donors	53%(48)
	Disagreement regarding suitability of potential deceased donors	52%(47)

326.2

GPS-Enabled smartphone integration into digitalDonor electronic donor record produces double-digit increase in donation by ensuring rapid response to assist donor families and physicians

Thomas Mone[1], Prasad Garimella[2], Tom Seto[3].
[1]Administration, OneLegacy, Los Angeles, CA, United States; [2]Operations, OneLegacy, Los Angeles, CA, United States; [3]Information Technology, OneLegacy, Los Angeles, CA, United States.

Organ donation authorization and organ transplantation are most successful in regions and programs with dedicated, trained, specialized, professional organ recovery staff who are available to collaborate with hospital staff and MDs and meet with families when they are the critical decisions of continued care are being made. However the very low frequency of brain dead donation opportunities prevents scheduling these staff at each potential donor hospital 24 hours per day. Thus, most donation programs dispatch staff in combinations of scheduled rounds and individual case referral response. In a well-staffed and well-scheduled recovery program this methodology ensures timely availability 80-90% of the time. However, families in crisis are often unbound by clocks and workday schedules and can appear at the hospital at any time. Further, the combination of the increase in DCD donor candidates and economic and resource contention that prompts hospitals and MDs to discontinue "terminal" care has resulted in doctor-family end of life conversations earlier than in years past.

Thus, OPOs must adapt their potential donor referral response practices to ensure early detection, collaboration with hospital staff, and availabilty to families. At OneLegacy in Los Angeles, California, whcih serves a populaiotn of 19.5 million, 210 donor hopsials, and 11 local transplant centers, this challenge has been addressed with a combination of human and technological innovations in referral repsonse that has increased donation from 418 donors per year to 460, independent of increases inautorizaiton rates.

The first of these innovations was recruiting and training dedicated Referral Response Coordinators, who routinely are Emergency Medical Technicians who are trained to be "first responders" to potential donor referrals.

The second innovation was to re-train hospitals to refer cases, not at a specific Glasgow Coma Scale number, but rather any time a patient has a sever neurological injury and is on a ventilator.

The third innovation is the creation of "geo-fences" around each donor hospital that allows OPO supervisors to monitor the presence of each OPO staffmember who is approaching a donor hospital and coordinate the activities of different OPO specialists. This coordination is made possible through the OneLegacy Electronic Donor Record and its "digitalDonor Response" module that identifies individual staff on-screen and visbile to our donation team as soon as they arrive at the donor hospital, lets the staff know who they will be working with, and identifies other potential donor referrals in the hospital. The system makes the entire donor record immediately available to all OneLeacy staff, faciltiating a common understanding of the state of the case. This allows the Supervisor and Administrator on-call and the Intensivist on-call to collaborate with the coordinators, ensure they are available to treating MDs, hospital staff, and most of all families; and enables the supervisors to re-deploy staff to meet wiht hospital, MD and family whenever they are ready to address the donation opportunity. SInce digitalDOnor Response was deployed in late 2014, OneLegacy has seen a 10%/ 40+ donors per year.

This combination of technological and human resource investment has enabled OneLegacy to facilitate more than 100 additional transplant sin teh past year and is scalable to any organ recovery program.

326.3

Living liver donors may under-report mental health comorbidities

Hillary Braun[1], Garrett R.Roll[2], John PRoberts[2], Nancy L.Ascher[2].
[1]School of Medicine, University of California, San Francisco, San Francisco, United States; [2]Department of Surgery, University of California, San Francisco, San Francisco, United States.

Introduction: Psychiatric implications are the focus of many concerns about living organ donation. At UCSF, potential donors first complete a screening questionnaire that addresses medical history, current medications, and a review of systems. Once donors pass the initial screen, they are interviewed by an independent living donor advocate (ILDA) and/or social worker (SW). Donors deemed to be at increased psychological risk are subsequently evaluated by the consulting psychiatrist. The purpose of this study was to examine the prevalence of psychiatric comorbidities in our living liver donors and to determine the relationship between self-reported conditions and those identified during interviews with medical professionals.

Materials and Methods: We conducted a retrospective chart review of living liver donors at UCSF from January 2012-February 2016. Basic demographic data was obtained. Screening questionnaires were reviewed to determine whether the donor disclosed current or prior psychiatric conditions and/or psychiatric medications. Documented medical and social histories along with SW, ILDA, and psychiatry notes were reviewed for past psychiatric history, past psychiatric medications, current psychiatric diagnosis, current psychiatric medications, and current or prior therapeutic relationship with a psychiatrist or psychologist. Data are presented as median (interquartile range (IQR)) and differences were evaluated using Wilcoxon rank sum tests.

Results: 72 patients underwent living donor hepatectomy at our institution between 2012-2016. Donors were 53% female (38/72) with a median age of 32.7 (16-49.4) years. On self-reported questionnaire, 14% (10/72) of donors disclosed a current/prior psychiatric condition and 11.1% (8/72) reported psychiatric medication use. According to chart documentation, 33% (24/72) of donors had a current or prior psychiatric condition, 24% (17/72) had previously required psychiatric medication, and 39% (28/72) had seen a psychiatrist or psychologist at some point prior to donation (Figure 1).

At the time of donation, 24% (17/72) of donors had an Axis I psychiatric diagnosis, 8% (6/72) were on psychiatric medication, and 9.7% were in a therapeutic relationship. Of those with an Axis I diagnosis, 35% (6/17) reported a psychiatric condition on screening questionnaire. One hundred percent (6/6) of donors on psychiatric medications at the time of donation reported the medication previously. There was no significant association between psychiatric diagnosis and gender (p=0.867), relationship to recipient (related vs. unrelated, p=0.271), or marital status (p=0.402).

	Self-Reported	Reported During Interviews	Present at Donation
Psychiatric Condition	14%	33%	24%
Psychiatric Medication	11.1%	24%	8%
Current/Prior Therapeutic Relationship	N/A	39%	9.7%

Discussion and Conclusion: Potential living donors appear to under-report psychiatric conditions at the time of screening. This may be due to concern over candidacy for donation. These findings underscore the critical importance of thorough mental health screening carried out via in-person interviews prior to donation. It will be vitally important to examine the relationships among pre-donation psychiatric condition, post-operative course, and any complications.

326.4

Kidney transplant patient preferences and trade-offs for outcomes after transplantation.

Martin Howell[1,2], Germaine Wong[1,2,3], John Rose[4], Allison Tong[1,2], Jonathan C.Craig[1,2], Kirsten Howard[2].
[1]Centre for Kidney Research, The Children's Hospital at Westmead, Sydney, Australia; [2]School of Public Health, University of Sydney, Sydney, Australia; [3]Centre for Transplant and Renal Research, Westmead Hospital, Sydney, Australia; [4]Institute for Choice, University of South Australia, Sydney, Australia.

Background: Patient preferences in clinical decisions are increasingly relevant, yet transplant patient perspectives through structured, quantified preference elicitation methods are unknown. We aimed to evaluate preferences and trade-offs patients may accept to avoid adverse outcomes of long-term immunosuppression.
Method: Preferences and trade-offs between graft duration and the risk of dying, cancer, cardiovascular disease (CVD), diabetes, infection, anxiety/depression, diarrhoea/nausea, and weight gain, was assessed using a best-worst scaling survey. Participants, recruited from three clinics, were shown 10 scenarios with varying outcome probabilities and years of graft duration and asked to choose best, next-best, worst and next-worst outcomes. Responses were analysed with multinominal-logit models.
Results: Ninety-three patients (age 18-69 years, transplant duration 0.9-31 years) completed the survey. The least desirable outcome was graft loss after one year with a preference value of 0.0 (95% CI: -0.05,0.05) and worse than any other outcome including 100% risk of dying with a preference value of 0.19 (95%CI:0.13,0.24). The most desirable outcome was a 0% risk of dying before graft loss with a preference value 1.0 (95% CI:0.92,1.08) which was more important than all other outcomes including 25 years of graft survival with a preference value 0.8 (95% CI:0.64,0.78). A low risk of CVD, cancer and serious depression/anxiety is of equal or greater importance to 15 years of graft duration. A high risk of serious adverse outcomes is equivalent to 5 to 15 years graft survival. Preferences varied with age, gender, comorbidities, years on dialysis years since transplant duration, and number of transplants. To achieve zero risk of cancer, dying, and anxiety/depression participants were willing to trade 3.1 (95%CI:2.10,4.69), 1.66 (95%CI:1.12,2.51), 1.21 (95%CI:0.82,1.83) years of graft survival, respectively, while to achieve a 10% risk, patients were willing to trade less than 1 year of graft survival for all adverse outcomes.
Conclusion: Elicitation of preferences and trade-offs for outcomes after transplantation has demonstrated a focus on long-term adverse outcomes that tend to be poorly addressed in clinical trials. This limits the ability for clinicians to effectively engage with patients in developing individualized care. Graft survival of 15 years represents a 'tipping' point in terms of the relative importance of adverse outcomes compared to duration of graft survival. Beyond 15 years, a low risk of CVD, cancer and serious depression/anxiety is of equal or greater importance than graft survival, while high risks of serious adverse outcomes including dying, are equivalent to (i.e. as bad as) graft survival of 5 to 15 years. The willingness to trade years of graft survival to avoid serious adverse outcomes is contingent on achieving a low, essentially zero risk of the adverse outcome.

326.5

"You killed him so that you can take the organs!" – Exploring novel factors influencing low cadaver organ donor rates in South Africa: Beyond religious beliefs and cultural practices

Harriet Etheredge[1], Claire Penn[2], Jennifer Watermeyer[2].
[1]Wits Donald Gordon Medical Centre, Johannesburg, South Africa; [2]School of Human and Community Development, University of the Witwatersrand, Johannesburg, South Africa.

South Africa (SA) is a low-to-middle-income country of great complexity and diversity. It is multi-lingual[1] and multi-cultural, characterised by significant socio-economic disparities[2] and SA people hail form a plethora of backgrounds. All these factors bring themselves to bear in the healthcare setting, and organ transplantation is no exception. A small number of transplants are performed across SA annually, but the country is faced with very low donor numbers relative to other parts of the world. Often, low donor numbers in SA are generalised to religious beliefs or cultural practices which are said to be fundamentally in opposition to the notion of organ donation[3]-[8]. This research challenged these assumptions, by exploring other potential explicators for low donor numbers, and associated ethical issues, in the unique SA context.
The study took place across 6 institutions using qualitative methods. Thirty in-depth interviews with transplant professionals, two focus groups with coordinators, two interviews with cadaver donor families, and one focus group with living kidney donors were conducted. Thematic analysis was undertaken.
Hospital staff and members of the public were found to sometimes be suspicious of biomedicine, and a perception that donors may be killed for their organs was held amongst both these groups. At the bedside, this translated into healthcare staff possibly influencing the family of a potential donor against organ donation before the transplant coordinator arrived. This could lead to bias and negatively affected meaningful informed consent. Suspicions about biomedicine and transplant were additionally complicated by sensational reporting of transplant happenings in the SA media, which further diminished trust in the institution. Socio-economic challenges in accessing healthcare were highly relevant, and sometimes resulted in a failure to identify potential donors. Moreover, even when they were in the system, it was found that physicians may be unwilling to refer brain-dead patients as potential donors, as this was seen as an admission of failure to fulfil one's professional duty to 'cure' the patient.
Religious beliefs and cultural practices may not influence organ donation decisions as much as published literature suggests, and a combination of multi-layered factors must be considered. To appreciate the reasons for low donor rates, the interplay of suspicions about biomedicine, sensational media-reports, socio-economic issues and clinical factors all require consideration. The complexity of these issues results in a situation where patient-centered care and informed consent may be compromised. The situation is further compounded by an overwhelming lack of donor referral policy or regulation. Recommendations include the need for a paradigm shift when discussing low donor numbers; nationwide, uniform donor policy and the necessity of greater governmental support for organ donation and transplant education.

References:

[1] • Mesthrie, R. (2002). South Africa: A sociolinguistic overview. In Mesthrie, R. (Ed.) Language in South Africa (11–27). Cambridge, United Kingdom: Cambridge University Press.
[2] • Baldwin-Ragavan, l., De Gruchy, J. & London, L. (1999). *An ambulance of the wrong colour*. Rondebosch, South Africa: University of Cape Town Press.
[3] • Pike, R. E., Odell, J. A. & Kahn, D. (1993). Public attitudes to organ donation in South Africa. *South African Medical Journal*, 83(2), 91–94.

[4] • Bhengu, B. R. & Uys, H. H. (2004). Organ donation and transplantation within the Zulu culture. *Curationis*, 27(3) 24–33.
[5] • Van den Berg, L. (2005). *Organ and Tissue Donation and Transplantation: A perspective of South African Baptists from the Baptist Northern Association and its implications for preaching*. Retrieved from UPSpace (Electronic thesis and dissertation database of the University of Pretoria) http://hdl.handle.net/2263/28354
[6] • Buthelezi, N. & Ross, E. (2011). Gift of life or cultural taboo: Effects of an educational pamphlet on young adult's knowledge and attitudes regarding organ donation. *Social Work and Healthcare*, 50, 719–738.
[7] • Etheredge, H. R., Turner, R. E. & Kahn, D. (2014). Attitudes towards organ donation amongst a sample of urban South Africans remain unchanged: A cross-sectional study (1993–2013). In *South African Medical Journal*, 104(2), 133-137. doi:10.7196/SAMJ.7519.
[8] • Etheredge, H. R., Turner, R. E. & Kahn, D. (2013). Public attitudes to organ donation among a sample of urban-dwelling South African adults: a 2012 study. *Clinical Transplantation*, 27 (5), 684–692.

326.6

Mobile apps to improve organ donation rate

Sunil Shroff, Sujatha Suriyamoorthi, Koti Reddy, Pallavi Kumar, Sumana Navin, Hemal Kanvinde.
Trustee, MOHAN Foundation, Chennai, India.

Introduction: Mobile apps have made rapid advances in healthcare and their popularity has been is due to the ease of their use and the dynamic portability on various mobile devices.
MOHAN Foundation, a NGO from India identified key areas where these app could make a difference and help in contributing to the overall increase of deceased donation rate in the country.
Material and Methods: *MOHAN Foundation have developed three apps in the field and include:*
a. e-donor card - for consumers,
b. Donor Optimization app- for ICU staff
c. Organ Registry / Allocation app - for administrators & transplant coordinators
These app were developed for both Android and iphone platform and are available free of cost on their store & on www.mohanfoundation.org website. The brain death app was developed in association with National Health Services & Blood Transfusion, UK .
The app have the following functionality:
The e-donor card app allows users to express their desire to be an organ donor, informs next of kin of their wishes, provides a list of 'FAQ's' related to organ donation, provides the latest news related to organ donation and can be used to invite friends to sign up for pledging organs
The donor optimization app allows an ICU staff to manage brain death and use it as a ready reckoner to manage various clinical scenarios that develop in its management.
The recipient registry /organ allocation app allows easy sharing of info with multiple transplant teams and keeps them updated of the progress of brain death.
Results: The number of downloaded of the app have been increasing from these stores. There have been over 1000 downloads of the e donor card app. The donor optimization app has undergone over 500 downloads. The recipient registry /organ allocation app is currently only for private network.
The app have been easy to use and has had positive feedback and a five star rating on the stores. Intensive care doctors have used the donor optimization app to manage brain deaths iin the odd hours of night. The allocation staff have found the app useful and has saved them from sending multiple sms's. The e-donor card is a popular app with youths and they have used it to popularize organ donation.

Discussion: Technology can be used effectively in the complex feild of transplantaion. The current mobile apps have been developed in India

to address and overcome some of the challenges that were encountered in the programme. India has almost 300 million smart phone users and the e-donor card is looking into tapping this vast pool to popularize the concept of organ donation. Donor optimization was required as many ICU staff who had never managed a brain death. Organ registry as mobile app simplifies access to computers in odd hours for organ allocation.

Conclusion: The three apps were developed to ease some of the key areas in this field and to facilitate the organ donation process with the objective to contribute towards increasing the organ donation rate.

Apps were developed for MOHAN Foundation by www.medindia.net - a leading health website from India

326.7

The "Doha model of organ donation" from adoption of the principles and recommendations of the "Declaration of Istanbul " in 2010 to inclusion of the implemented strategies in the new national transplant legislation - 2015

Riadh Fadhil.
Director of Qatar Organ Donation Center , Hamad Medical Corporation , Doha, Qatar.

Introduction: Qatar is a small Gulf country of 2 million people, mostly expatriate workers from South East Asia and the Middle East. Historically, Lack of awareness of organ donation and poor rate of donation in Qatar, compelled many wealthy patients to seek organs in foreign markets, returning to Qatar with high rates of morbidity and mortality. In response to these challenges, Qatar's major provider of health services, Hamad Medical Corporation (HMC) collaborated in 2009 with The Transplantation Society and the Declaration of Istanbul Custodian Group in development of the Doha Donation Accord (DDA). The DDA is an ethical framework that implements the recommendations of the Declaration of Istanbul designed to increase organ donation and prevent transplant commercialism. The DDA together with its strategies is now known as the "Doha Model of Organ Donation".

Materials and Methods: Implementation of the ethical principles and the evolving strategies of the Doha Model like the public education campaign, establishment of the deceased donation program, creation of the Donor Registry, formation of the ethical committee for living donor's evaluation, have increased domestic transplant activity from living related and deceased donors, and reduced travel from Qatar for commercial transplantation.

Results and Discussion: In August 2015, a new national transplant law (No.15) was introduced to ensure maintained implementation of the core strategies of the Doha model, the previous Qatar Organ Transplant Law 21 of 1997 insufficiently addressed the legal and ethical challenges of donation and transplantation. The new complementary sections of law 15 regulate the full implementation of the DDA e.g. recruitment for the donor registry; authority of the ethical committee for psychosocial evaluation of living donation, equitable access to deceased donor organs and transplant services, prohibition of financial incentives to donors and donor's family, removal of disincentives to donation, defining the authority of the Minister of Health in approving the DDA's provisions to the donors and donor families, fight commercialism, and prevention of commercial transplantation arising from Qatar. In addition to reconfirming sections imported from law 21 that regulate altruistic living donation, deceased donation and combat commercialism.

Conclusion: the Doha Model succeeded in adopting the principles and recommendations of the Declaration of Istanbul, customized it to address local challenges successfully and finally secured sustained future implementation through inclusion of the strategies in the local transplant legislation. This couldn't have been achieved without the persistence and commitment of all administrative and professional stakeholders.

Thanks to the support, partnership and guidance of the Declaration of Istanbul Custodian Group, the Doha Model has become a good example to all countries willing to develop successful ethical organ donation programs.

326.8

A qualitative study into the modus operandi, investigation and prosecution of organ trafficking networks in South Africa, Israel and Kosovo

Frederike Ambagtsheer[1], Jessica De Jong[2,3], Martin Gunnarson[4], Susanne Lundin[4,5], Ingela Byström[4], Willem Weimar[1].
[1]Department of Internal Medicine, Section Transplantation and Nephrology, Erasmus MC, Rotterdam, Netherlands; [2]Analysis and Research Dpt, Central Division of the National Police of the Netherlands, Driebergen, Netherlands; [3]Willem Pompe Institute for Criminal Law and Criminology, Utrecht University, Utrecht, Netherlands; [4]Department of Arts and Cultural Sciences, Lund University, Lund, Sweden; [5]Stellenbosch Institute of Advanced Study (STIAS), Wallenberg Research Centre , Stellenbosch University, Stellenbosch, South Africa.

Background: In 2010 a South African hospital plead guilty for allowing its doctors and other employees to conduct over 100 illegal kidney transplants. In 2013 in Kosovo doctors were convicted for participating in 24 illegal transplants. Both cases have a strong connection with Israel. In 2012 and 2013 we travelled to South Africa, Kosovo and Israel to study and compare a) the modus operandi of the criminal networks, and b) the successes and hurdles of authorities in the investigation and prosecution of the networks. This study formed part of the EU-funded HOTT Project on trafficking in human beings for the purpose of organ removal that aims to improve non-legislative responses to the crime.
Methods: We conducted fieldwork in each country. We performed 37 interviews with 49 persons. These were: police, prosecutors, legal experts, policy-makers, ministry representatives, health inspectors, patients, nephrologists, transplant surgeons, health insurance company representatives, defense attorneys, representatives of international organizations and national transplant coordinators. Interview transcripts were analyzed qualitatively. In addition, we studied case materials, including charge sheets, witness statements, indictments, closing statements and legislation.
Results: Using sophisticated modes of operation, both trafficking networks successfully conducted illegal transplants in an organized manner. Most of the recipients were of Israeli descent and were charged up to US$120,000 for a transplant. 'Donors' were recruited from Romania, the Russian Federation, Brazil, Israel, Kazakhstan and other Eastern European countries. They were (sometimes falsely) promised amounts between US$3,000 and $26,000. Both networks operated for a long time with relative impunity because of legal loopholes and corruption, and were discovered and prosecuted very differently. The local disruption of the criminal networks can be defined as the most important success, while the biggest hurdle in both cases comes down to international collaboration.
Conclusion: The prosecutions in these cases were successful but leave room for improvement. First, prosecutions could have been more successful if the appropriate laws would have been in place at the time when the activities took place. Second, investigations and prosecutions could have been initiated earlier if available signals were identified and picked up already at an early stage and if international collaboration would have occurred sooner. Based on this study, we developed indicators and recommendations that will benefit countries worldwide in recognizing, investigating and prosecuting organ trafficking.

326.9

Reporting organ trafficking networks: A survey-based plea to breach the secrecy oath

Frederike Ambagtsheer[1], Linde J.Van Balen[1], Wilma L.L.J.M.Duijst-Heesters[2], Emma K.Massey[1], Willem Weimar[1].
[1]Department of Internal Medicine, Erasmus MC, Rotterdam, Netherlands; [2]Department of Public Health, Radboud University , Nijmegen, Netherlands.

Introduction: The purchase of kidneys is prohibited in almost all countries. Nevertheless, patients buy kidneys and commonly travel overseas in order to do so. Transplant professionals (TPs) who treat these patients pre- and post-operatively can play a role in identifying and reporting kidney purchase. However, TPs report a tension between their obligations to provide medical care and maintain secrecy on the one hand and their duty to prevent harm on the other.
Methods: We present the results of a national survey that describes transplant professionals' experiences, attitudes, behaviors, conflicts of duties, legal knowledge and needs for guidelines towards patients who purchase kidneys abroad.
Results: Of the 546 TPs who were invited, 241 (44%) completed the survey. Between 2008 and 2013, 100 (42%) professionals treated patients that traveled to a country outside the European Union for a kidney transplant. Thirty-one of the 100 TPs (31%) were certain that patients purchased kidneys. Sixty-five (65%) had suspicions that patients had bought kidneys Whereas most professionals (85%) understood why patients bought kidneys, they also felt that they have a duty to prevent organ purchase (72%). Participants experienced a conflict of duties (65%) when suspicions of purchase occurred. Eighty percent reported a need for guidelines in dealing with patients who will or have purchased a kidney.
Conclusion: TPs can contribute to eradicating the harm and suffering inflicted on patients and donors by anonymously disclosing information about organ trafficking networks to law enforcement. Reporting information such as the names of hospitals, clinics, cities, hospital staff and other individuals who are involved in potentially illegal transplant activities, is a more effective way to disrupt and deter organ trafficking than reporting patients. We propose that guidelines are established for TPs to disclose information that supports the police and judiciary in investigating, disrupting and prosecuting the facilitators of illegal transplantations. We offer key components for guidelines on disclosure of these networks.

326.10

A systematic review on the prevalence of patients who purchase organ transplants abroad

Frederike Ambagtsheer[1], Jessica De Jong[2,3], Wichor Bramer[4], Willem Weimar [1].
[1]Department of Internal Medicine, Transplantation and Nephrology, Erasmus MC, Rotterdam, Netherlands; [2]Analysis and Research Dpt, Central Division of the National Police, Driebergen, Netherlands; [3]Willem Pompe Institute for Criminal Law and Criminology, Utrecht University, Utrecht, Netherlands; [4]Medical Library, Erasmus MC, Rotterdam, Netherlands.

Background: Over the years, the trade in human organs has become an object of international concern. Whilst first regarded as an organ theft rumour, organizations including such as the World Health Organization (WHO) the Council of Europe now present it as a lucrative and serious form of organized crime that exploits vulnerable donors worldwide. The WHO estimated in 2007 that 5-10% of the approximately 60,000 kidney transplants performed annually around the globe occur via organ trade. We present a systematic literature review about the number of patients that purchased organs. With this information we discuss whether a scientific basis exists for the claim that organ purchase is a frequent occurrence.
Methods: We searched Embase, Medline, Web of Science, Scopus, PubMed, Cochrane and Google Scholar. The methodology and results of this systematic literature review are in line with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines. All English studies published in full text after 1 January 2000 were included. Presentations, abstracts, opinion papers, non-English records and publications lacking methodology were excluded.
Results: Between 2000 and 2015, 86 studies were published. Seventy-six of these presented patients who traveled and 42 stated that the transplants were commercial. Only 11 studies reported that patients paid and 8 described to what or whom patients paid. In total, over a period of 42 years, 6002 patients have been reported to travel for transplantation. Of these, only 1238 were reported to have paid for their transplants. Patients paid between $10,000 and $200,000 for kidney transplants. Liver transplants were more expensive (range $40,000-$300,000). Eight articles describe to what or whom patients paid. These papers report 158 patients in total. Of these, 129 paid their donors, 22 paid brokers, five paid to hospitals, two paid to private companies and an unknown number paid doctors for a transplant. An additional unknown number of patients paid for their transplants in their native countries.
Conclusion: We conclude that the literature is speculative and anecdotal about patients buying organs: their purchases are more often assumed than determined. This hampers the drawing of firm conclusions about the scale of patients' involvement in the trade. The empirical data published in the literature does not provide a basis for the claim that large numbers of patients purchase organs worldwide on a frequent basis. More rigorous quantitative and qualitative research is needed to enable a more reliable picture of the trade's scale. A reporting code for transplant professionals to report organ trafficking networks is a potential strategy to collect and quantify cases.

326.11

A kidney paired donation standard acquisition charge pilot program

Michael A. Rees[1,2], Laurie J.Reece[2], Susan E.Rees[2], Betty Crandall[3], Alan B.Leichtman[4].
[1]Urology, University of Toledo Medical Center, Toledo, United States; [2]Alliance for Paired Donation, Perrysburg, United States; [3]Transplant Surgery, Wake Forest Baptist Health, Winston Salem, United States; [4]Arbor Research Collaborative for Health, Ann Arbor, United States.

Background and Purpose: A United States non-profit Kidney Paired Donation (KPD) organization undertook a demonstration project to redesign payment for KPD transplants with a payment model similar to the current approach for deceased donor cost recovery—utilizing a standard acquisition charge (SAC). A 2012 Consensus Conference on KPD concluded a national SAC would remove many of the financial barriers and create cost certainty for transplant (Txp) centers. Aim One of the grant: Collect actual financial data from a clinically active KPD program to allow calculating the real-life cost per KPD transplant.
Methods: Over a number of months, stakeholders agreed on what costs should be reimbursed. Notably, the group decided on the appropriate tests included in the donor evaluations. Further, they compiled de-identified data on the current costs for the nephrectomy facility charges; the surgeon and anesthesiologist professional fees; and costs to pack and ship a living donor kidney. The de-identified data included the Medicare reimbursement rate for their geographic area plus average commercial insurance reimbursement calculated from 3 commercial payers of their choosing. The study started slowly, as it was necessary not only to achieve consensus, but for the Centers for Medicare and Medicaid Services (CMS) to give the awardee permission to move forward, as the project had direct implications for a Txp center's Medicare cost report. Approval was given in June 2014, and Txp centers formalized with their participation via a written agreement.
Results: The initial study was designed to provide reimbursement to Txp centers only for donor evaluations related to KPD; but it became obvious that in order to attract more centers to participate (thus broadening access for patients desiring KPD), paying centers for KPD donor nephrectomy was highly desired. The project evolved to one that paid participating centers for the cost of: donor evaluation, nephrectomy, surgeon & anesthesiologist's fees, and packing and shipping a kidney donated for KPD. In return, the Txp center receiving the donated kidney agreed to pay the organization a KPD SAC following transplantation of such a kidney. To date, 49 transplants have been performed using the KPD SAC mechanism since initiation in 2015.
Conclusion and Implications: This Agency for Healthcare Research and Quality (AHRQ)-funded project overcomes barriers to creating additional living donor kidney transplants in the United States, leading to improved quality of care, increased access to care, and reduced cost. A similar strategy could be used to facilitate international kidney exchanges involving shipped kidneys.
Grant funding provided by the Agency for Healthcare Research and Quality in the United States, grant number R18 HS-020610

326.12

Research priority setting in solid organ transplantation: a systematic review

Claudia Harper[1,2], Jonathan C.Craig[1,2], Jeremy R.Chapman[3], Allison Tong[1,2].
[1]Sydney School of Public Health, The University of Sydney, Sydney, Australia; [2]Centre for Kidney Research, The Children's Hospital Westmead, Sydney, Australia; [3]Centre for Transplant and Renal Research, Westmead Hospital, Sydney, Australia.

Introduction: The lack of transparency and exclusion of patients in determining research priorities can lead to wasteful allocation of limited resources into areas that may not be important to stakeholders, particularly patients. The purpose of this study is to evaluate approaches to research prioritization for solid organ transplantation and to identify the research priorities of patients in need of an organ transplant, their caregivers, the healthcare providers involved in their care, and policy makers.
Materials and Methods: Electronic databases were searched from inception to December 2015. Studies that elicited patient, caregiver, healthcare provider, or policy maker priorities for research in solid organ transplantation were included.
Results: We identified 26 studies (n=1582 participants) conducted in the United States, the Netherlands, Australia, Canada and internationally. The studies focused on kidney (8 studies), heart (7 studies), liver (3 studies), lung (1 study), and non-specified solid organ transplantation (7 studies). Various priority setting methods were used including the Delphi technique, expert panels, consensus conference, ranking or voting surveys, focus groups and interviews, of which the process was described in detail by 16 (62%) studies. Only eight (31%) studies reported patient involvement. The priority areas for research were: organ allocation (19 studies); immunosuppression (15 studies); transplant complications (15 studies); donor and recipient quality of life (12 studies); organ donation (12 studies); and improving patient-provider communication (5 studies).
Discussion: The priorities identified by organ transplant research priority setting exercises are broad ranging. However, patients explicitly set few of the priorities. Further, the methods used to establish the research priorities in most of the studies lacked clarity.
Conclusion: To ensure that research is done in areas that are relevant to the users of the research, research priorities should instead be set using a prespecified and transparent process that engages patients, caregivers, and healthcare providers, not solely transplant experts.

326.13

Health-related quality of life improves further after liver transplantation and is influenced by length of stay

Louis Onghena[1], Wouter Develtere[1], Xavier Rogiers[2], Roberto Troisi[2], Frederik Berrevoet[2], Aude Vanlander[2], Anja Geerts[1], Hans Van Vlierberghe[1], Xavier Verhelst[1], Carine Poppe[3].
[1]Gastroenterology and Hepatology, University Hospital Ghent, Ghent, Belgium; [2]Hepatobiliary Surgery and Transplantation Medecine, University Hospital Ghent, Ghent, Belgium; [3]Transplantation Center, University Hospital Ghent, Ghent, Belgium.

Background and Aims: Liver transplantation (LT) is the only curative treatment for end-stage liver disease (ESLD) with excellent long-term outcomes. However, an important outcome parameter is health-related quality of life (QoL). Improvement of QoL has been described after liver transplantation. We wanted to investigate the evolution in QoL in a period of two years after LT and identify factors influencing this evolution.
Methods: We performed a cross-sectional study in a liver transplant unit. Self-report questionnaires (SF36, ICQ and NEO-FFI) were conducted in 177 patients with ESLD: 60 patients pre-transplantation, 60 post-transplantation and control group of 57 patients without perspective of transplantation. Data were analyzed using the Mann-Whitney U test, Spearman's rank correlation coefficient and correlation matrices.
Results: The post-LT patient group consists of 63,0% male individuals and 20,0% had no complications. Mental QoL shows a 19,0% elevation in two years post-LT whereas physical QoL knows a 54,8% elevation in two years post-LT, of which 46,5% the first six months. We observed a significant increase in physical QoL >3 months compared to £3 months after LT (p=0,027), as well as a significant increase in disease benefits (p=0,018).
In a period of two years post-LT a significant correlation (p<0,001) was found in QoL (physical and mental) compared with length of stay in intensive care (rs=-0,359 and rs=-0,324), length of stay in total (rs=-0,327 and rs=-0,347), helplessness (rs=-,0771 and rs=-0,797), acceptance (rs=0,679 and rs=0,710) and disease benefits (rs=0,514 and rs=0,436). Length of stay on intensive care >6 days post-LT shows a significantly worse physical QoL up to two years post-LT (p=0,023).
Conclusions: These data confirm an increase of QoL during two years post-LT. A better acceptance of illness, a reduction in helplessness and more benefits of the illness after transplantation result in a better QoL. We assume that these findings indicate that patients' illness cognitions are of great influence on QoL. A longer stay in intensive care implies longer immobilization, which negatively influences physical QoL. Patients who spent more than six days on intensive care post-LT deserve a more intense physical rehabilitation. These aspects could give new directions in the approach of liver patients after transplantation.

327.1

CD4+CD25+FoxP3+ Treg cells are critical to tolerance induction by SA-FasL-engineered porcine islets transplanted into mice

Esma S.Yolcu[1], Kyle Woodward[1], Hong Zhao[1], Feng Wang[1], Melanie Graham[2], Bernhard Hering[2], Haval Shirwan[1].
[1]Institute for Cellular Therapeutics and Department of Microbiology and Immunology, University of Louisville, Louisville, KY, United States; [2]Schulze Diabetes Institute and Department of Surgery, University of Minnesota, Minneapolis, MN, United States.

Introduction: We have shown that porcine islets engineered to transiently display SA-FasL protein on their surface establish tolerance in mice following transplantation under the kidney capsule or intraportally. This study was designed to elucidate the mechanistic basis of the observed tolerance.
Methods: Islet grafts, graft-draining LNs, and spleen were collected from C57BL/6.FoxP3DTR/GFP recipients of SA-FasL-engineered porcine islets in a time course study (n=6/time point) on days 3, 6, 12 post-transplantation and at experimental end-points (>200 days). Lymphocytes were stained with antibodies to various cell surface markers to identify T effector, T effector memory, T central memory, induced and natural Treg cells, and various innate immune cells using flow cytometry. Lymphocytes were also analyzed for intracellular cytokines following ionomycin and PMA stimulation. Treg cells were depleted early or late post-transplantation using diphtheria toxin. Long-term mice were also challenged with BALB/c allogeneic skin to test the immune competency and islet-specific tolerance.
Results and Discussion: Long-term (>200 days) recipients of porcine islets rejected BALB/ skin grafts in an acute fashion (MST=11.3±1.0) without any detrimental effect on the survival of porcine islets, demonstrating immune competency and antigen-specificity of tolerance. Extensive lymphocyte phenotyping and intracellular cytokines established the increased ratio of Treg/T effectors and induced/natural Treg cells within graft draining LNs of long-term survivors as the correlate of tolerance for both intraportal and subrenal islet transplantation sites. Depletion of Treg cells early (15 days) or late (60 days) post-transplantation resulted in prompt graft rejection in both settings.
Conclusion: Immunomodulation with SA-FasL-engineered porcine islet grafts serves as an effective means of tolerance induction in a xenograft model. Tolerance appears to be localized to the graft and Treg cells are prerequisite for tolerance induction.

327.2

Dual phases and mechanistic basis of tolerance induced by allogeneic pancreatic islets engineered with SA-FasL protein

Esma S.Yolcu, Kyle Woodward, Hong Zhao, Haval Shirwan.
Institute for Cellular Therapeutics and Department of Microbiology and Immunology, University of Louisville, Louisville, KY, United States.

Introduction: We have recently shown that pancreatic islets engineered to display on their surface a chimeric form of FasL with streptavidin (SA-FasL) induce robust allotolerance under the transient cover of rapamycin. Tolerance at the maintenance phase was localized to the graft. We herein tested if tolerance is systemic at induction phase and elucidated underlying mechanisms.
Methods: C57BL/6 mice were transplanted with BALB/c SA-FasL-islets under the transient cover of rapamycin administered on the day of Tx daily for 15 days. BALB/c SA-islets served as control. Systemic tolerance was tested by simultaneously transplanting unmodified donor islets with SA-FasL-engineered islets separately under contralateral kidney capsules. Unmodified islets from C3H mice and skin and heart grafts from BALB/c mice were used to test antigen and tissue specificity of tolerance. To test the role of Treg cells in the systemic and localized tolerance, these cells were depleted early or late post-transplantation.
Results and Discussion: Tolerance was systemic and antigen-specific as recipients with BALB/c SA-FasL-engineered islets rejected unmodified islets from C3H, but not those from BALB/c. Tolerance was also tissue specific as the recipients of SA-FasL-engineered BALB/c islets rejected both skin and heart from the same donor. Importantly, rejection of BALB/c skin and heart grafts precipitated rejection of SA-FasL-engineered islets. Tolerance involved CD4+CD25+FoxP3+ Treg/phagocytes/TGF-b axes as depletion of phagocytes or Treg cells or blockade of TGF-b at the induction phase resulted in acute islet graft rejection. Most importantly, tolerance at the maintenance phase was localized to the graft and required the presence of graft-localized Treg cells.
Conclusion: Immunomodulation with SA-FasL-engineered allogeneic islets results in donor and tissue-specific systemic tolerance that becomes localized to the graft at the maintenance phase. The presence of graft was the key driving factor for retention of Treg cells within graft microenvironment and maintenance of tolerance.

327.3

The influence of dissociation enzymes on isolated islets during pancreatic islet isolation procedures

Yuki Miyazaki[1], Takehiro Imura[2], Youhei Yamagata[3], Hiroshi Maeda[3,4], Kazutaka Murayama[5], Kimiko Watanabe[4], Akiko Inagaki[2], Yasuhiro Igarashi[2], Noriaki Ohuchi[1], Susumu Satomi[1], Masafumi Goto[1,2].
[1]Division of Advanced Surgical Science and Technology, Tohoku University School of Medicine, Sendai, Japan; [2]Division of Transplantation and Regenerative Medicine, Tohoku University School of Medicine, Sendai, Japan; [3]Department of Applied Biological Chemistry , Graduate School of Agriculture, Tokyo University of Agriculture and Technology, Tokyo, Japan; [4]New Industry Creation Hatchery Center, Tohoku University, Sendai, Japan; [5]Division of Biomedical Measurements and Diagnostics, Graduate School of Medical Engineering, Tohoku University, Sendai, Japan.

Introduction: It has been reported that tissue dissociation enzymes, especially neutral proteases, impair the viability of isolated islets during pancreatic islet isolation procedures. Here, in the present study, we examined whether the dissociation enzymes *per se* cause damages on isolated islets using highly purified collagenases and neutral protease.
Materials and Methods: Islets isolated from male Lewis rats were co-cultured with Liberase MTF and thermolysin (enzyme group), or digested solutions of pancreatic tissues (solution group) at 37℃ for 120 minutes. The digested solutions were prepared after centrifuging and filtrating the dissociated pancreatic tissues at 30 minutes in digestion procedures. In the control group, islets were co-cultured with HBSS (Hanks' Balanced Salt Solution), and HEPES was added in all groups. After 2 hour co-culture, appearance of islets, ADP/ATP ratio, ATP/DNA, static glucose stimulation test and FDA/PI were evaluated. Furthermore, according to above results, we investigated whether trypsin or chymotrypsin deteriorated islet viability by means of co-culturing islets with five times higher concentration of trypsin or chymotrypsin than that in normal digested solutions of pancreatic tissues.
Results and Discussion: Islet appearance was remarkably aberrant in only the solution group. In the solution group, the ADP/ATP ratio was significantly higher than that of the HBSS group and enzyme group (0.176±0.040 VS 0.002±0.002, 0.012±0.012; p=0.01), and the ATP/DNA was significantly lower than that of the other groups (36.8±2.9 vs 56.1±3.0, 51.4±4.8; p=0.01). Stimulation index of static glucose stimulation test was considerably lower in the solution group than that of the other groups (4.46±0.59 vs 29.42±18.78, 15.66±6.56). These data suggest that isolated islets were damaged by digested solutions of pancreatic tissues, but not by dissociation enzymes. Therefore, the involvement of endogenous proteases, especially trypsin or chymotrypsin which have been considered detrimental to the islets, on islet damages was strongly inferred. However, of particular note, no detrimental effects of trypsin or chymotrypsin on islet viability were observed (ADP/ATP ratio: 0.014±0.008, 0.000±0.000, ATP/DNA: 62.2±2.5, 62.3±1.7).
Conclusions: The present study reveals that tissue dissociation enzymes *per se* seem to be harmless on the islets, but indirectly relevant to the islet damages by activating endogenous proteases derived from pancreatic exocrine tissues. These endogenous proteases, unlike trypsin or chymotrypsin, might be novel strategic targets towards improving the quality of isolated islets.

327.4

Pretreatment of donor islets with papain improves allograft survival without systemic immunosuppression in mice

Kenjiro Kumano[1,2], Hitomi Nishinakamura[1,3], Toshiyuki Mera[4], Takeshi Itoh[1,5], Hiroyuki Takahashi[1,4], Toshiyoshi Fujiwara[2], Shohta Kodama[1,5].
[1]Department of Regenerative Medicine and Transplantation, Fukuoka University, Fukuoka, Japan; [2]Department of Gastroenterological Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan; [3]Department of Pharmacology, Fukuoka University, Fukuoka, Japan; [4]Department of Gastroenterological Surgery, Fukuoka University, Fukuoka, Japan; [5]Center for Regenerative Medicine, Fukuoka University Hospital, Fukuoka, Japan.

Introduction: Current immunosuppression protocols improve the efficacy of clinical allogeneic islet transplantation (tx). However, high-dosage immunosuppressant therapy is a risk factor for the incidence of infection and oncogenesis in the recipients. Thus, the dosage of systemic immunosuppressant should be minimized. One possible approach to minimize the dosage of immunosuppressant is reducing the immunogenicity of donor islet cells. Major histocompatibility complex (MHC) class I on the donor islet cells play a crucial role in T cell-mediated allograft rejection after islet tx in human and mice. It has been previously reported that papain, a cysteine protease enzyme, has the unique ability to cut the extracellular domain of MHC class I heavy chain, and also reported that pretreatment of allogeneic donor lymphocytes with papain inhibited the activation of host human T cells in mixed lymphocyte reaction assay in vitro. From these backgrounds, we hypothesized that pretreatment of donor islets with papain may reduce the expression of MHC class I on islet cell surface, reducing allografts immunogenicity, and contributing to the prolongation of islet allograft survival in mice.
Materials and Methods: BALB/c islets were transplanted into C57BL/6 mice. Prior to the tx, 200 islets were pretreated with papain at 1 or 10 mg/mL for 15 minutes, or left untreated as control, and then transplanted beneath the kidney capsule of streptozotocin-induced diabetic recipient mice.
Results and Discussion: The graft survival of control (n=10), pretreatment with papain 1 mg/mL (n=11) and 10 mg/mL (n=5) was 20.4±2.0, 54.2±13.4 and 13.6±1.2 days, respectively (mean±se, $p<0.05$ between untreated control and papain 1 mg/mL), and papain 1 mg/mL group showed significantly higher graft survival rate in the three groups ($p<0.001$). Flow cytometric analysis revealed that the MHC class I positivity on islet cells was approximately 10% lower in papain 1 same as 10 mg/mL groups when compared to control, indicating that papain treatment reduces the expression of MHC class I on islet cells. To investigate the effects of papain pretreatment on the inhibition of host T cell activation, we performed in vitro T cell proliferation assays. Host T cell activation against allogeneic lymphocytes was inhibited by the pretreatment of donor lymphocytes with papain 1 or 10 mg/mL when compared to control. Furthermore, host T cell activation against allogeneic islet cells was also suppressed by the pretreatment of donor islet cells with papain 10 mg/mL in a mouse model.
Conclusion: Pretreatment of donor islets with papain suppresses MHC class I-mediated allograft rejection, and contributes to the prolongation of islet allograft survival without administrating systemic immunosuppressant in mice. These results suggest that pretreatment of human donor islets with papain may reduce the immunogenicity of the donor islets and minimize the dosage of systemic immunosuppressant required in a clinical setting.

327.5

Sustained euglycemia after islet xenotransplantation in diabetic NOD mice using novel purification and encapsulation technologies

Michael Alexander[1], Antonio Flores[1], Sarah Lee[1], Nick Neel[1], Shiri Li[1], Paul de Vos[3], Jonathan Lakey[1,2].
[1]Surgery, University of Calfornia Irvine, Orange, United States; [2]Biomedical Engineering, University of Calfornia Irvine, Irvine, United States; [3]University Medical Center Groningen, Groningen, Netherlands.

Background: Islet transplantation has been demonstrated as potential option for those patients suffering from diabetes. Limitation are lack of sufficient viable pancreatic islets and chronic immunosuppression. Encapsulation islets has been a potential option for decades, however this technology has been limited in its success, especially in autoimmune diabetes. We have addressed current limitations of material selection, purification, and addressed issues of manufacturing to produce consistent 400 µm diameter purified alginate capsules.
Experimental Methods: Sprague-Dawley rat islets were isolated using standard methods of enzymatic distension, collagenase digestion, and Ficoll purification. Aliquots of islets were counted using dithizone stained aliquots and viability was measured using FDA/PI. Islets were then encapsulated using chemically and mechanically purified UPLVM alginate (Novamatrix) (gp 1). Control groups of islets were encapsulated using standard published techniques (gp 2). Non-transplanted diabetic mice (gp 3) served as diabetic control. NOD mice, 9-10 weeks old, were made diabetic using a single injection cyclophosphamide (200 mg/kg). After hyperglycemia was confirmed (3+ days of non-fasting blood glucose from tail capillary over 350 mg/dL) mice received encapsulated islet transplant into the peritoneal cavity (groups 1 and 2, n=10 per group). Mice were monitored daily, with blood glucose 3x/wk, body weight weekly and OGTT at 1 month post-transplant before histological evaluation including CD68, Masson Trichrome, insulin/glucagon.
Results and Discussion: In vitro results: Viability of islets was 93±1% (mean±SEM, n=10) post isolation and 92±2% post-encapsulation (p=ns, t-test). Islets were functional, with a stimulated GSIR of 4.3±1.2, compared to post encapsulation GSIR of 2.4±0.6 (gp 1, p=ns) and 1.9±0.1 (gp 2, p=ns). (gp 2, $p<0.05$). In vivo results: Control mice remained hyperglycemic for >30 days after diabetes induction (n=7). In diabetic NOD mice transplanted with our novel alginate purification methods, 8 of the 10 mice gp 1 showed immediate reversal of hyperglycemia and sustained euglycemia (bg < 200 mg/dL) within 2 days of transplant. Gp 2 mice also reversed hyperglycemia, but within 3-5 days retuned back to the diabetic state ($p<0.05$). OGTT in euglycemic mice at 30 days post txp (gp1) showed equivalent function to non-transplanted controls (p=ns). Histological evaluation of peritoneal cavity flush showed islets within intact capsules (gp 1), compared to free but damaged islets surrounded by immune cells, and debris from destroyed capsules (gp 2).
Conclusion: Using our novel alginate purification strategies an encapsulation system is capable of protecting encapsulated xenogeneic islets in the autoimmune diabetic NOD mouse model, significantly better outcomes than using standard alginate. Our goal is to adapt this novel purification and encapsulation system into large animal model and clinical trial of patients with diabetes.
Juvenile Diabetes Research Foundation, grant # 17-2013-288; Department of Surgery, UC Irvine, California

327.6

Cotransplantation of preactivated mesenchymal stem cells inhibits liver natural killer cell–induced islet graft injury during instant blood-mediated inflammatory reaction after syngeneic intraportal islet transplantation

Nobuki Ishida, Kohei Ishiyama, Fumihiro Hirata, Yoshihiro Saeki, Yuka Tanaka, Hideki Ohdan.
Department of Gastroenterological and Transplant Surgery, Hiroshima University, Hiroshima, Japan.

Introduction: Cotransplantation of islets with mesenchymal stem cells (MSCs) improves graft survival by enhancing graft revascularization and causing secretion of antiapoptotic signaling molecules. We previously showed that activation of cytokine-stimulated liver natural killer (NK) cells, which play a crucial role in transplanted islet destruction, was significantly suppressed on coculture with MSCs in vitro. We also found that liver NK cells were activated under instant blood-mediated inflammatory reaction (IBMIR), which occurred immediately after islet transplantation (IT). Inflammatory cytokine stimulation is known to enhance the immunomodulatory function of MSCs; however, little is known about phenotypical and functional alterations of MSCs during IBMIR after cotransplantation with islets. Here, we examined whether cotransplantaion of MSCs preactivated with IFN-γ, TNF-α, and IL-1β, which are actively produced under IBMIR conditions, could suppress liver NK cell activation after intraportal IT.
Methods: To analyze phenotypical alterations in liver NK cells after syngeneic intraportal IT, liver mononuclear cells were collected 3 days after intraportal administration of 300 islets with or without bone marrow-derived MSCs (0.5×10^6) from C57BL/6 mice. Furthermore, to examine the suppressive effect of cotransplantation of preactivated MSCs, MSCs were cultured for 24 hr with inflammatory cytokines (IFN-γ, TNF-α, and IL-1β) before IT.
Results: Liver NK cells are classified into two subsets based on DX5 expression. $DX5^-$ NK cells are considered as liver resident population which expresses high levels of TRAIL and CD69, while $DX5^+$ NK cells are considered as conventional population. $TRAIL^+$ $DX5^-$ NK cells proportion at 3 days after intraportal MSC administration was significantly lower than that in the naive condition (43.1% ± 14.3% vs. 71.9% ± 9.6%, $p < 0.05$). CD69 and TRAIL expression on liver NK cells after intraportal IT was significantly higher than that in the naive condition (72.3% ± 10.1% vs. 29.0% ± 5.6%; $p < 0.01$, 43.2% ± 6.7% vs. 37.4% ± 6.6%; $p < 0.01$, respectively). In contrast, CD69 expression on liver NK cells after cotransplantation of islets with preactivated MSCs was significantly lower than that in IT alone (52.1% ± 4.4%, $p < 0.01$); however, TRAIL expression on liver NK cells did not show a significant difference (36.1% ± 10.7%, $p = 0.21$). CD69 and TRAIL expression on liver NK cells was somewhat suppressed when naive MSCs were cotransplanted with islets ($p = 0.08$, $p = 0.99$, respectively). Furthermore, the $DX5^-$ NK cell proportion after cotransplantation of islets with preactivated MSCs was significantly lower than that in IT alone (37.7% ± 4.4% vs. 50.2% ± 8.7%, $p < 0.05$).
Conclusion: This is the first report to show that IBMIR conditions after IT potentially enhance the immunomodulatory function of cotransplanted MSCs, although IBMIR conditions themselves are considered harmful for islet grafts. Our findings imply that cotransplantation of islets with preactivated MSCs could improve islet graft survival after IT by suppressing NK cell activation in the liver.

327.7

SA-FasL-engineered porcine islets induce tolerance in mice following intraportal transplantation

Esma S.Yolcu[1], Hong Zhao[1], Kyle Woodward[1], Melanie Graham[2], Bernhard Hering[2], Haval Shirwan[1].
[1]Institute for Cellular Therapeutics and Department of Microbiology and Immunology, University of Louisville, Louisville, KY, United States; [2]Schulze Diabetes Institute and Department of Surgery, University of Minnesota, Minneapolis, MN, United States.

Introduction: We have recently demonstrated that allogeneic islets engineered to transiently display on their surface a novel form of FasL protein chimeric with streptavidin (SA-FasL) induce tolerance in allogeneic mouse models. In this study, we tested the efficacy of this approach in inducing tolerance to porcine islets transplanted into mice using two different transplant sites; subrenal and intraportal.
Methods: Porcine islets were modified with 5 μM biotin and engineered with SA-FasL protein (~200 ng/1000 islets). SA-FasL-engineered islets were transplanted under the kidney capsule (~2000 islets/transplant) or intraportally (2,500 islets/transplant) in streptozotocin diabetic C57BL/6 mice under a transient cover (20 days) of rapamycin. Unmodified pancreatic islets with rapamycin treatment served as controls.
Results and Discussion: Porcine islets were effectively engineered with SA-FasL protein without a detrimental effect on their viability and function. SA-FasL-engineered islets induced apoptosis in responding mouse T cells in in vitro co-culture experiments. All control grafts with transient rapamycin treatment were rejected within 30 days post-transplantation. In marked contrast, all SA-FasL-engineered porcine grafts showed prolongation and ~60% of intraportal (n=37) and ~80% of subrenal (n=22) grafts survived for a 300-day observation period. Intraperitoneal glucose tolerance test demonstrated normal function of long-term islets, with the subrenal model performing better than the intraportal model. There was no detectable signs of acute or chronic toxicity of the procedure. The long-term euglycemia was due to the transplanted porcine islets as the surgical removal of the grafts resulted in prompt hyperglycemia.
Conclusion: SA-FasL as an immunomodulatory molecule is effective in inducing tolerance to porcine islet grafts transplanted subrenally as a standard site in rodents and intraportally as a clinically applicable yet immunologically more challenging site.

327.8

Anti-inflammatory CD169+ TIM-4+ islet resident macrophages induce Tregs and prolong allograft survival

Periklis Kyriazis, Thomas Thornley , Krishna Agarwal , Lingzi Ma, Vaja Chipashvili, Sarantis Korniotis, Terry Strom , Maria Koulmanda.
Department of Medicine - Surgery, Beth Israel Deaconess Medical Center/Harvard Medical School, Boston, MA, United States.

We previously characterized an immunoregulatory islet resident macrophage subset that expresses CD169 and TIM-4, dwells in heart and skin, promotes FoxP3+ Treg generation, and promotes allograft survival. Now, we report on the existence of a kindred CD169+TIM-4+ islet resident macrophage subset that is located within pancreatic islets from C57BL/6 mice and control human subjects. Compared to islet resident DCs (IRDCs), islet resident macrophages (IRMs) express higher levels of immunoregulatory CD39, CD73, and galectin-9. In vitro, bm12(I-Abm12) IRMs induced much greater Foxp3+ Treg conversion (20.9+/-6.12%) as compared to bm12 IRDCs (3.41+/-1.61%, $p<0.001$) in naïve CD4+ABM TCR transgenic T cells that express an alloreactive clonotypic anti I-A bm12 TCR. Surprisingly, genetic TIM-4 deletion increases the survival and absolute number of tissue resident macrophages that migrate to the draining lymph nodes following oxidative stress. Donor TIM-4 deletion promotes long-term islet allograft survival (>110 days) in 2/5 islet transplant recipients as compared to 0/4 mice that received wild type control islets. As CD169+ TIM-4+ IRMs are present in large numbers in donor islets, possess a potent immunoregulatory phenotype, powerfully induce FoxP3+Tregs, and promote islet allograft survival, we believe that CD169+TIM-4+ IRMs are a potential therapeutic target in islet allograft transplantation.
References:
[1] [1] Thornley et al. J Clin Invest. 2014; 124(8): 3443–3454.

327.9

Endoscopic biopsy of islet transplants in the gastric submucosal space provides evidence of islet graft rejection in diabetic pigs.

Hidetaka Hara[1], Takayuki Tanaka [1], Minoru Fujita [1,5], Rita Bottino [2], Jon D.Piganelli[2], Kevin McGrath[3], Jiang Li[1], Whayoung Lee[1], Hayato Iwase[1], Martin Wijkstrom[1], Suzanne Bertera[2], Cassandra Long[1], Douglas Landsittel[4], Ken Haruma[5], David K.C.Cooper[1].
[1]Surgery, University of Pittsburgh, Pittsburgh, PA, United States; [2]Pediatrics, University of Pittsburgh, Pittsburgh, PA, United States; [3]Gastroenterology, Hepatology and Nutrition, University of Pittsburgh, Pittsburgh, PA, United States; [4]Biostatistics and Clinical and Translational Science, University of Pittsburgh, Pittsburgh, PA, United States; [5]Internal Medicine, Kawasaki Medical School, Kurashiki, Japan.

Introduction: Transplantation of islets into the gastric submucosal space (GSMS) has several advantages (e.g., avoidance of the instant blood-mediated inflammatory response [IBMIR], ability to biopsy). The aim of this study was to determine whether endoscopic biopsy of islet allografts transplanted into the GSMS in diabetic pigs can provide histopathological and immunohistochemical information that correlates with the clinical course (e.g., blood glucose level, insulin requirement).
Materials and Methods: Islet allografts (Group1: 10,000IEq /kg [n=4]; Group2: 15,000IEq /kg [n=2]) were transplanted into the GSMS of streptozotocin-induced diabetic pigs under immunosuppression. In Group2, the anti-oxidant, BMX-001 was applied during preservation, isolation, and culture of the islets, and at the time of transplantation. Endoscopic biopsies of the islet grafts were obtained one or two weeks after transplantation, and histopathological features were compared with the clinical course (e.g., blood glucose, insulin requirement).
Results: While Group1 insulin-positive islets were fragmented, Group2 grafts showed preserved islet morphology. Cell infiltration was observed in biopsies and necropsies (4 weeks) from both groups, and most of the infiltrating cells were positive for CD3 and CD68. In Group1, in the absence of anti-oxidant therapy, there was no reduction in exogenous insulin requirement. In Group2, with an increased number of transplanted islets in the presence of BMX-001, more healthy insulin-positive islet masses were obtained, and these correlated with reductions in both blood glucose level and insulin requirement.
Conclusions: After islet transplantation into the GSMS, endoscopic biopsy can provide information on graft rejection as well as the presence of healthy islet grafts, which would be an immense advantage in clinical islet transplantation.

327.10

Sphingolipids regulate the podocyte insulin signaling in diabetic kidney disease

Alla Mitrofanova[1], Sandra Merscher[1], Alessia Fornoni[1,2], Linda Chen[3], George Burke[2,3].
[1]Medicine/Division of Nephrology and Hypertension, University of Miami Miller School of Medicine, Miami, United States; [2]Peggy and Harold Katz Discovery Center, University of Miami Miller School of Medicine, Miami, United States; [3]Miami Transplant Institute/Surgery, Jackson Memorial Hospital/University of Miami Miller School of Medicine, Miami, United States.

Disruption of physiological insulin signaling occurs in podocytes in experimental diabetic kidney disease (DKD). Podocytes are insulin sensitive and podocyte-specific deficiency of insulin receptor (IR) in in vivo mouse model recapitulates many features of DKD and affects podocyte actin cytoskeleton. Our studies have demonstrated that podocytes become insulin resistant in the early stages of experimental DKD. Sphingolipids are known to be important regulators of insulin signaling. We have identified sphingomyelin-phosphodiesterase-acid-like-3b (SMPDL3b) as a key lipid raft associated protein that is upregulated in DKD. We hypothesize that SMPDL3b overexpression suppresses pro-survival insulin signaling in podocytes via decreased ceramide kinase dependent C1P production.
Wild type (WT) or SMPDL3b overexpressing (SMPOE) human podocytes were utilized and their lipid content was analyzed by mass spectrometry technique. Further, we tested the response of SMPOE podocytes to insulin (1 nM, 30 min, Sigma), and analyzed changes in ceramide metabolites. We determined the levels of ceramide species, sphimgomyelin, and ceramide-1-phosphate in WT and SMPOE podocytes. Amounts of phospho- and total protein kinase B (AKT) and p70S6 kinase (p70S6K) were measured by Western blotting. The ability of SMPDL3b to immunoprecipitate both insulin receptor subtypes (IRA and IRB) was also tested.
SMPDL3b immunoprecipitated both IR isoforms. SMPOE podocytes are unable to phosphorylate AKT, but demonstrated increased phosphorylation of p70S6K in response to insulin stimulation. Ceramide C16:0 species was primarily detected in podocytes and remained unchanged by SMPDL3b overexpression. Content of total sphimgomyelin was higher in SMPOE podocytes when compared to WT cells. However, SMPOE cells demonstrated lower amounts of C1P ($p<0.05$). Pre-treatment with C1P 16:0 (100uM, 1h, Avanti) restored the ability of SMPOE podocytes to phosphorylate AKT in response to insulin stimulation. Our data indicate that SMPDL3b overexpression suppresses pro-survival insulin signaling in podocytes and might affect C1P production, leading to podocyte insulin resistance in DKD. C1P replacement strategies may protect podocytes in DKD.

327.11

Sphingosine-1-phosphate promotes the migration of intraislet vascular cells: implications for post-transplant islet function

Darling Rojas-Canales[1,2], Daniella Penko[1,2], Heshan Peiris[3,7], Stuart Pitson[4], Chris Drogemuller[1,2], Damien Keating[3,7], Shane Grey[5], Patrick T.Coates[1,2], Claudine S.Bonder[4], Claire F.Jessup[1,6].
[1]Central Northern Adelaide Renal Transplantation Service , The Royal Adelaide Hospital, Adelaide, Australia; [2]Discipline of Medicine, The University of Adelaide, Adelaide, Australia; [3]Department of Human Physiology, Flinders University of South Autsralia, Bedford Park, Australia; [4]Centre for Cancer Biology, University of South Australia, Adelaide, Australia; [5]Transplantation Immunology, Garvan Medical Institute , Darlinghurst, Australia; [6]Anatomy & Histology, Flinders University of South Autsralia, Bedford Park, Australia; [7]Centre for Neuroscience, Flinders University of South Autsralia, Bedford Park, Australia.

Introduction: Pancreatic islet transplantation is a promising clinical treatment for type I diabetes, but success is limited by extensive beta cell death in the immediate post-transplant period, and impaired islet function in the longer term. Following transplantation, appropriate vascular remodelling is crucial to ensure the survival and function of engrafted islets. The ubiquitous sphingosine kinase pathway is an important regulator of vascular beds, however its expression and function in islets is largely undefined. The aim of this study was to investigate the expression and role of sphingosine kinase 1(SK1) in the function of islets in vitro and post transplantation.
Methods: Islets were isolated and density-purified from B6 (WT) or SK1 knockout (SK1KO) mice. SK1 gene expression and SK enzyme activity was determined by quantitative real-time PCR and thin-layer chromatography separation respectively from isolated WT islets. In vitro function of islets was determined by a glucose stimulation insulin release assay, where insulin secretion was determined by ELISA. Flow cytometry was used to enumerate endothelial cell number per islet. In vivo islet function was assessed in WT streptozotocin-induced diabetic mice transplanted with a minimal mass of 200 islets under the kidney capsule (cure=consecutive BGL<11.1mM). All cured animals were subjected to an IP glucose tolerance test (IPGTT).
Results and Discussion: Isolated islets have SK1 gene expression that increases with time in culture, this correlated with the presence of SK1 enzyme activity. Here, we show that sphingosine kinase 1 (SK1) promotes the engraftment of pancreatic islets. We found that donor islets from mice deficient in SK1 (SK1-KO) contain a reduced number of resident intraislet vascular endothelial cells compared to WT ($p=0.0165$). Furthermore, we demonstrate that the main product of SK1, sphingosine-1-phosphate, controls the migration of intraislet vascular endothelial cells in vitro, where SK1 specific inhibitor JTE-013 inhibits MS-1 cell migration by over 50% ($p<0.05$). We found in vivo that SK1-KO islets have an impaired ability to cure diabetes in recipient mice, compared to wildtype controls. Thus SK1-deficient islets not only contain fewer of the building blocks required for revascularisation, but also have a reduced ability to function post transplantation.
Conclusion: Our data suggest that the sphingosine kinase axis is important for islet endothelial cell migration, and islet function following transplantation, thus represents a novel clinical target for improving transplant outcomes.
Medvet funding source

327.12

Hypothermic machine perfusion preservation of non-heart-beating donor pancreas for islet transplantation

Naotake Akutsu[1], Takemichi Ishida[1], Michihiro Maruyama[1], Kazunori Otsuki[1], Tomonori Saitoh[1], Kenichi Saigo[1], Masayuki Hasegawa[1], Hiromichi Aoyama[1], Ikuko Matsumoto[1], Takashi Kenmochi[2], Hirofumi Noguchi[3].
[1]Surgery, Chiba-East National Hospital, Chiba, Japan; [2]Transplantation Surgery, Fujita Health University School of Medicine, Toyoake, Japan; [3]Regenerative Medicine, Ryukyu University School of Medicine, Nishihara, Japan.

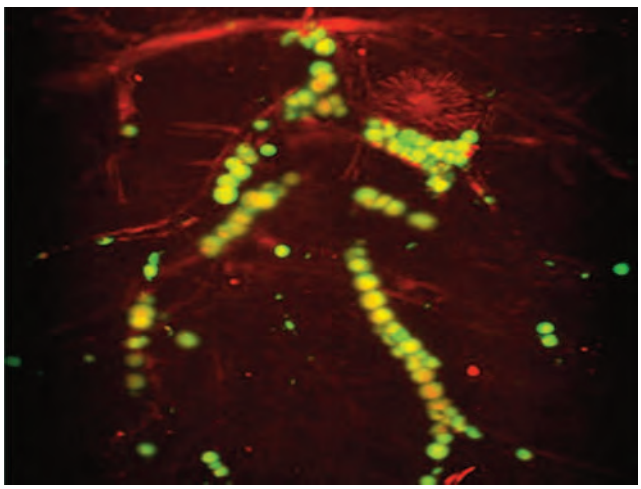
Background: In Japan, brain-dead donors have been increased by revised Act of Organ Transplantation, however, insufficiency of deceased donor's number is still serious problem. In islet transplantation in Japan, it is more serious, because we can only procure the pancreas that could not harvest for pancreas transplantation. Then, it is important to use the pancreas from marginal donors such as non-heart-beating donors for solving this problem. Improvement of preservation method would be necessary to use the pancreas from non-heart beating donor. Static cold storage preservation (SCS) with UW or two layer preservation method (TLP) is the most widely used method for islet transplantation. However, hypothermic machine perfusion (HMP) preservation technique has had a major impact in circumventing ischemic injury in kidney transplantation in western countries. In this presentation, we report efficiency of HMP method for preservation of long ischemic pancreas grafts for islet transplantation in beagle.
Methods: Young beagles were used as auto-transplantation model. After general anesthesia of beagles, pancreatic artery and vein were made ligation, after 30 min warm ischemia time (WIT) in situ. Each harvested pancreas was assigned to one of three preservation treatment groups; SCS group (storage in University of Wisconsin solution at 2-4°C for 24h, n=4); TLP group (storage on two-layer preservation method at 2-4°C for 24h, n=4); HMP group (storage on LifePort® (Organ Recovery Systems), a portable hypothermic machine perfusion apparatus, at 2-4°C on pulsatile pressure (10 mmHg) for 24h with KPS-1 solution, n=4). After the storage, wedge biopsies were taken for histological evaluation. With the preserved pancreas islets were isolated by standard technique involving ductal distension of the gland with liberase enzyme, normothermic digestion and density gradient purification. After purification, the islets were evaluated for the yield and function, auto-transplanted to the same beagle in portal vein, and checked graft qualities in the recipients (serum glucose and insulin, IVGTT test).
Results: Each islet yield after purification was 182±175IEQ/g (SCS), 509±222IEQ/g (TLP), and 655±496IEQ/g (HMP). Stimulation index of each group was 1.28±1.22 (SCS), 1.43±1.14 (TLP) and 1.72±1.10 (HMP). Without statistically significant, yield and function of isolated islet of HMP group were better than that of SCS or TLP group. In histological findings, no difference was indicated with each group. Serum glucose level and IVGTT test in auto-transplanted recipient was not significantly different from each group.
Discussions and Conclusions: In this experiment, we indicate that HMP should be better for long term WIT pancreas preservation model for islet transplantation compared with SCS and TLP method in beagles. It suggested that preservation of pancreas on HMP technique should be useful for islet transplantation with non-hear-beating donor in Japan.

327.13

Analysis of post-transplant islet by organ transparency and macro three-dimensional image

Koya Shinohara, Masayuki Shimoda.
Pancreatic Islet Cell Transplantation, National Center,Global Health and Medicine, Tokyo, Japan.

Islet transplantation is a potential treatment for brittle type 1 diabetic patients. The principle of islet transplantation is at first to excise pancreas from donor body, and islets are isolated from the pancreas, then they are implanted into in the liver via portal vein. Then, the engrafted islets become to secrete insulin according to blood glucose levels. However, the detail of how the transplanted islets actually in the portal veins of the liver distribute, survive and function remains unclear. One of the reasons of the difficulty of observation of islets after transplantation is because they are not visible in the liver. Therefore, if the whole liver could be transparentized and the status of the islets after transplantation could be observed comprehensively, it must contribute to understanding of islet transplantation and improving it.
Recently, whole-organ imaging at single-cell resolution and tissue clearing methods have been developed[1]. These methods enables us to analyze the tissue structure of solid organs at the cellular, circuit, and organ-wide scale.
We have recently developed a method to transprantize a mouse liver by the techniques with our modification. As a result, it is possible to check the transparency of the liver, further also visible localization of fluorescent beads of the transplanted islets model in the liver on wide scale.



However, it was still difficult to perfectly clear the center of the organ. In this study, we aimed further improvement. For transparency of tissues, it has been found that treatment with a surfactant agent is very important. It is a process for removing cells from the tissue, which is often used when the artificial organ creation of biological origin. Therefore, we focused on the decellularization of the liver. We investigated some surfactant agents as a pretreatment of Scale / CUBIC, we could reduce the time to clear the liver. Then, we applied the method to islet transplantation model. The islets isolated from GFP transgenic mice were transplanted into nude mice via portal vein, and then the whole liver was transparented. Then, the whole liver was observed by 3D microscope. This method should be useful for the research of islet transplantation.

References:

[1] Tainaka K, Ueda HR, et al. Cell. 2014 ;159:911-24

328.1

Maintenance of long-term tolerance by T regulatory cells in a mouse model of heart transplantation

Ning Jin, Weili Chen, Fangna Lu, Maoshu Zhu, Feifei Du, Wei Shao, Chang Gao, Tianshu Lan, Chen Dai, Shuo Han, Qing Li, Shifu Hong, Junjie Xia, Zhongquan Qi.
Xiamen University, Organ Transplant Institute, Xiamen, People's Republic of China.

Background: Regulatory T cell therapy is generally used for bone marrow transplantation and autoimmune diseases but is less frequently applied for transplantation of the heart. In this work, we adopted a drug regimen developed by Battaglia et al. to induce CD4+Foxp3+Tregs and T regulatory type 1 cells in vivo to maintain long-term tolerance after heart transplantation.
Methods: Hearts from BALB/c mice were transplanted into C57BL/6 mice. We then administered (hu)IL-10 and rapamycin to generate CD4+Foxp3+Tregs and Tr1 cells (CD4+IL-10+IL-4-) in vivo. Levels of rejection were analyzed using hematoxylin and eosin (H&E) staining and ELISA, and cell subtypes were identified using flow cytometry.
Results: Combined treatment with (hu)IL-10 and rapamycin induced tolerance to the heart allografts in all recipient mice, and levels of IL-2 and INF-γ were also clearly decreased in the rapa+IL-10 group. Compared to the rapa group, the subset of Th1 was decreased in the combined group. The proportion of CD4+Foxp3+Tregs induced by this treatment continued to be high during the administration period and recovered to normal levels on the 100th day after transplantation, yet the proportion of Tr1 cells remained high after drug withdrawal. Mixed lymphocyte reactions and adoptive transfer experiment showed that the recipient's T cells were tolerant (showed no response) to the donor's antigen presenting cells (APCs) compared to the third-party donor's APCs, and it was the induced regulatory T cells that inhibited this proliferation.
Conclusion: The combination of (hu)IL-10 and rapamycin was able to induce CD4+Foxp3+Tregs and Tr1 cells in vivo . The two subsets showed different regulatory effects and antigen specificity in the mouse model of heart transplantation and were able to maintain a low immune response environment to facilitate long-term graft survival.

328.2

Neonatal tolerance in a mouse model of ABO-incompatible heart transplantation

Bruce Motyka[1,2,3], Brendon Lamarche[1], Katrina Labonte[1], Szu-I Wang[1,2,3], Jean Pearcey[1,2], Kesheng Tao[1,2], Michael Mengel[2,3,4], Banu Sis[2,3,4], Peter J.Cowan[5], Lori J. West[1,2,3,6].
[1]Dept. Pediatrics, University of Alberta, Edmonton, AB, Canada; [2]Alberta Transplant Institute, University of Alberta, Edmonton, AB, Canada; [3]Canadian National Transplant Research Program, University of Alberta, Edmonton, AB, Canada; [4]Dept. Laboratory Medicine & Pathology, University of Alberta, Edmonton, AB, Canada; [5]Immunology Research Centre, St Vincent's Hospital, Melbourne, Australia; [6]Depts. Surgery and Medical Microbiology & Immunology, University of Alberta, Edmonton, AB, Canada.

Introduction: ABO-incompatible heart transplantation (ABOi HTx) can be performed safely in infants due to lack of natural anti-A/B antibodies, in contrast to adults. Following ABOi HTx, B cell tolerance to donor A/B blood group antigen(s) develops by mechanisms not well understood. For detailed study we developed a model using mice transgenic for expression of human A-antigen as donors (A-Tg, C57BL/6 (B6) background) and B6 wild-type (WT) mice as recipients. We showed that A-Tg heart grafts undergo antibody-mediated rejection (AMR) in adult WT mice with anti-A antibody, whereas A-Tg HTx into juvenile WT mice (4 weeks old) results in tolerance to A-antigen. The present study investigated whether tolerance can be induced in WT mice following administration of A-antigen in a form other than a transplant.
Methods: Neonatal WT mice (<1 day) were injected i.v. with A-antigen expressing splenocytes/bone marrow cells (n=17) from adult A-Tg mice, or were untreated (n=15). As adults (7 weeks), mice were injected i.p. with human A erythrocytes (RBC) in an attempt to induce anti-A antibody. Subsequently mice (neonatal-treated n=9; untreated n=6) received heterotopic A-Tg HTx. Grafts were monitored for function (beating) and assessed for AMR by histology at the experimental endpoint 14-21 days post-Tx or when beating ceased. Serum anti-A and third-party antibodies were assessed by hemagglutination (with A-Tg and reagent human A/O RBC).
Results: Anti-A antibodies were induced to high levels in all mice not treated as neonates (median titre 1:1024, range 1:256-2048) and were undetectable-low in neonatal-treated mice (median titre ≤1:2); anti-human non-A RBC antibodies (third-party) were comparable in both groups. In untreated mice, 5/6 grafts survived to day 14-21 post-Tx despite abundant anti-A antibody, however C4d deposition in capillaries (3/6) and morphologic features of AMR (4/6) were present. In contrast, in neonatal-treated mice, all grafts (9/9) survived to day 14-21 with no C4d deposition; 6/9 grafts had distorted morphology that may be artifact or related to ischemia.
Conclusion: The inability to elicit abundant anti-A antibodies in adult neonatal-treated mice by either injection of A-RBC or Tx of A-Tg heart grafts, together with third-party antibody production, suggests that robust A-antigen-specific tolerance was induced by neonatal exposure to A-expressing cells. This model will prove useful for addressing mechanisms of ABO-tolerance.
Supported by Heart and Stroke Foundation of Canada; Women and Children's Health Research Institute, University of Alberta; Alberta Innovates Health Solutions; and the Canadian Institutes of Health Research (CIHR) through the Canadian National Transplant Research Program (CNTRP)

328.3

Interleukin-5 therapy prevents chronic allograft rejection by induction of antigen specific CD4+CD25+ T regulatory cells

Suzanne J. Hodgkinson[1,2], Bruce M.Hall[1,2], Rachael M.Hall[1,2], Catherine M.Robinson[1,2], Paul Wilcox[1,2], Giang T.Tran[1,2], Chuanmin Wang[3], Alexandra Sharland[3].
[1]Immune Tolerance Laboratory, Department of Medicine, University of New South Wales, Liverpool, Australia; [2]Ingham Institute, Liverpool Hospital, Liverpool, Australia; [3]Australian National Liver Transplantation Unit, Royal Prince Alfred Hospital & Department of Surgery, University of Sydney, Sydney, Australia.

Aim: Naïve $CD4^+CD25^+Foxp3^+$T regulatory cells (nTreg) activated by specific alloantigen and IL-4, not IL-2, express the IL-5 receptor (IL5Rα) and we call these Ts2 cells[1],[2]. Ts2 cells are more potent allo-antigen (Ag) specific Treg than nTreg. Here we examined if IL-5 treatment activated alloAg specific Ts2 cells to prevent chronic rejection.
Methods: Rats recipients of F344 strain with an adult heterotopic heart allografts from Lewis rats received 5000units rIL-5 ip daily for 10d from 7d after grafting, all groups n=5-6. Rejection was scored on a semi-quantitative scale. Changes in the $CD4^+CD25^+$T cells were examined for their numbers, their response in vitro to donor antigen with and without IL-5 and their cytokine and cytokine receptor expression by RT-PCR
Results: Sham treated rats developed rejection at 18d and all grafts were totally rejected by 28d (n=5). IL-5 treatment prevented rejection until cessation of IL-5 ($p<0.01$ vs sham) at 18d post-transplant. At that time $CD4^+CD25^+FoxP3^+$ Treg from IL-5 treated rats, compared to controls, were increased in number (6-8% vs 3-4%), expressed IL-5Rα and proliferated to specific donor antigen in MLC. After cessation of IL-5 treatment, a rejection crisis occurred but all grafts recovered and survived for 60d when they all had good function. In another group, IL-5 treatment was continued and it had less rejection and at 60d had $CD4^+CD25^+$Treg that expressed IL-5Rαand proliferated to specific donor antigen in the presence of IL-5.
Pre-treatment with anti-CD25 or anti-IL-4 abolished benefit of IL-5 treatment consistent with host $CD25^+$T cells being activated to Ts2 cells that were then expanded by IL-5. After 10d of IL-5 treatment, $CD4^+CD25^+$T cells responded to Lewis but not to F344 or third party PVG heat grafts in MLC, showing activation of alloAg specific Treg by IL-5 treatment. $CD4^+CD25^+$T cells from rats treated for 50d with IL-5 had their response to Lewis enhanced by IL-5, consistent with a Ts2 cell. RT-PCR of host $CD4^+CD25^+$T cells found IL-5 treated groups had more IL-5Rα, but remained $FoxP3^+$.
Conclusion: IL-5 prevented heart graft rejection by activation of $CD4^+CD25^+FoxP3^+$ Treg that had been activated by host IL-4 and donor alloantigen. This effect was consistent with induction of Ts2 cells. This study shows promotion of Th2 cytokine induced Treg by IL-5 therapy may have potential to prevent chronic allograft rejection. Chronic rejection remains an unsolved problem in clinical transplant, and induction of antigen specific Treg may be able to control this pathology[2]. Human $CD4^+CD25^+FoxP3^+$Treg activated by IL-4 and alloantigen also are induced to express IL-5Rα. Thus, this IL-5 pathway of activation of antigen specific Treg may be useful if activated in chronic rejection.
References:
[1] Verma ND. Research Paper. *Blood*. 2009: 113: 479-487.
[2] Tran GT. IL5 promotes induction of antigen specific CD4+CD25+ Tregulatory cells that suppress autoimmunity. *Blood*. 2012;119:4441-4450.

328.4

Contrasting effects of tacrolimus and everolimus on regulatory T cell activation and mixed hematopoietic chimerism induced by natural killer T cell stimulation

Toshihito Hirai[1], Satoshi Miyairi[1], Yusuyuki Ishii[2], Masayoshi Okumi[1], Kazunari Tanabe[1].
[1]Department of Urology, Tokyo Women's Medical University, Tokyo, Japan; [2]Laboratory for Vaccine Design, RIKEN IMS-RCAI, Yokohama, Japan.

Introduction: Calcineurin-dependent IL-2 production is crucial for regulatory T cells (Tregs) to demonstrate suppressive activity. The mTOR signal is not required for Treg function because IL-2-signaling stimulated Tregs preferentially induce the JAK/STAT signaling pathway rather than PI3K/AKT signaling. Therefore, calcineurin inhibitors and mTOR inhibitors should have different effects on Treg performance. We previously reported Treg-dependent mixed hematopoietic chimerism induction therapy comprising of iNKT cell activation by a liposomal formulation of α-galactosylceramide (lipo-aGC) and CD40/CD40 ligand blockade (MR1). Here, we characterized the impact of tacrolimus (TAC) and everolimus (EVL) on Tregs and donor cell engraftment.
Methods and Results: 3 Gy-irradiated BALB/c ($H2^d$) mice were administered bone marrow cells (2×10^7) from C57BL/6(B6; $H2^b$) mice, followed by a single dose of lipo-aGC plus MR1. The mice showed early expansion of splenic Tregs with high Ki67 expression on day 7 after BMT and establishment of robust permanent mixed chimerism. When TAC was administered consecutively from day 1 until day 14 after BMT, Ki67 expression on splenic Tregs significantly decreased, whereas that on the conventional T cell counterpart did not. On cocultured with naive $H2^{d+}$T cells, $H2^{d+}CD4^+CD25^+$cells isolated from the splenocytes of control chimeric mice suppressed proliferation of naive $H2^{d+}$T cells against $H2^{b+}$cells, whereas those from TAC mice attenuated this suppressive potential against the donor antigen. Although TAC mice showed transient chimerism, the engrafted donor cells dwindled, which resulted in a chimerism brake (fig 1). This abrogation of mixed chimerism induction was reinforced by TAC in a dose-dependent manner. In contrast, when EVL was administered between day 1 and day 14, abrogation of Treg expansion and mixed chimerism induction was not observed (fig 2).
Conclusion: These data collectively suggest that TAC, but not EVL, precluded Treg activation by stimulated iNKT cells.

Fig 1.

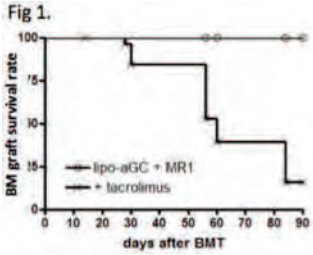
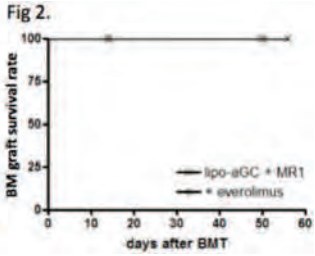


Fig 2.



328.5

IFN-gamma/B7-H1 signaling pathways in graft mesnechymal cells mediate liver transplant tolerance

Miwa Morita[1], Daniel Joyce[2], John J. Fung[2], Shiguang Qian[1,2], Lina Lu[1,2].
[1]Immunology, Lerner Reaerch Institute, Cleveland Clinic, Cleveland, OH, United States; [2]General Surgery, Transplant Center, Digestive Disease Institute, Cleveland Clinic, Cleveland, OH, United States.

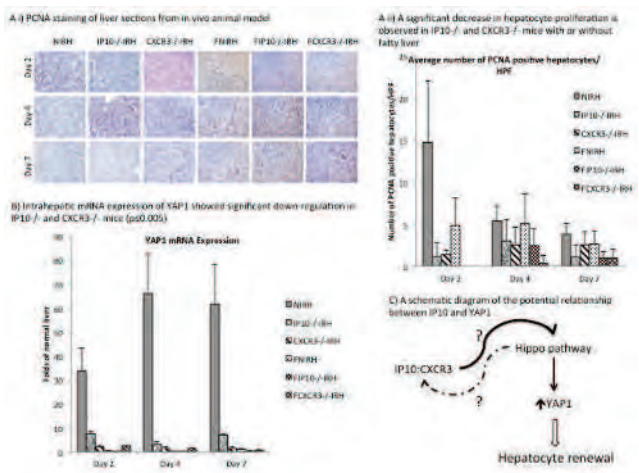
Induction of transplant tolerance, an ideal solution for organ transplantation, remains elusive in clinic practice. Indeed, transplant tolerance occurs naturally. Thus, 1/4 liver transplants (LT) in farmer pigs were spontaneously accepted. This phenomenon was reproduced in rats and mice. World-wide data showed that ~25% LT patients successfully underwent totally weaning off immunosuppression. Establishment of a mouse LT tolerance model in our lab allowed to scoop out the behind mechanisms. LT tolerance is not related to T cell clonal deletion (as seen in central tolerance) because recipient T cells respond normally to donor antigens in vitro, rather related to apoptotic death of activated T cells. We were inspired by an unexpected observation that LT tolerance absolutely requires IFN-γ, an inflammatory cytokine, mainly produced by Tef cells that should promote rejection. The aim of this study was to determine the underlying cellular and molecular mechanisms. The experiments were performed in the recipients (C3H) of B6 WT (tolerance) or IFN-$\gamma R1^{-/-}$ (rejection) liver graft. We focused on analyzing graft non-parenchymal cells (NPC), because, unlike the whole liver, hepatocyte allografts are acutely rejection, suggesting the tolerance is mediated by NPC. We divided the graft NPC into hematopoietic ($CD45^+$) and mesenchymal ($CD45^-$) groups for analysis, and found that graft $CD45^+$ NPC were rapidly replaced by cells of recipient origin (WT) following transplantation, while $CD45^-$ cells remained IFN-$\gamma R1^{-/-}$, therefore, rejection of tolerance largely depends on the behavior of graft mesenchymal cells (GMC), not hematopoietic cells. Comparison of GMC in WT and IFN-$\gamma R1^{-/-}$ grafts revealed that ligation of IFN-γR on GMC triggered the upregulation of their B7-H1 expression leading to the apoptosis of the infiltrating Tef cells, but did not affect MDSC and Treg activities, indicating a key role of Tef elimination in induction of tolerance. We tested immune regulatory activity of hepatic stellate cells (HpSC) and liver sinusoid endothelial cells (LSEC) in vitro and in vivo. Addition of either HpSC or LSEC markedly suppresses T cell response in vitro (MLR). Co-transplantation with HpSC or LSEC significantly prolongs survival of islet allografts, while HpSC from IFN-$\gamma R^{-/-}$ or B7-$H1^{-/-}$ mice failed to protect islet allografts. These results illustrate a mesenchyme-mediated immune control (MMIC) mechanism in graft to respond to the host immune attacks, leading to LT tolerance. It is likely that graft plays a key role in the equipoise between tolerance and rejection and warrants attention in the search for biomarkers of tolerance. MMCI activity is unlikely exclusive to the liver, as spontaneous acceptance has also been reported in kidney transplants, although less common, reflecting the variant MMIC activities in different organs.

328.6

Liver regeneration was attenuated by deletion of CXCL10 or its receptor CXCR3 via YAP1 signaling

Yuen Yuen Ma, Changxian Li, Wei Geng, Kevin Ng, Chungmau Lo, Kwan Man.
Surgery, The University of Hong Kong, Hong Kong , Hong Kong.

Background: Acute phase liver graft injury after transplantation often causes delayed liver regeneration. Our recent study showed that chemokine (C-X-C motif) ligand 10 (IP10), a pro-inflammatory chemokine, is a specific gene signature of acute phase liver graft injury that promotes tumor cell proliferation and angiogenesis[1],[2]. While other reports suggested IP10 is also hepatoprotective during liver injury[3]. Here, we aimed to investigate the effects and molecular mechanisms of IP10 in liver regeneration and repair.
Material and Methods: IP10-/- and CXCR3-/- mice models with or without fatty liver were subjected to partial hepatectomy (50%) and hepatic ischemia/reperfusion injury. Mouse hepatocyte proliferation was assessed at day 2, 4 and 7 by PCNA staining. Hepatic mRNA expression profiles of PCNA, hepatocyte growth factor (HGF), and Yes-Associated protein 1 (YAP1), Cyclin E1, Cyclin E2, Cyclin A2, MCM2, MCMBP and other cell cycle associated genes. Intragraft expression of IP10, CXCR3 and YAP1 mRNA were evaluated in small-for-size rat orthotopic liver transplantation model and further validated in clinical samples.
Results: Proliferation of hepatocytes in non-fatty liver is significantly higher compared with fatty liver. Wild type mice of the non-fatty liver group also exhibited significantly higher number of PCNA positive hepatocytes in comparison to IP10-/- and CXCR3-/- groups on day 2 (14.7/HPF vs 1.1/HPF vs 1.4/HPF respectively, $p \leq 0.005$), day 4 (5.4/HPF vs 3/HPF vs 2.6/HPF respectively, $p \leq 0.01$) and on day 7 (3.8/HPF vs 1.1/HPF vs 2.6/HPF respectively, $p \leq 0.5$). A significant decrease in YAP1 expression is found in IP10-/- and CXCR3-/- compared to wild type in the non-fatty group ($p \leq 0.005$) while different expression patterns were presented in the fatty group. No significant difference was found in expressions of Cyclin D1, Cyclin E2 and Cyclin A2.
Conclusion: IP10 and its receptor CXCR3 play critical roles in liver regeneration through regulating Yap1 signaling.



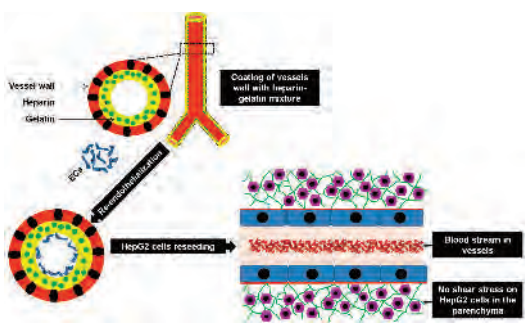
References:
[1] Geng et al. Oncotarget 2015
[2] Ling et al. The Journal of Hepatology 2014
[3] Bone-Larson et al. The Journal of Immunology 2001

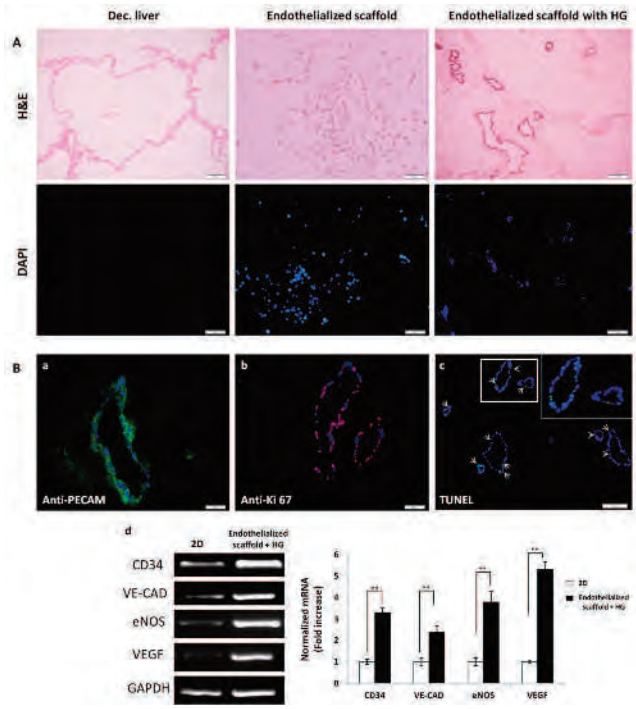
328.7

Heparin-gelatin mixture improves vascular reconstruction efficiency and hepatic function in bioengineered liver

Kamal Hussein[1,2], Kyung M.Park[1,2], Yun S.Lee[1], Kyung S.Kang[3], Heung M.Woo[1,2,4].
[1]Stem Cell Institute, Kangwon National University, Chuncheon, Korea; [2]College of Veterinary Medicine, Kangwon National University, Chuncheon, Korea; [3]College of Veterinary Medicine, Seoul National University, Seoul , Korea; [4]Harvard Stem Cell Institute, Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, United States.

Introduction: Whole organ decellularization is a cell removal process that creates a natural extracellular matrix for use in transplantation. A lack of an intact endothelial layer in the vascular network of decellularized organs results in blood clotting even with anti-coagulation treatment. Furthermore, shear stress caused by blood flow may affect reseeded parenchymal cells. We hypothesized that a heparin-gelatin mixture (HG) can act as an antithrombotic coating reagent and induce attachment and migration of endothelial cells (ECs) on vascular wall surfaces within decellularized livers, with subsequent parenchymal cell function enhancement.
Materials and Methods: Portal vein (PV) perfusion was performed for right lateral lobe decellularization of porcine livers. We tested if HG-precoating of isolated decellularized PV could increase EC attachment and migration. Additionally, we coated PV and hepatic artery walls in decellularized liver with HG, and then repopulated it with ECs and maintained it under vascular flow in a bioreactor for 10 days. Re-endothelialized scaffolds were perfused with porcine blood for thrombogenicity evaluation. We then co-cultured hepatocellular carcinoma (HepG2) cells and ECs to evaluate the effect of endothelialization on parenchymal cells. Finally, we transplanted these scaffolds heterotopically in pigs.
Results: HG improved ECs' ability to migrate and adhere to vessel discs. ECs efficiently covered the vascular compartments within decellularized scaffolds and maintained function and proliferation after HG-precoating. No thrombosis was observed after 24 h blood perfusion in HG-precoated scaffolds, indicating a fully endothelialized vascular tree. HepG2 cells displayed a higher function in scaffolds endothelialized after HG-precoating compared to uncoated scaffolds *in vitro* and after *in vivo* transplantation.
Discussion and Conclusion: We have shown that re-endothelialization of a porcine liver scaffold after precoating of blood vessels using HG can be achieved. Blood vessel surface modification can improve ECs attachment and migration with anti-platelet prosperity. *In vivo* results indicated that no coagulation occurred and parenchymal cell functions were maintained. Our results are promising to the pursuit of generating a fully functional human-sized tissue engineered liver scaffold with non-thrombogenic, patent vessels that can be used as liver assist-systems or for *in vivo* transplantation. This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ0110022015)", Rural Development Administration, Republic of Korea.





This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ0110022015)", Rural Development Administration, Republic of Korea

328.8

Supercharging mesenchymal stem cells with Interleukin-17A: An alternative to interferon-gamma enhancement of mesenchymal stem cell immunomodulation

Kisha Sivanathan[1,2,3], Darling Rojas-Canales[1,2], Stan Gronthos[4,5], Shane Grey[6], Patrick T.Coates[1,2,3,7].
[1]Centre for Clinical and Experimental Transplantation, Royal Adelaide Hospital, Adelaide, Australia; [2]School of Medicine, Faculty of Health Sciences, University of Adelaide, Adelaide, Australia; [3]Centre for Stem Cell Research and Robinson Institute, School of Medical Sciences, University of Adelaide, Adelaide, Australia; [4]Mesenchymal Stem Cell Group Laboratory, School of Medicine, Faculty of Health Sciences, University of Adelaide, Adelaide, Australia; [5]South Australia Health and Medical Research Institute , Adelaide, Australia; [6]Transplant Immunology Group, Garvin Institute of Medical Research, New South Wales , Australia; [7]Central Northern Adelaide Renal Transplantation Service (CNARTS), Royal Adelaide Hospital, Adelaide, Australia.

Human bone marrow derived mesenchymal stem cells pre-treated with interleukin-17A (MSC-17) are superior T cell immunomodulators for allotransplantation. We have previously shown that human MSC-17 potently suppressed PHA-induced T cell proliferation (3H-thymidine) and T cell activation (downregulated CD25, IFN-γ, TNF-α, IL-2) by inducing Tregs expressing functional Treg markers (CD39, CD73, CD69, OX40, CTLA-4, GITR). Here we aimed to identify potential mechanisms by which human MSC-17 mediate their superior immunomodulatory function. Untreated-MSC (UT-MSC), IFN-γ treated MSC (MSC-γ) and MSC-17 were assessed for their gene expression profile (microarray, 3 human MSC donors). Significantly regulated genes ($p<0.05$, fold change (FC)<-2 or >2) were identified for their biological functions (Database for Annotation, Visualisation and Integrated Discovery, DAVID). Microarray analysis identified 1278 differentially regulated genes (902 upregulated; 376 downregulated) between MSC-γ and UT-MSC; and 67 genes (39 upregulated; 28 downregulated) between MSC-17 and UT-MSC. MSC-γ were enriched for gene sets involved in the regulation of programmed cell death, apoptosis, TGF-β receptor signaling pathway (eg. FMOD. CCL2, MAPK3K1, SMAD6, GDF15 and TGFB2) and in positive regulation of defense response (eg. IL-6, TLR3, TLR4 and IDO). MSC-γ genes were also enriched for cell migration (eg. CXCL10, CXCL16, ICAM1, IL-6 and VEGFA). Despite the enhanced immunosuppressive and migratory properties, MSC-γ therapy is likely to be immunogenic in allogeneic hosts. MSC-γ exhibited the highest enrichment of genes associated with antigen processing and presentation via MHC class I. Enriched genes sets included aminopeptidases that hydrolyses antigenic peptides for MHC class I peptide binding and antigen presentation (eg. ERAP1 and ERAP2), peptide transporter genes (eg. TAP2) and other genes involved in the antigen processing and presentation pathways (eg. TAPBL, B2M and CD74). Other biological processes enriched in MSC-γ included immune response activation, humoral response and complement pathways activation. This microarray data is consistent with the upregulation of MHC molecules in MSC-γ (flow cytometry) and in other studies showing increased MSC-γ immunogenicity in vivo. MSC-17 genes were associated with chemotaxis responses, which may be involved in T cell recruitment for MSC-17 immunosuppression. MSC are known to express MMP molecules with chemotaxis and immunosuppressive properties. MMP13 was highly expressed specifically in MSC-17 (FC 15.6) and was validated by RT-PCR. Hence, MMP13 may mediate the superior immunomodulatory function of MSC-17. MSC-17 represent a potential cellular therapy to suppress immunological T cell responses in allotransplantation, with minimal immunogenicity. Studies on the functional role of the key candidate molecule MMP13 in MSC-17 immunomodulation are currently underway.

References:
[1] Sivanathan KN, Rojas-Canales D, Hope CM, et al. Interleukin-17A induced human Mesenchymal Stem Cells are superior modulators of immunological function. Stem Cells. 2015.

328.9

An increase in CD8+ effector memory T cells precedes rejection in a primate cardiac transplant model

Natalie A. O'Neill, Tianshu Zhang, Wenji Sun, Gheorghe Braileanu, Ivan Tatarov, Wessam Hassanein, Selin Sendil, Richard NPierson, Agnes M.Azimzadeh.
Department of Surgery, University of Maryland, Baltimore, MD, United States.

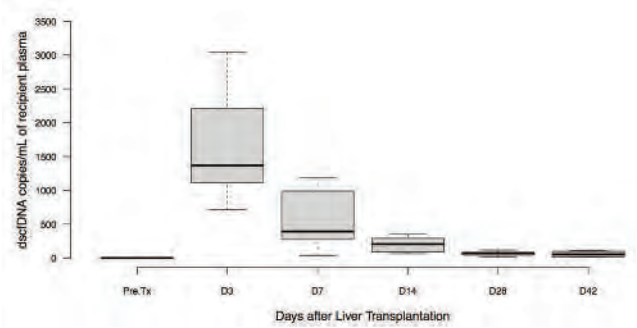
Purpose: Costimulation blockade prolongs graft survival but does not induce tolerance in primates. The mechanisms of rejection are incompletely understood, but costimulation blockade resistant rejection may be due to $CD28^-$ T cells. Here we evaluate changes in the proportion of $CD28^-$ T cells and naïve, central memory, and effector memory T cell populations over time in a non-human primate (NHP) transplant model in the context of αCD28, αCD40, and αCD80/86 immunomodulation.
Methods: Cynomolgus heterotopic (intraabdominal) cardiac transplant recipients were treated with either αCD80/86 (Belatacept, n=3), αCD28 (FR104, n=7), αCD40 (2C10R4, n=6), or αCD28 + αCD40 (n=2) for 90 days. Primary graft survival was defined by diminished pulse pressure or a drop in heart rate (by telemetry), and confirmed by histology. Peripheral blood $CD4^+$ and $CD8^+$ T cells were analyzed for CD45RA, CD95, CD28, and CD62L using flow cytometry. $CD4^+$ and $CD8^+$ T cells were categorized as naïve (N: $CD45RA^+CD62L^+$ or $CD95^-CD62L^+$ phenotype), central memory (CM: $CD45RA^-CD62L^+$ or $CD95^+CD62L^+$), or effector memory (EM: $CD45RA^{+/-}CD62L^-$ or $CD95^{+/-}CD62L^-$).
Results: Survival of grafts treated with αCD80/86 (median 122 days, 8 – 125), αCD28 (164 days, 52 – 181), αCD40 (125 days, 90 – 179), and αCD28 + αCD40 (138 days, 102 – 172) was similar. Proportion of $CD8^+CD28^-$ T cells increased from baseline to day of rejection in the αCD28 (1.66±0.43) and αCD40 (1.42±0.77) groups, but not with αCD80/86 (0.75±0.44; p=0.24) therapy. CM $CD4^+$ and $CD8^+$ T cells were a low proportion and exhibited minimal changes in these treatment groups. Two EM distribution patterns were found at baseline, with either a low (n=4, $CD45RA^{+/-}CD62L^-$; mean: 38.9%, SD±4.9) or high (n=10, $CD45RA^{+/-}CD62L^-$; 65.1%±8.8; p=0.0001) proportion of EM among $CD3^+CD8^+$ T cells. The proportion of T cell subsets was similar whether phenotyping was based on CD45RA or CD95. The proportion of $CD8^+$ EM increased significantly from baseline (58.3%±14.6) to time of rejection (75.3%±11.2; $p<0.0001$) across all drugs and profiles. Two of the four low CD8+ EM phenotypes switched to a high phenotype within three weeks of transplantation and subsequently experienced rejection during immunomodulation therapy, but no correlation was observed between baseline $CD8^+$ EM proportion and graft survival.
Conclusions: $CD8^+$ effector T cells significantly increased before graft rejection, and $CD8^+CD28^-$ T cells trended upwards in the αCD28 group. Monitoring these phenotypic changes could allow for early intervention prior to allograft rejection, and specifically targeting this "escape" mechanism of co-stimulation-independent effector T cells may help promote tolerance in transplantation.

328.10

Donor-specific cell-free DNA as a non-invasive marker of organ rejection after liver transplantation: A pilot study

Su Kah Goh[1,2], Hongdo Do[2], Vijayaragan Muralidharan[1], Alexander Dobrovic[2], Christopher Christophi[1].
[1]Department of Surgery, Austin Health, Melbourne, Australia; [2]Translational Genomics and Epigenomics Laboratory, Olivia Newton-John Cancer Research Institute, Melbourne, Australia.

Introduction: Despite modern immunosuppressive agents, up to 20% of patients will develop an episode of rejection in the first 12 months after liver transplantation (LTx)[1]. Conventional means to diagnose organ rejection is either inaccurate or invasive. Clinical findings and liver function tests are non-specific and do not necessary correlate with the severity of organ rejection[2]. Although liver biopsy is the gold standard for the diagnosis of organ rejection, this procedure is invasive and carries reasonable risks[3]. Better biomarkers for the non-histological diagnosis of organ rejection are required. Recent studies have proposed the use of donor-specific circulating cell-free DNA (dscfDNA) as a non-invasive marker of organ rejection[4],[5]. Unlike current methodologies adopted to evaluate dscfDNA, we have developed a novel digital PCR approach based on deletion/insertion polymorphisms to accurately measure dscfDNA levels. Using this technique, we monitored a cohort of patients who underwent LTx.
Materials and Methods: Eight patients who underwent LTx were prospectively recruited. Genotyping of a set of deletion/insertion polymorphisms was performed to identify donor-specific alleles. Droplet digital PCR was then utilized for the precise serial quantification of dscfDNA in the circulation of the recipient. Levels of dscfDNA were measured in pre-transplant and post-transplant bloods for each recipient at days 1, 3, 7, 14, 28 and 42. Findings were correlated with routine liver function tests and clinicopathological factors.
Results and Discussion: Levels of dscfDNA were reflective of graft health. In six recipients who underwent uneventful transplantation, levels of dscfDNA markedly reduced within 3 days and rapidly plateaued to a very low level 7 days after LTx. We also found that dscfDNA levels were independent of cholestasis in a separate recipient. On the other hand, dscfDNA levels significantly increased in a patient who developed an episode of acute cellular rejection 7 days after LTx. These findings of dscfDNA as a biomarker for organ rejection after LTx are promising. Furthermore, as the measurement of dscfDNA is attainable under 5.5 hours, our workflow will be supportive of same-day clinical decision-making.
Conclusion: In this pilot study, we demonstrated the robustness of our methodology for the detection and quantification of dscfDNA. We also highlighted the potential of dscfDNA as a clinically applicable biomarker of organ rejection. However, larger validation studies are required to confirm the diagnostic performance of dscfDNA, especially in the setting of other common post-transplant complications.



Victorian Liver Transplantation Unit, Australia; DonateLife, Australia; Professor Robert Jones; Professor Peter Angus

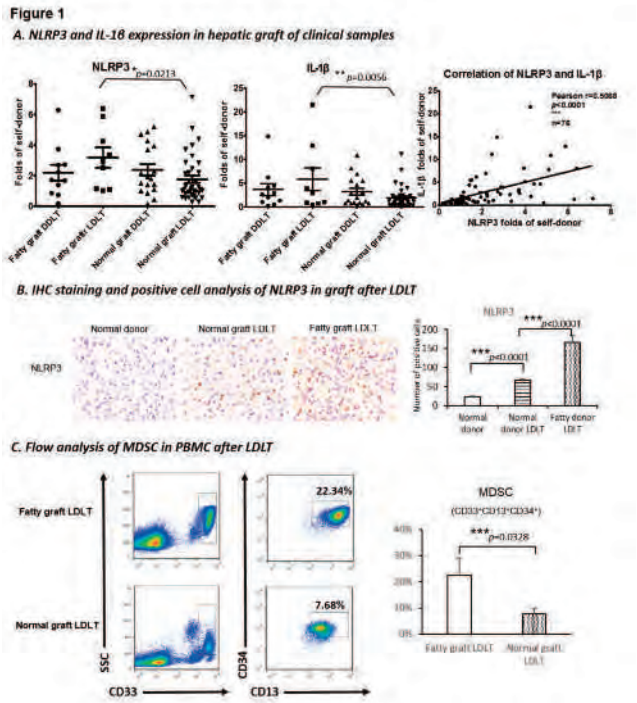
References:
[1] Lerut J, Mathys J, Verbaandert C et al. Tacrolimus monotherapy in liver transplantation: one-year results of a prospective, randomized, double-blind, placebo-controlled study. Annals of surgery 2008; 248: 956.
[2] Hickman PE, Potter JM, Pesce AJ. Clinical chemistry and post-liver-transplant monitoring. Clinical Chemistry 1997; 43: 1546.
[3] Grant A, Neuberger J. Guidelines on the use of liver biopsy in clinical practice. Gut 1999; 45: iv1.
[4] Vlaminck I De, Valantine HA, Quake SR, Khush KK. Circulating Cell-Free DNA Enables Noninvasive Diagnosis of Heart Transplant Rejection. Science translational medicine 2014; 6: 333.
[5] Beck J, Bierau S, Balzer S et al. Digital Droplet PCR for Rapid Quantification of Donor DNA in the Circulation of Transplant Recipients as a Potential Universal Biomarker of Graft Injury. Clinical Chemistry 2013; 59: 1732.

328.11

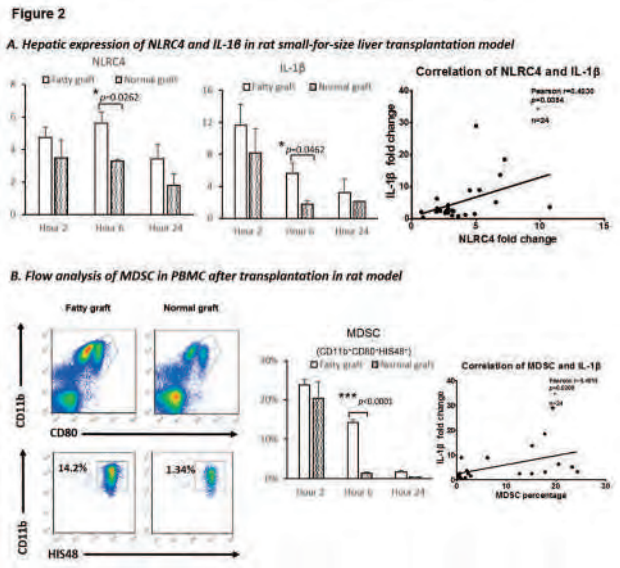
Inflammasome exacerbated fatty graft injury with accumulation of myeloid-derived suppressor cells after living donor liver transplantation

Hui Liu, Nancy Kwan Man, Jiang Liu, Oscar Wai Ho Yeung, Yuen Yuen Ma, Yan Shao, See Ching Chan, Chung Mau Lo.
Surgery, The University of Hong Kong, Hong Kong, Hong Kong.

Introduction: Acute phase fatty liver graft injury will deteriorate when graft is small-for-size, which is common in living donor liver transplantation (LDLT). Inflammasome plays critical roles in inflammation initiation. Hepatic steatosis promotes myeloid-derived suppressor cells (MDSC) accumulation. Here, we aimed to explore the regulatory role of inflammasome on MDSC in fatty graft injury after LDLT.
Materials and Methods: Expression pattern of inflammasome and its association with IL-1β was investigated in 76 transplant recipients and a rat orthotopic liver transplantation model using small-for-size fatty graft. The regulatory role of inflammasome on MDSC accumulation and mobilization was examined both in rat model and in vitro.
Results and Discussion: Clinically, the expressions of NLRP3 and IL-1β in fatty living graft were significantly higher and associated with poor liver function after transplantation. Intragraft NLRP3 positive cells were associated with more neutrophils infiltration in fatty living graft ($p<0.0001$). The circulatory MDSC in fatty graft LDLT group was significantly accumulated.



In rat model, hepatic expression of NLRC4 and IL-1β was significantly increased in fatty graft group. NLRC4 overexpression was positively correlated with IL-1β and poor liver function. Consistently, MDSC was also increased in fatty graft group and correlated with IL-1β expression ($p=0.02$).IL-1β administration induced the MDSC mobilization.
Conclusion: Inflammasome, associated with MDSC accumulation, played an important role in exacerbating acute phase inflammatory injury in fatty graft after LDLT.



328.12

Optimizing immunomodulatory properties and immunogenicity of MSC for immunotherapy following organ transplantation

Samantha de Witte[1], Marcella Franquesa[1], Tanja Strini[1], Sander S.Korevaar[1], Franka Luk[1], S. J.Elliman[2], P. N.Newsome[3], M. Gargesha[4], D. Roy[4], Ana M.Merino Rodriguez[1], Carla C.Baan[1], Martin J.Hoogduijn[1].
[1]Nephrology and Transplantation, Department of Internal Medicine , Erasmus MC, Rotterdam, Netherlands; [2]Orbsen Therapeutics Ltd, Galway, Ireland; [3]Department of NIHR Liver Biomedical Research Unit and Centre for Liver Research , University of Birmingham, Birmingham, United Kingdom; [4]BioInVision Inc., Mayfield Village, OH, United States.

Mesenchymal stromal cells (MSC) are attractive candidates for immunotherapy after organ transplantation as well as targeting immune mediated disorders. MSC contain promising immunomodulatory capacities and are low immunogenic, due to low MHC I and lack of MHC II expression. These properties are favorable for their applicability as immunotherapy, however there is evidence that MSC are more immunogenic than previously thought. Design strategies which improve these qualities would be advantageous for their application within the transplantation field. This study aims to optimize MSC into less immunogenic cells with improved immunomodulatory capacities.
Umbilical cord-derived MSC were treated for 3 days *in vitro* under various conditions, i.e. in the presence of pro-/anti-inflammatory cytokines, vitamins and serum-deprivation. Their immunogenicity and immunosuppressive capacity were examined by gene-expression analysis, surface-marker expressions, IDO-activity and inhibition of Tcell proliferation. Subsequently, the susceptibility to NK cell and CD8 Tcell lysis was investigated via CD107a expression. Furthermore, the immunomodulatory capacity as well as the survival of the treated MSC was examined in a CCL4-induced liver disease mouse model.

Treatment	Immunogenicity		Immune modulation	
IFNγ	↑ HLA type-I ↑ HLA type-II ↑ protection NK lysis	** * *	↑ PD-L1 ↑ IDO activity ↑↑ inhibition CD4 and CD8 proliferation	*** * *
IFNβ	↑ HLA type-I ↑ Protection NK lysis	* *	↑ PD-L1	*
TGFβ1	↑ Protection NK lysis	*	↑ PD-L1 ↑ inhibition CD4 and CD8 proliferation	*
Serum deprivation (Starv)	↓ HLA type-II	**	↓ PD-L1 ↑ inhibition CD4 and CD8 proliferation	*
Vitamin B6 (VitB6)	↑ HLA type-I ↑ HLA type-II	** *		
Retinoic Acid (RA)			↑ inhibition CD4 and CD8 proliferation	
Starvation + Vitamin B6 (StarvVitB6)	↑ HLA type-II	**	↑ inhibition CD4 and CD8 proliferation	

Table 1. Effects of treatments on ucMSC for three days compared to unstimulated ucMSC. Significance levels p<0.5 (*), p<0.01 (**) and p<0.005 (***)

In vitro results show increased immunomodulatory capacity of IFNγ-, IFNβ- and TGFβ- treated MSC. IFNγ-MSC were the most potent inhibitors of Tcell proliferation plus their IFNγ production. Furthermore, increased HLA levels were observed in IFNγ-, IFNβ-, VitB6- and StarvVitB6- treated MSC. These MSC were significantly protected against NK lysis, which correlates to increased HLA type I levels. In addition, TGFβ-MSC were significantly protected against NK lysis while HLA levels remained unchanged. Table 1 displays *in vitro* effects of the most prominent factors on immunogenicity and immunomodulatory capacity. *In vivo*, TGFβ-MSC had improved persistence after infusion while clearance of VitB6- and StarvVitB6-treated MSC was accelerated compared to untreatedMSC. Nonetheless, *in vivo*, a trend of improvement in liver function after TGFβ- and Starv-treated MSC administration was observed.
These data show the versatility of MSC to culture conditions and the possibility of optimizing MSC into less immunogenic cells with improved immunomodulatory properties, which is important for further application of MSC as immunotherapy for immune mediated disorders and after organ transplantation.

329.1

Transplantation of graft from older donors: does age still matter?

Nicholas Gilbo, Ina Jochmans, Mauricio Sainz, Jacques Pirenne, Diethard Monbaliu.
Abdominal Transplantation Surgery and Coordination, University Hospitals of Leuven, Leuven, Belgium.

Background: Improved outcome after Liver Transplantation (LT) has led to an imbalance between patients waiting and availability of suitable grafts. As an attempt to reduce this gap, selection criteria have become less restrictive including elderly donors (>70y). However, registry data have previously raised concerns that advanced donor age is associated with impaired graft and recipient survival.
Methods: We retrospectively compared donor and recipient demographics, transplant and outcome data between recipients of liver grafts procured from donors >70y (D>70) and <70y (D<70) in a cohort of 643 consecutive LT in (01/2003-12/2014). The influence of LT of elderly grafts on recipient and graft survival was investigated by means of multivariable regression. Data presented as median and IQR.
Results: 98/643 (15%) LT were performed with grafts procured from D>70. D>70 [71-90y] were all brain dead and when compared to D<70, peak AST/ALT was lower, most were locally procured (71.4% vs 45.3%, p<0.0001), had shorter graft extraction time [30min (24-36) vs 38min (28.5-50), p<0.0001] and higher DRI [2.6 (2.4-2.8) vs 2.1 (1.7-2.4), p<0.0001]. Recipients of D>70 were older [62y (56-68) vs 57y (48-64), p<0.0001] with similar MELD but higher BAR score [4 (2-4) vs 2 (1-4), p<0.0001] and more frequently transplanted for HCC (44.9% vs 28.3%, p=0.002). Cold ischemia [7.24h (6-9.5) vs 7.98h (6.2-9.5), p:0.1] and anastomotic time [80min (67-91) vs 78min (67-90), p=0.3] was similar. Peak AST post-LT was lower in D>70 [527IU/L (298-1071) vs 721IU/L (383-1386), p=0.025]. No difference in early allograft dysfunction (D>70 26.3% vs D<70 28.9%, p=0.71) or acute kidney injury (D>70 22.7% vs D<70 22.7%, p=1) was observed. The incidence of non-anastomotic biliary strictures within 1y post-LT was lower in D>70 (6.2% vs 13.4%, p=0.045). One and five year graft survival were similar between D>70 and D<70 (89.7% vs 87.7%, 73.5% vs 70.6% respectively, p=ns); likewise 1 and 5 y patient survival did not differ (92.8% vs 91.1%, 76.1% vs 75.9% respectively, p=ns). Multivariable Cox regression did not reveal an impact of donor age on patient and graft survival (D>70 HR:0.95, 95%CI:0.55-1.63 and HR:0.78, 95%CI:0.46-1.33) in contrast to CIT which was an independent risk factor of impaired recipient survival (HR: 1.11, 95%CI:1.04-1.2, p=0.004).
Conclusion: In our experience, transplantation of grafts >70y did not increase post-LT morbidity and achieved survival comparable to that one reached by younger grafts in the long-term. Careful selection of graft from D>70 to be procured by experienced team and stable recipients seems essential to safely use these elderly grafts.

329.2

Dynamic Liver Compliance- A new radiological metric to predict peri-operative course of donor hepatectomy

Mettu Reddy, Abdelrrhaim Dabora, Sripriya Srinivas, Ilankumaran Kaliamoorthy, Rajesh Rajalingam, Gomathy Narasimhan, Venugopal Kota, Mohamed Rela.
Institute of Liver Disease & Transplantation, Global Health City, Chennai, India.

Background: Minimising donor morbidity remains the cornerstone of successful LDLT. The most important variable predicting donor outcome after right lobe donation is how the partial liver remnant copes with portal hyperperfusion. Multiple biochemical, radiological and histological parameters have been used to evaluate this aspect. We devised a new radiological metric- dynamic liver compliance (ΔLV) and evaluated its utility in predicting peri-operative course after donor right hepatectomy.
Methods: Potential liver donors underwent contrast enhanced triple phase CT as part of standard donor assessment. Donors underwent plain CT after overnight fasting. This was followed by intravenous infusion of normal saline (10ml/kg) over 30 minutes. The patient then underwent standard triphasic CT after intravenous contrast injection. Liver volumes in the plain scan and the hepatic portal phase were compared. Dynamic liver compliance (ΔLV) was calculated as (Portal phase volume- Plain volume)*100/Plain volume. The intra-operative and post-operative course of these donors was prospectively followed. Relationship between ΔLV and standard intra-operative and post-operative variables was explored.
Results: 16 donors completed the protocol CT and proceeded to right lobe donation. Post hydration volumes increased by a mean of 82±56ml. Mean dynamic liver compliance (ΔLV) was 5.9%±3.4%. ΔLV showed significant negative correlation (Pearson's correlation) with intra-operative blood lactate levels (p=0.004) and day 1 transaminase levels (AST: p=0.044, ALT: p=0.033). Donors with ΔLV less than 6% had significantly higher end intra-operative blood lactate (3.4 vs 2.1, p=0.008) and day 1 transaminase levels (ALT: 151 vs 244, p=0.049, AST: 133 vs 214, p=.054). There was no difference in peak bilirubin, peak INR, ICU or hospital stay or morbidity between the two groups.
Discussion: Donors who have a compliant liver as measured by a high ΔLV appear to have better intra-operative blood lactate and post-operative transaminase profiles, suggesting lesser peri-operative hepatocyte injury. In this pilot study, ΔLV appears to be an interesting new metric to predict peri-operative course of liver donors. Larger studies are necessary to identify the ability of this metric to predict clinical course of these donors.
Conclusion: Dynamic Liver Compliance (ΔLV) has the potential as a new non-invasive radiological metric to predict donor liver quality & the peri-operative course after donor hepatectomy.

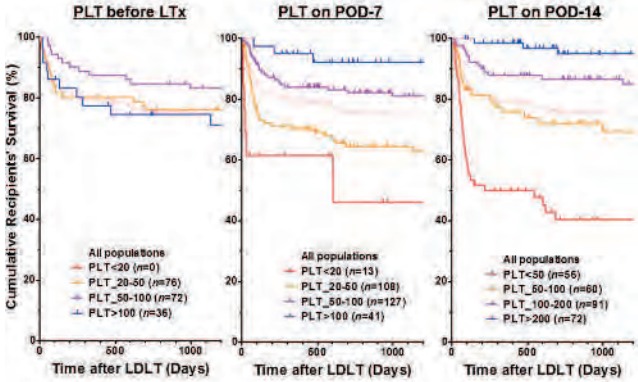
329.3

Post-operative thrombocytopenia determines the fate of adult-to-adult living donor partial liver transplantation; Proposal of "Thrombotic Microangiopathy (LTx-TMA) Score"

Koichiro Hata[1], Hirokazu Tanaka[1], Kayoko Endo[2], Hirofumi Hirao[1], Yusuke Okamura[1], Toyonari Kubota[1], Osamu Inamoto[1], Ichiro Tamaki[1], Yermek Nigmet[1], Hideaki Okajima[1], Toshimi Kaido[1], Akira Shimizu[2], Shinji Uemoto[1].
[1]Department of Surgery, Division of Hepato-Biliary-Pancreatic Surgery and Transplantation, Kyoto University Graduate School of Medicine, Kyoto, Japan; [2]Institute for Advancement of Clinical and Translational Sciences, Kyoto University Hospital, Kyoto, Japan.

Background: It has recently been reported that peri-operative thrombocytopenia may predict the outcome of liver transplantation (LTx), however, the underlying pathophysiologies have not been elucidated. Here we propose the concept of "LTx-associated thrombotic microangiopathy (LTx-TMA) ", as a crucial pathology in LTx.
Methods: We retrospectively analyzed the clinical characteristics/data in the consecutive 290 cases of primary adult-to-adult living donor partial LTx (LDLT) in our single center between April 2006 and March 2013, with special interest to the diagnostic criteria of TMA, as follows: i) thrombocytopenia (<50,000/mm), ii) hemolytic anemia (Hb<8.0g/dl), iii) LDH elevation (>500 IU/L), and iv) manifestation of schistocytes. Baesd on these, we calculated "LTx-TMA" score, as detailed in the lower table in Fig.2, which was analyzed in correlation with recipients' survival.
Results: Among various clinical parameters, only post-LTx platelet count was significantly associated with 6-months mortality. Moreover, patients' survival was clearly deteriorated according to the degree of thrombocytopenia (p<0.0001, Fig.1). Regarding the TMA criteria, thrombocytopenia occurred in 253 out of 290 cases (87.2%), hemolytic anemia was observed in 271 (93.4%), LDH elevation in 166 (57.2%), and schistocytes in 205 (70.7%). Of note, 95.2% (276 cases) exhibited at least 2 characteristics of TMA, and surprisingly, 112 cases (38.6%) met all 4 criteria by 60 post-operative days, indicating that almost all LDLT cases were accompanied by TMA-like disorder. Furthermore, the recipients' survival in 21 cases whose LTx-TMA score was only 0 point (4 cases) or 1 (17 cases) was 100% with significantly less morbidity and shorter hospital stay, while 17 out of 25 cases (68%) whose score reached 7 points died within 3 months after surgery (p<0.0001). As summarized in Fig.2, the higher the score was, the more deteriorated the patients' survival was.

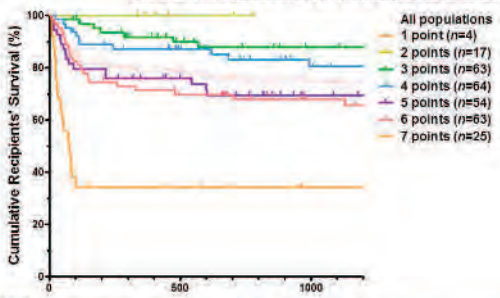
Fig.1 ***Post-operative thrombocytopenia determines Patients' Survival after LDLT***



Discussion: Almost all ADAMTS13 is produced by hepatic stellate cells, therefore, lowered activity of ADAMTS13 is always concurrent with up-regulation of von Willebrand factor (vWF) multimers just after

LTx. This imbalance, in turn, leads to platelet thrombus formation both in hepatic and in systemic microcirculation, resulting in prolonged dysfunction of transplanted livers, as well as in functional disorder of remote organs. Thus, we would like to propose such critical pathology as "LTx-TMA", which seems not only to be an important predictor of prognosis, but to be a novel therapeutic target for improving high mortality and morbidity after LDLT.

Fig.2 ***Impact of "LTx-TMA Score" on Patients' Survival after LDLT***



LTx-TMA Score

Points	Platelet Counts ($x10^3$/L)	Schistocytes	Hemolytic anemia	LDH elevation
3	<20			
2	20≤ , <50	+		
1	50≤ , <100		+	+
0	≥100	-	-	-

329.4

Impact of the Share 35 liver allocation policy on candidates with MELD exception scores

Elaine Cheng[1,2], Matthew Everly[2,3], Douglas Farmer[1], Fady Kaldas[1], Ali Zarrinpar[1], Vatche Agopian[1], Ronald Busuttil[1].
[1]Division of Liver and Pancreas Transplantation, University of California Los Angeles, Los Angeles, CA, United States; [2]Terasaki Research Institute, Los Angeles, CA, United States; [3]Department of Medicine, University of California Los Angeles, Los Angeles, CA, United States.

Introduction: Liver allocation policies in the United States have typically favored candidates listed with MELD exception scores, and the use of exceptions has increased yearly since the inception of the MELD era. A change to the allocation system was implemented on June 18, 2013 which mandates the regional sharing of deceased donor (DD) livers for candidates with MELD scores of 35 or higher. Our aim is to investigate the impact of the Share 35 policy on liver transplant (LT) candidates with exception points, with a focus on patients with hepatocellular carcinoma (HCC).

Methods: We reviewed the 73,508 LT candidates added to or removed from the United Network for Organ Sharing (UNOS) waitlist between June 18, 2010 and June 5, 2015. Candidates listed for multiple organs (except liver-kidney), pediatric patients and those with fulminant liver failure are excluded, and the remaining 67,119 candidates are included in the analysis. Patient data and donor characteristics are analyzed on a yearly basis (Years 1-5).

Figure. Reasons for Removal from the Waitlist by Year in the PRE-Share 35 and POST-Share 35 eras for **(A)** All waitlisted candidates and **(B)** Candidates with HCC exceptions only.

Results: The proportion of candidates added to the waitlist with exception scores have increased each year (21.5% in Year 1 vs. 24.4% in Year 5), with approximately 70% of the exception candidates carrying the diagnosis of HCC. The percentage of DD organs being used for exception cases have also increased over time (34.4% in Year 1 vs. 41.3% in Year 5). Candidates with HCC exceptions are more likely to receive DD transplants compared with the overall waitlisted population. After implementation of the Share 35 policy, however, a lower percentage of HCC candidates received DD transplants (82.9%

POST vs. 84.8% PRE, P=0.017), while a higher percentage died or experienced medical deterioration while on the waitlist (7.4% vs. 6.2%, P=0.032). These observations are in contrast to the overall cohort which has witnessed higher transplant rates and a lower waitlist mortality since Share 35 has taken effect.

Time on the waiting list for HCC candidates has increased with each given year. There is a significant rise in the proportion of HCC candidates with allocation MELD scores (including exception points) ≥35 at the time of LT in the POST-Share 35 era (2.5% vs. 0.8%, P<0.001), and a decrease in the proportion of HCC patients transplanted within 6 months of listing (45.7% vs. 50.5%, P<0.001). Additionally, high-risk donor organs are being preferentially used in exception cases, as shown by the increased median donor risk index in the POST-Share 35 era (1.40 vs. 1.38, P=0.010). Nevertheless, 1-year patient and graft survival rates have not been affected with implementation of Share 35.

Table. Summary of deceased donor liver transplant recipients with HCC exceptions during the PRE-Share 35 (Years 1, 2, and 3) and POST-Share 35 (Years 4 and 5) eras. AMELD = allocation MELD score (with exception points); IQR = interquartile range.

	PRE-Share 35			POST-Share 35	
	Year 1	Year 2	Year 3	Year 4	Year 5
Median [IQR] AMELD at time of transplant	29 [22, 29]	29 [22, 29]	29 [22, 29]	29 [22, 29]	29 [22, 29]
Number [%] AMELD ≥35 at time of transplant	5 [6.2%]	10 [9.3%]	11 [9.5%]	12 [9.5%]	30 [9.5%]
Median [IQR] time on waitlist (days)	130 [64, 331]	181 [83, 363]	190 [84, 395]	193 [80, 378]	205 [104, 406]
Number [%] transplanted in 6 months	677 [51.4%]	712 [49.8%]	695 [46.4%]	661 [44.8%]	617 [45.1%]
Median [IQR] Donor Risk Index	1.38 [1.15, 1.67]	1.38 [1.15, 1.65]	1.35 [1.12, 1.65]	1.40 [1.15, 1.85]	1.39 [1.16, 1.70]
Allocation Type					
Local	1080 [81.8%]	1176 [82.2%]	1170 [81.8%]	1181 [81.4%]	1116 [77.8%]
Regional	198 [15.7%]	239 [20.0%]	202 [14.2%]	217 [15.0%]	227 [15.8%]
Median [IQR] distance travelled (miles)	50 [6, 147]	45 [6, 143]	42 [6, 142]	50 [6, 135]	49 [7, 149]
Median [IQR] cold ischemia time (hours)	6.2 [5.0, 8.0]	6.0 [4.7, 7.6]	6.0 [4.6, 7.6]	5.8 [4.5, 7.3]	5.8 [4.4, 7.3]

Conclusions: The Share 35 policy for liver allocation in the United States has reduced the relative advantage of MELD exception candidates, as evidenced by reduced transplant rates, a rise in waitlist mortality, prolonged waiting times, and the use of higher-risk donor organs. These recent changes have not affected short-term post-LT outcomes in these patients.

329.5

Live donor champion program for liver transplantation: Time to redefine the strategy

Jingzhou Wang [1], Melissa Donovan[1], Curtis Argo[2], Anita Sites[1], Daniel Maluf[1].
[1]Transplantation, UVA, Charlottesville, VA, United States; [2]Medicine, UVA, Charlottesville, VA, United States.

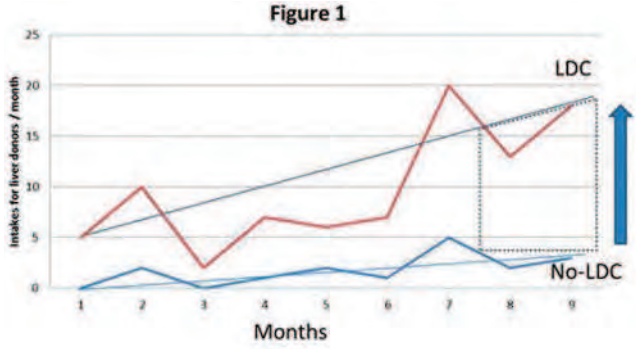
Background: Live Donor Champion (LDC) Programs promote the advocacy for living donor transplantation and has not been tested yet in liver transplantation. We conducted a prospective 9-month pilot trial of this novel educational intervention.

Patients and Methods: Patients evaluated for liver transplantation (LT) and without contraindications for living donor liver transplantation (LDLT) were invited to voluntarily attend the LDC training session designed to establish a collaborative approach from patients and health care team members (HCT), to develop an environment to promote donation, and test the feasibility of this pilot program. The LDC intervention includes (a): an initial face-to-face meeting (focus groups) with a presentation that enhances the knowledge on living donation, (b): personalized education provided by HCT to target all levels of health literacy, (c): follow-up phone calls for ongoing patient education, and video-conference consults. A historic control group was included. Metrics for evaluation of the intervention were established.

Results: From a total of 167 evaluated end stage liver disease (ESLD) patients, 72 were enrolled and 57 (79.2%) ESLD patients identified a care giver / donor champion interested to advocate for them. During this period of time, a total of 72 intakes (67 directed and 5 non-directed donors) were received on behalf of 38 participants (1.76 donors per ESLD patient). Data show that LDC program increased the number of intakes for liver donors during the study period (p-<.05). Furthermore, the number of completed donor evaluations increased.

Conclusion: LDC Programs improve patient knowledge of the live donor LT and increase access to living donation.

Figure 1



329.6

Factors associated with worse outcome in Korean split-liver transplantation: analysis of the 10-year Korean Network for Organ Sharing data base

Nam-Joon Yi, Sanghee Song, Ok-Kyung Kim, Hyeyoung Hong , Kyung Chul Yoon, Hyo-Sin Kim, Hyo-Sin Kim, Kwang-Woong Lee, Kyung-Suk Suh.
Surgery, Seoul National University College of Medicine, Seoul, Korea.

Background and Aim: Organ shortage has been a hot issue especially in the field of liver transplantation (LT) in Asian countries including Korea. In order to increase the donor pool, the policy of split LT (SLT) has been recently changed and recently number of SLT has been increased in Korea. However the outcome of SLT in Korea has not been reported.
Methods: This study evaluated the outcomes of SLT using the Korean Network for Organ Sharing (KONOS) between January 2005 and December 2014. Cases with two recipients from one deceased-donor were considered as SLT. A total of 200 cases of 100 pairs of SLT were examined.
Result: The recipient population was 107 adults and 93 children. The type of SLT composed of adult/children pair in 87 (87.0%). The median donor age and body weight were 25.0 (9 ~ 48) years and 66.3 (38 ~ 120) kg. Adult recipients' median age was 53.1 (25~77) years, and body weight 62.2 (35~101) kg. UNOS status 1 and 2A was 57 (53.3%) in adult recipients. The median donor / recipient weigh ratio (DRWR) was 1.12 (0.56 ~ 1.95); the DRWR < 1.0 was 37.4%. Pediatric recipients' median age was 2.82 (0~16) year and body weight was 13.2 (range, 3.6~55) kg. The median DRWR was 6.80 (1.09 ~ 17.04); the DRWR > 10.0 was 22.6 %. UNOS status 1 in pediatric recipients was 10 (10.8%). The overall patients' survival outcome was 75.5 %, but it was worse in adult recipients (67.3% vs. 84.9%) (Fig.1) ($p=0.004$). In adult recipients, prolonged prothrombin time (INR >1.5) of the donor and center sharing were poor prognostic factors in multivariate analysis ($p<0.05$).
Conclusion: Although the outcome of SLT in Korea was acceptable, the outcome was worse in urgent and big adult recipients, especially in cases of marginal donor with prolonged INR and low volume center. Further evaluation should be performed to make a good guideline for allocation of the deceased donor for SLT.

329.7

Principles from high volume living donor liver transplantation centre doubles the donor pool in deceased donor liver transplantation

Shishir Pareek, Nilesh Patil, Neerav Goyal, Subash Gupta.
Centre for Liver and Biliary Sciences, Indraprastha Apollo Hospital, New Delhi, India.

Aim: To study the contribution and need of LDLT experience in increasing the donor pool in DDLT.
Material and Method: Deceased donor transplantation is very uncommon in the south Asian countries due to ethical reasons.
With vast experience of over 1900 LDLT at our centre from Sept 2006 till date, steps helpful in havesting two Hemiliver in special circumstances from single deceased donor for two adult patients were analysed *without* the need for preoperative CT angiogram or intra-operative Doppler.
Results: LDLT differs from DDLT in following principles and Hemiliver grafts are utilized for donation.
1. Miticulous porta dissection with retrieval of first and second order vascular branches as inflow vessels.
2. Division of first and second order biliary radicles for anastomoses with recipient ductal opening with idea of maintaining a non obstructed biliary continuity in the remnant Hemiliver.
3. Miticulous bloodless insitu parenchymal transection is performed with- out the preoperative CT scan or intra-operative Doppler with the end point of keeping the MHV to Left lobe and retrieving sizeable reconstructable segment five and eight veins for anterior sector drainage of right lobe, hence two healthy grafts can be retrieved.
4. Main portal vein as well as CHD is kept with Right hemiliver whereas CHA is kept towards left hemiliver as greater anatomical variation of portal vein and biliary radicles are found in right Hemiliver and multiple segmental arterial branches are found in left Hemiliver.
5. The Inferior vena-cava along with caudate lobe is kept with the Left lobe.
6. Inflow vessels and ducts with small size and caliber are joined under magnification by keeping the recipient stumps long and with multiple openings with high porta dissection.
7. GRWR as low as 0.6 is acceptable with favourable recipient outcomes
Adult recipients with this insitu splitting technique had no difference in post-operative recovery and complication, day of normalization of LFT, coagulation profile, platelets, drain output, hospital stay.
Conclusion: Principles and experience from high volume LDLT centre can double donor pool in DDLT centre as well outcomes
Transplant surgeons in DDLT centres should have adequate exposure in LDLT setting as well.
More people will become motivated for organ donation as twice as many lives can be saved.

329.8

Changes in liver allograft steatosis and its impact on early graft function and long term survival

Jason Davis, Spencer Fuller, Chandrashekhar A.Kubal, Jonathan A.Fridell, A. J.Tector, Richard S.Mangus.
Department of Surgery, Transplant Division, Indiana University School of Medicine, Indianapolis, IN, United States.

Background: Deceased organ donor liver transplant allografts with steatosis have an increased risk of primary non-function and initial poor function post-transplant. A large percentage of donor livers have significant steatosis.Previous research suggests improvement in steatosis in the immediate post-transplant period. This study compares reperfusion and early post-transplant surveillance biopsies, and correlates the results with initial graft function and long-term outcomes.
Methods: Records of all liver transplants performed at a single center over a 14-year period were reviewed.The original biopsies were reviewed by experienced liver pathologists. Liver biopsies are obtained at the time of transplant and 3 days after transplant. Total steatosis is calculated as the sum of both micro- and macrovesicular steatosis, and is categorized into four study groups: (1) none (0%), (2) mild (<10%), (3) moderate (10-20%) and (4) severe (>20%). For this analysis, change in liver steatosis is calculated as moving from one study group to another. Early post transplant liver function is assessed by biochemical analysis of liver enzymes (alanine aminotransferase (ALT); liver injury), total bilirubin (TB; excretion), international normalized ratio (INR; synthesis). Long-term survival is assessed using Cox regression analysis.
Results: Data were available for 1572 adult subjects. Among the patients with steatosis, there was a significant and rapid decrease in steatosis.The median group change was greatest for severe steatosis groups (Group 3, >20%: -1.54; Group 2, 10 to 20%: -0.93; Group 1, 1 to 10%: -0.47 ($p<0.001$).Moderate and severe steatosis was associated with more acute liver injury ($p<0.05$ for days 1 to 6), and delayed graft function (higher TB and INR ($p<0.05$ on days 1, 3)). These values decreased for all study groups until they were similar by day 7 (ALT) and day 14 (TB and INR). Systemically, steatotic groups demonstrated an acute decrease in glomerular filtration rate from 1 to 3 days post transplant, ranging from -12 to -22% change, compared to only a -5% change for the nonsteatotic group. Graft survival was worse at all time periods for moderate and severe steatosis livers. Subgroup analysis was employed to identify groups that have a more dynamic decrease in steatosis. Those groups with better clearance of severe steatosis included recipients who were younger, more obese, male, and those with fatty liver disease.

Table 1 Study group demographics

		Transplant Liver Percent Steatosis at day 0				
	Overall	0% (none)	1-10% (mild)	11-20% (moderate)	> 20% (severe)	p-value
Number	1572	994 (63%)	382 (24%)	135 (9%)	61 (4%)	
Donor characteristics						
Age (years, median)	41	39	43	44	44	0.001
Male (%)	57%	56%	58%	58%	61%	0.87
Race (% white)	1278 (81%)	79%	84%	86%	87%	0.09
BMI (median)	26	25	28	30	28	<0.001

Table 2 Change in median liver steatosis from Day 0 to Day 3 post transplant

Day 0 biopsy		63%	24%	9%	4%	
Day 3 biopsy		71%	20%	6%	3%	
Median group change		0.19	-0.47	-0.93	-1.54	0.001

Conclusion: These results confirm a marked post-transplant decrease in allograft steatosis that occurs within 3 days of transplant. Subgroup analysis suggests that younger male patients who are obese or have fatty liver disease are more able to clear steatosis in this period. Allografts with moderate to severe steatosis have worse early injury, delayed graft function and worse early and late survival. Steatotic grafts are associated with a substantial acute decrease in renal function early post transplant.

Table 3 Subgroup Analysis (BMI, Age, Gender)

	Group Percent steatosis	Overall	0 0% (none)	1 1-10% (mild)	2 11-20% (moderate)	3 >20% (severe)	p-value
			994 (63%)	382 (24%)	135 (9%)	61 (4%)	
Recipient BMI							<0.001
< 25.0	Normal	-0.12	0.18	-0.5	-1.1	-1.2	
25.0 to 29.9	Overweight	-0.1	0.22	-0.43	-0.89	-1.5	
30.0 to 34.9	Obese	-0.22	0.15	-0.49	-1	-2.1	
> 35.0	Very obese	-0.09	0.26	-0.44	-0.67	-0.75	
Recipient age							<0.001
18 to 39 years		-0.05	0.19	-0.28	-1	-2	
40 to 59 years		-0.17	0.17	-0.49	-1.05	-1.6	
60 years and older		-0.08	0.25	-0.46	-0.68	-1.35	
Recipient gender							<0.001
Male		-0.018	0.18	-0.47	-0.95	-1.81	
Female		-0.04	0.22	-0.46	-0.87	-1.27	

329.9

Evaluation of the potential impact of inter-regional sharing for high MELD patients waiting for liver transplantation

Michael A. Fink[1,2], Glenda A.Balderson[3], Christopher Christophi[1], Robert M.Jones[1,2].
[1]Department of Surgery, Austin Health, The University of Melbourne, Melbourne, Australia; [2]Liver Transplant Unit Victoria, Austin Health, Melbourne, Australia; [3]Australia and New Zealand Liver Transplant Registry, Brisbane, Australia.

Background: Patients with high MELD or PELD scores have a risk risk of death on the liver transplant waiting list and a short window of opportunity during which rescue by liver transplantation is possible. A system of inter-regional sharing for patients with high MELD or PELD scores might enable a greater chance of transplantation
Aim: To evaluate the potential impact of an inter-regional sharing program in Australia and New Zealand for patients with high MELD or PELD scores.
Method: Prospective data of patients on the waiting lists of the six liver transplant units in Australia and New Zealand with a MELD or PELD score of at least 25 were collected during the period 1 October 2013 to 30 April 2015. The MELD or PELD score at the time of liver transplantation was obtained for all non-urgent patients transplanted using deceased donor grafts in Australia and New Zealand during the study period using data from the Australian and New Zealand Liver Transplant Registry. Simulation of inter-regional sharing of blood group compatible livers was performed using Filemaker Pro 13.0. The variables assessed in the model were the MELD/PELD threshold at or above which the patient would be eligible to receive an inter-regional offer and the minimum difference in MELD/PELD between the potential recipient of an inter-regional offer and the local recipient (who was transplanted in reality). The outcomes of the models that were assessed included the number of livers that would be required to be shipped, the number of patients potentially rescued from waiting list death by inter-regional sharing, the additional cold ischaemia time and the additional distance between donor and recipient hospital.
Results: During the 19-month prospective study period, 388 liver transplants, excluding urgently listed patients and those transplanted using living donor livers, were performed in Australia and New Zealand. During the same period across Australia and New Zealand, 102 patients had a MELD or PELD score of at least 25 at some time while waiting. Of these, 27 (26%) died waiting. Simulation of models of inter-regional sharing revealed that the lower the MELD/PELD threshold and the lower the MELD/PELD difference in the model, the greater the number of patients rescued from waiting list death. However, this is at the expense of a greater number of livers shipped, increased transport distance and cold ischaemia time.
Conclusion: Simulation of models of inter-regional sharing for patients with high MELD and PELD scores has demonstrated that this approach has the potential to rescue patients at a high risk of waiting list death. A trial of voluntary inter-regional sharing of deceased donor livers for patients with a MELD or PELD score of at least 35 has begun in Australia and New Zealand.
Liver and Intestinal Transplantation Advisory Committee of the Transplantation Society of Australia and New Zealand

329.10

Histologic change after body weight reduction of potential living liver donors with fatty liver

Young Rok Choi, Suk-Won Suh, Jeong-Moo Lee, Hyeyoung Kim, Hae Won Lee, Nam-Joon Yi, Kwang-Woong Lee, Kyung-Suk Suh.
Surgery, Seoul Nation University College of Medicine, Seoul, Korea.

Background: Fatty liver is critical for donor selection, which has been associated with a risk of complication for both donor and recipient after liver transplantation. After living donor weight reduction for a short-term, the change of liver fat percentile and pathologic findings were investigated.
Methods: A total of 356 living donor candidate were between January 2011 and November 2013 at a single center. Of them, 18 donors tried to lose their weight reduction. Fat fraction was estimated on preoperative non-invasive MR spectroscopy. And liver biopsy findings were analyzed before and after weight reduction.
Results: Eighteen donor candidates lose mean 3.6 ± 3.1 kg of their weight for mean 43.9 ± 31.0 days (range 7-107 days) and BMI was significantly decreased to 25.9 ± 3.1 kg/m2 from 27.1 ± 3.2 kg/m2 ($p < 0.001$). Their fat fractions were also significantly decreased to 6.8 ± 4.5% from 11.4 ± 4.7% ($p < 0.001$). Although preoperative liver biopsy showed that ballooning change (n=3, 23.1%), inflammation (n=9, 69.2%), fibrosis (n=2, 15.4%) and necrosis (n=3, 16.7%) before weight reduction, intraoperative biopsy showed no fibrosis, no necrosis, decreased inflammation (n=2, 17.6%) and improved ballooning change of hepatocyte (n=1, 5.9%) after weight reduction [table 1]. Although one candidate could not donate his liver finally because of steatohepatitis, the others recovered uneventfully.
Conclusions: Donor body weight reduction can expand donor pool and contribute to improving donor safety.

329.11

Prediction of nonalcoholic fatty liver in apparently healthy living liver donors: a large histology based comparison to normal controls

Narendra S. Choudhary[1], Sanjiv Saigal[1], Neeraj Saraf[1], Rahul Rai[1], Tarun Piplani[2], Dheeraj Gautam[3], Lipika Lipi[3], Amit Rastogi[1], Sanjay Goja[1], Prashant Bhangui[1], Sumana K.Ramchandra[1], Yalakanti R.Babu[1], Vikram Raut[1], Thiagrajan Srinivasan[1], Arvinder S.Soin[1].
[1]Institute of Liver Transplantation and Regenerative Medicine, Medanta The Medicity, Gurgaon, India; [2]Department of Radiodiagnosis, Medanta The Medicity, Gurgaon, India; [3]Departments of Histopathology, Medanta The Medicity, Gurgaon, India.

Background: Patients with milder grades of non-alcoholic fatty liver (NAFL) are often not picked up by ultrasound abdomen. Conventionally LDLT centers use CT based liver attenuation index (LAI) to rule out fatty liver in living related liver donors.
Materials and Methods: The study group included all prospective liver donors who had a liver biopsy during work-up due to high body mass index (>28 Kg/M^2), dyslipidemias, presence of metabolic syndrome or borderline remnant/graft to recipient weight ratio. Non-alcoholic fatty liver was defined as >5% hepatocytes having steatosis and no changes of steatohepatitis and/or fibrosis. Data is shown as mean (SD) or median (25-75 IQR). Following metabolic risk factors were calculated: BMI>25 Kg/M^2 (Asian Indian cut-off for obesity), diabetes/impaired fasting glucose, triglycerides >150 mg/dl, high-density lipoprotein (<40 in males and <50 mg/dl in females) and blood pressure>130/85 mmHg or hypertension.
Results: The study group comprised of 521 prospective liver donors (280 females), age 35.1±10.3 years, BMI 25.7±3.8 Kg/M^2. A total of 243 donors (46.6%) were diagnosed as having nonalcoholic fatty liver, the amount of steatosis ranged from 5-40%. When subjects with NAFL were compared with subjects with normal histology; NAFL group had higher BMI (26.4±3.6 versus 25.1±3.8 kg/M^2, p=0.000), higher values of ALT [33(24-41) versus 27(21.2-32.7), p=0.000], GGT [23(16-33.5) versus 18(13.2-26), p=0.000], triglycerides (135.5±65.3 versus 111.6±52.2 mg/dl, p=0.000), low density lipoprotein (108.5±31.5 versus 102.4±29.4 mg/dl, p=0.017), lower high-density lipoproteins (40.5±14 versus 42.9±10.7 mg/dl, p=0.029) and CT LAI values [9(4-13) versus 12(8-16), p=0.000]. NAFL group had higher number of metabolic risk factors [2(1-3) versus 1(1-2), p=0.000]. Presence of CT LAI<5 had a sensitivity of 25.7% and specificity of 91.4% for prediction of NAFL. Presence of ≥2 metabolic risk factors predicted NAFL with a sensitivity of 67%.
Conclusion: Presence of ≥2 metabolic risk factors has better sensitivity for prediction of NAFL than CT LAI in living liver donors and thus combination of both is better.
Yogesh Saini; Payal Arora

329.12

Significance of living domino donor liver transplantation in the context of limited number of deceased organ donors in Japan - A single center experience

Yukihiro Inomata, Shintaro Hayashida, Yasuhiko Sugawara, Hidekazu Yamamoto, Masaki Honda, Kaori Isono, Daiki Yoshii, Kohei Miura, Keita Shimata, Shinnichi Kawabata, Koushi Uchida. Transplantation and Pediatric Surgery, Kumamoto University Hospital, Kumamoto, Japan.

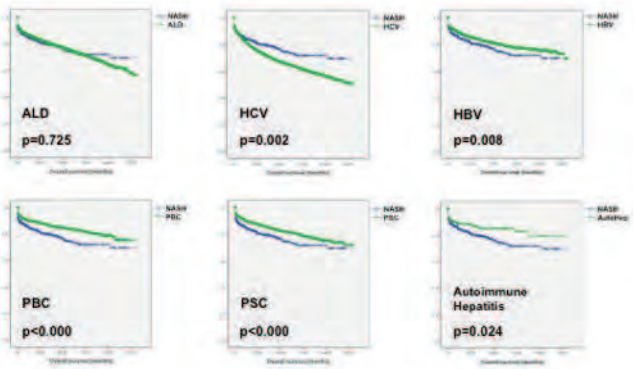
Background: Domino liver transplantation (DLT) using the liver from familial amyloid polyneuropathy (FAP) can expand the donor pool, although there is substantial risk of de novo onset of FAP in the recipient. With the reform of the organ transplant law in Japan, annual umber of deceased donor (DD) increased around 5 times, from 10 to 50. There is no nation-wide principle of the selection criteria of domino liver recipient using FAP liver. Benefit and risk of living domino donor liver transplantations (LDDLT) was analyzed in the cohort of one institution in the situation of still-limited number of deceased donors.
Patients and Methods: From 2003 to 2010, fifteen cases were done before the reform of the law (pre-group), and the other 6 were done after (post-group), from 2014 to February 2016. Selection process of the domino recipients (whether the candidate was on the DD liver transplant list or not), outcome of the LDDLT, and the onset of FAP, according to the timing of the transplant were analyzed.
Results: The original indication for LDDLT were; end-stage liver diseases in 12 (4 with HCC), re-transplant in 2, and congenital absence of portal vein with encephalopathy in 1, and in the post-group, ELD in 5 (3 with HCC), and re-transplant in the other 1. The mean age of the pre-, and post-group was 45.1 (18-58 yo) and 57.5 (51-62 yo), respectively. Only 4 out of 15 in the pre-group, and all of the post-group had been registered on the waiting list for DLT in Japan. Six-month post-transplant survival was 100% in both groups. Three patients of the pre-group died of malignancy. In 2 patients of the 8 long survivors in the pre-group, there are objective signs of FAP with least subjective symptoms.
Discussion: In the pre-group, younger patient were selected in consideration of hopeless possibility for DDLT. In the post-group, older patients with HCC were more common, as the traditional standards of the domino LT in the world, although, highly urgent patients (status 1) were not included.
Conclusion: Domino liver transplant had results of excellence and significance in the limited number of DD liver transplants. However, small increase of DD in Japan made the selection criteria a little difficult because the highly urgent patients will have substantial possibility of DD liver transplantation without the domino.

329.13

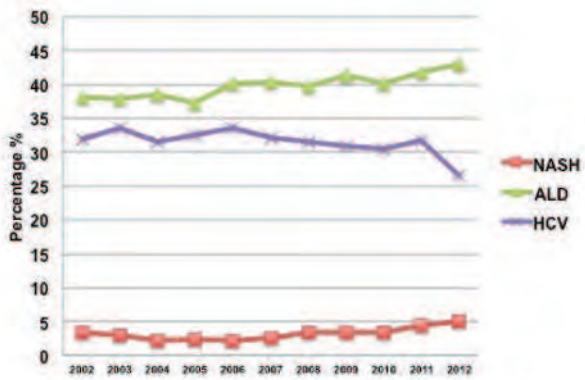
Nonalcoholic steatohepatitis as indication for liver transplantation in Europe. Clinical analysis of over 37.000 patients

Barbara Kern[1], Philip Newsome[2], Vincent Karam[3], Rene Adam[3], Gabriela Berlakovich[4], Josef Fritz[1], Benedikt Feurstein[1], Paolo Muiesan[5], Dietmar Öfner[1], Stefan Schneeberger[1].
[1]Operative Medicine, Innsbruck Medical University, Innsbruck, Austria; [2]Institute of Immunology and Immunotherapy , University of Birmingham, Birmingham, United Kingdom; [3]Centre Hépatobiliaire, Hôpital Paul Brousse, Villejuif, France; [4]Transplant Surgery, Medical University of Vienna, Vienna, Austria; [5]Queen Elizabeth Hospital, University of Birmingham, Birmingham, United Kingdom.

Background: Over the past years, nonalcoholic steatohepatitis (NASH) has emerged to become the third leading cause for liver transplantation (LT) in the US. Since 2002, chronic liver failure due to NASH has increased constantly.
Aim: The aim of this study was to investigate all adult patients with NASH, who underwent LT in Europe between 2002 and 2012.
Methods: We analyzed the European dataset of over 37.000 adult liver transplant recipients in collaboration with the European Liver Transplant Register (Paris – France) between 2002 and 2012. NASH was defined by clinical and histopathological findings. Patients with cryptogenic cirrhosis and a BMI over 30kg/m2 were also included.
Results: 37.612 adult patients underwent orthotopic LT in Europe. The most common indication for LT was alcoholic cirrhosis with 39.8% (14956/37612), the incidence of NASH was documented with 3.3% (660/37612). Except for ALD and HCV, overall survival was poor compared to other indications (HBV: p=0.0; PBC p<0.00; PSC: p<0.00; Others: p=0.02).



The male percentage accounted for 73.7% (27727/37612), female patients were only 26.1% (9855/37612; in less than 0.1% no sex was documented). Female patients and gender mismatch were identified as independent risk factor within the NASH cohort. Donor age over 55 years showed decreased overall survival in the nonNASH cohort, but not in the NASH group (p<0.00; p=0.5; respectively). Recipient BMI >40 did not impact on overall survival in the nonNASH patient group, but was identified as significant risk factor in the NASH cohort (p=0.1; p=0.02; respectively). Donor steatosis stratified as steatosis to mild, moderate and severe had no influence on the outcome in the NASH group, whereas in the nonNASH patient cohort, the difference was significant (p=0,01). In the multivariate analysis, donor BMI over 30m2/kg was identified as risk factor (p=0.023). The incidence of NASH reached 5%, ALD showed a slight increase, whereas HCV dropped below 30% by the year 2012.
Conclusion: Patients, who underwent LT due to NASH, did have decreased survival rates when compared to other indications except for ALD and HCV. Within the NASH group, female patients, gender mismatch, donor BMI over 30m2/kg and recipient BMI over 40m2/kg were identified as significant risk factors. A precise donor selection and the reduction of risk factors within the female population should be considered in patients with NASH undergoing LT.



350.1

Utilization and clinical outcomes of belatacept-based immunosuppression in U.S. renal transplant recipients

Xuerong Wen[2], Michael JinCasey[2], Alfonso H.Santos[2], Abraham Hartzema[1], Karl Womer[2].
[1]Department of Pharmaceutical Outcomes and Policy, University of Florida, Gainesville, FL, United States; [2]Nephrology/Medicine, University of Florida, Gainesville, FL, United States.

Introduction: In the BENEFIT and BENEFIT-EXT trials[1],[2], belatacept demonstrated non-inferior incidence of acute rejection and improved renal function in kidney transplants compared to a cyclosporine-based regimen. However, the drug's performance in a real clinical setting compared to tacrolimus has not been reported. A retrospective cohort study based on the Scientific Registry of Transplant Recipients data was conducted to compare 1-year utilization and outcomes of belatacept- and tacrolimus-treated U.S. kidney transplant recipients (KTR).

Methods: Solitary adult KTR who received belatacept+tacrolimus- or belatacept- (without CNI, termed belatacept alone) or tacrolimus (without belatacept, termed belatacept alone)-based regimens at hospital discharge from 6.1.2011 through 12.1.2014 were included. The primary outcome for this study included: one-year composite patient death or graft loss and one-year incidence of biopsy-proven acute rejection (BPAR). The secondary outcomes included mean estimated glomerular filtration rate (GFR) at 1 year and the one-year incidence of new-onset diabetes after transplantation (NODAT). Recipient demographic and clinical characteristics were adjusted in a multivariate Cox proportional hazard model. Further subgroup analyses were conducted in patients with high panel reactive antibody (PRA), with or without lymphocyte-depleting (LD) induction, and in individuals who met the same inclusion/exclusion criteria as the BENEFIT and BENEFIT-EXT trials[1],[2].

Results and Discussion: 50 244 adult recipients underwent solitary kidney transplantation during the study period, with 417 receiving belatacept+tacrolimus, 458 receiving belatacept alone, and 49 369 receiving tacrolimus alone at hospital discharge. The rates of 1-year composite patient death or graft loss in the two belatacept regimens were noninferior to that in the tacrolimus group. The rates of 1-year BPAR were numerically similar between belatacept+tacrolimus and belatacept alone in all recipients, BENEFIT-eligible patients, and BENEFIT-EXT-eligible patients (Figure 1A). However, these rates were significantly higher than those in the tacrolimus alone group for all three patient groups. The use of LD induction drugs was associated with lower 1-year BPAR rates in the recipients who received belatacept alone (14.6% vs 23.1%, P=0.02) (Figure 1B). In multivariate analyses, increased risk of BPAR associated with belatacept alone was identified in the overall study cohort compared to tacrolimus alone, (aHR: 2.36; 95%CI:1.82 – 3.05, P<.0001) with highest rates associated with non-LD induction (aHR: 2.65; 95%CI: 1.90-3.70, P<.0001), in BENEFIT-eligible patients (aHR: 2.51; 95%CI: 1.79 – 3.52, P<.0001) and in recipients with high PRA (aHR: 2.36, P<.0001). No significant difference in BPAR was demonstrated between belatacept+tacrolimus and tacrolimus alone. Renal function was significantly greater in belatacept- versus tacrolimus-treated BENEFIT-EXT-eligible patients (Figure 2). The risk of NODAT was significantly lower in the two belatacept groups than in the tacrolimus alone group (1.7% vs 2.2% vs 3.8%, respectively, P=0.01).

Conclusion: Despite improved graft function and fewer metabolic complications, it may be advisable to add short-term tacrolimus to belatacept the first year post-transplant and/or consider LD induction in high-risk patients, as the risk to benefit ratio allows.

Figure 1 A. 1-Year BPAR in All Recipients, BENEFIT-eligible Recipients, BENEFIT-EXT-eligible Recipients.
1 B. 1-Year BPAR in All Recipients with or without LD induction.

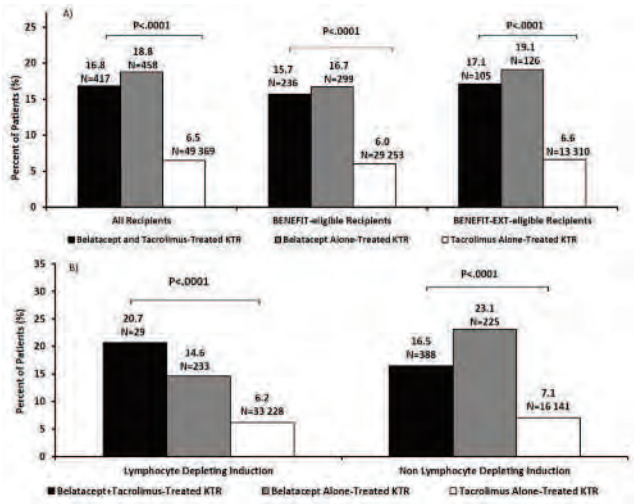
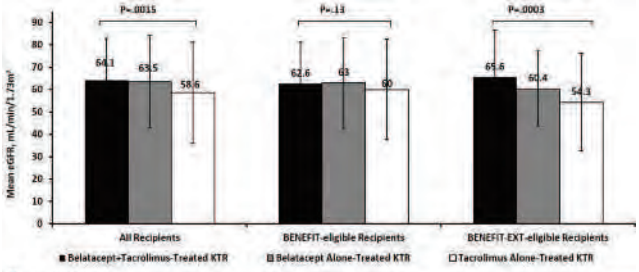


Figure 2. Renal Function Outcomes at 1 Year.



Recipients at 1 Year

Groups	All Recipients	BENEFIT-eligible Recipients	BENEFIT-EXT-eligible Recipients
Belatacept+Tacrolimus-Treated KTR	195	115	46
Belatacept Alone-Treated KTR	218	143	58
Tacrolimus Alone-Treated KTR	32 480	19 339	8530

This work supported by the Central Florida Kidney Center, Inc. Eminent Scholar Chair in Nephrology and Hypertension and the Gatorade Trust Fund distributed by the University of Florida, Division of Nephrology, Hypertension, and Renal Transplantation

References:

[1] Vincenti F, Charpentier B, Vanrenterghem Y, Rostaing L, Bresnahan B, Darji P, et al. A phase III study of belatacept-based immunosuppression regimens versus cyclosporine in renal transplant recipients (BENEFIT study). Am J Transplant. 2010;10(3):535-46.
[2] Durrbach A, Pestana JM, Pearson T, Vincenti F, Garcia VD, Campistol J, et al. A phase III study of belatacept versus cyclosporine in kidney transplants from extended criteria donors (BENEFIT-EXT study). Am J Transplant. 2010;10(3):547-57.

350.2

Outcomes of de novo belatacept versus tacrolimus in kidney recipients of high risk donors

Mahmoud Alameddine, Jeffrey Gaynor, Linda Chen, Zhobin Moghadamyeghaneh, Gaetano Ciancio, Mohammed Osman, Rodrigo Vianna, George Burke, Mahmoud Morsi, Vincent Chia, Warren Kupin, Adela Mattiazzi, Giselle Guerra.
Transplant Surgery, Miami Transplant Institute, Miami, United States.

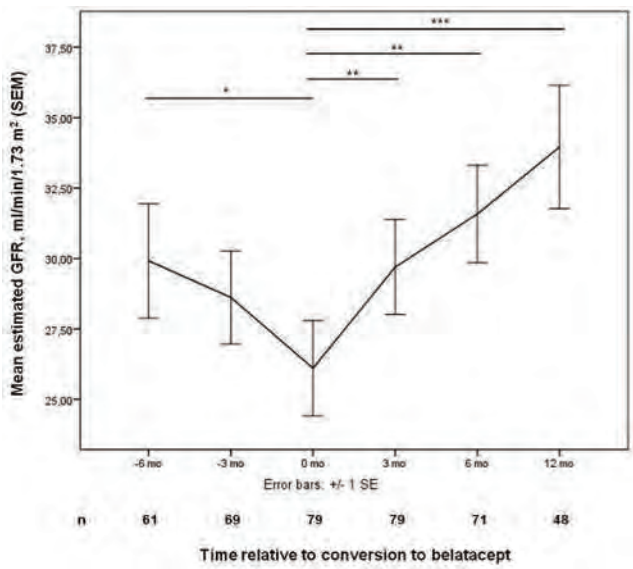
Background: The new kidney allocation system (KAS) has increased the incidence of delayed graft function (DGF) or slow graft function (SGF) in the United States. The benefit of a calcineurin sparing regimen using Belatacept (Bela) in this population may be offset by the reduced quality of the allograft. We report our preliminary experience with Bela vs Tacrolimus (TAC) in patients with kidneys from high risk donors (HRD) in KAS.
Material and Method: 24 patients received Bela from 1/2014 – 3/2015 after obtaining a kidney with one or more of the following: KDPI≥85%, Donation after Cardiac Death, terminal creatinine >2, cold ischemia time CIT > 30 hrs, donor age ≥ 70 year, or imported kidneys. We compared them to a similar control group (n=35) who received steroid free TAC regimen. Both groups received Thymoglobulin induction for three days after transplantation and maintenance with Myfortic. The Bela group remained on steroids. Outcomes were assessed for 6 months post-transplant (eGFR, white blood cell count (WBC), cytomegalovirus (CMV) and polyoma (BKV) viremia, and rejection).
Results: Mean eGFR was higher in the Bela vs TAC arm at months 2 (63.8 mL/min vs 50.2 mL/min) and 3 (62.8mL/min vs 51.7mL/min) P=0.0004 and P=0.007, respectively; however this decreased by 6 months, 56.3 vs. 54.7mL/min, respectively (P=0.73). The percentage of patients with DGF was higher with Bela 58.3% vs. TAC 17.1%, (P=0.001). Freedom-from-CMV Viremia was worse with Bela 11/24 events vs. 5/35 with TAC (P=.008). Freedom-from-BK Viremia was also poorer in Bela (P=.04), with 7/24 observed events vs. 3/35 in TAC. The occurrence of biopsy proven acute rejection (BPAR) was higher in Bela (4/24) vs. TAC (3/35), with actuarial BPAR at 12 months being 14.4% % + 7.9% vs. 6.8% + 4.7% in the Bela and TAC groups, respectively (P=0.20).
Conclusion: After 6 months, allograft function is similar for Bela and TAC but concerns exist with rejection, CMV, and BK viruses in the Bela for recipients receiving HRD from KAS.

350.3

Belatacept rescue therapy in kidney transplant recipients with severely impaired renal function

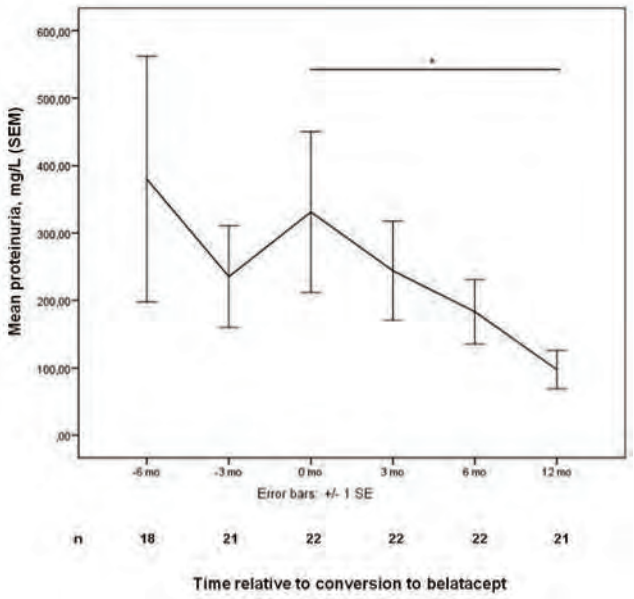
Klemens Budde[1], Michael Wiesener[2], Dennis Kannenkeril[2], Michael Dürr[1], Tobias Braun[2], Friederike Bachmann[1], Susanne Brakemeier[1].
[1]Nephrology, Charité, Berlin, Germany; [2]Nephrology, University Erlangen-Nürnberg, Erlangen, Germany.

Background: In kidney transplant recipients (KTRs) with chronic graft dysfunction, long-term immunosuppression with calcineurin inhibitors (CNIs) or mTOR inhibitors (mTORi) can be challenging due to adverse effects, such as nephrotoxicity and proteinuria.
Methods: 79 KTRs treated with CNI-based or mTORi-based maintenance immunosuppression who had CNI-induced nephrotoxicity or severe adverse events were switched to Belatacept. Mean time from transplantation was 69 months. The most common reason for conversion to belatacept was biopsy-confirmed CNI-induced nephrotoxicity (58.2%). The majority of patients (pts) were converted from tacrolimus (50.6%) followed by cyclosporine (21.5%), everolimus (20.2%), and sirolimus (7.6%). Prior to conversion, 50 (63.3%) pts were receiving steroids, 73 (92.4%) were receiving mycophenolate, and 6 (7.6%) azathioprine. Twenty-seven (34.2%) patients were DSA-positive at the time of conversion.



Results: Patient survival at 12 months post-conversion was 96.2%; two pts died (n=1 myocardial infarction and n=1 non-small–cell lung carcinoma (NSCLC). Graft survival at 12 months post-conversion was 86.1%, including 3 pts who died with a functioning graft. Of the 8 pts who lost their graft in the first year after conversion, 5 (62.5%) were DSA-positive at baseline and had a baseline estimated GFR of 21.9 mL/min/1.73 m2 and baseline proteinuria of 697±273 mg/L. Following conversion to Belatacept, 9 (11.4%) pts experienced biopsy-confirmed acute rejection (grade IB, n=4; grade IIA, n=1; grade IIB, n=3; grade III, n=1), with 2 graft losses due to fulminant rejection after conversion. Of the 9 pts presenting with acute rejection, 3 had switched to belatacept because of compliance issues. Mean eGFR (Figure 1) at baseline was 26.1 increasing to 34.0 mL/min/1.73 m2 at 12 months ($p<0.001$). Renal function improvements were also seen in pts with either low eGFR (<25 mL/min/1.73 m2) or high proteinuria (>500 mg/L) at conversion. Among the 22 pts who were using an mTORi prior conversion, mean proteinuria decreased significantly ($p<0.05$) from 331±119 mg/L at baseline to 244±74, 183±48, and 98±28 mg/L at 3, 6, and 12 months post-conversion, respectively (Figure 2). Discontinuation rate due to adverse events was 7.9%. During treatment

with Belatacept 46 pts (58.2%) were not hospitalized, but 33 (41.8%) pts were hospitalized for a total of 57 serious AEs, most commonly infection (n=19). Twelve of the 19 reported infections were severe (urosepsis n=6; CMV n=2; Pneumocystis jirovecii pneumonia n=1; miliary tuberculosis n=1; phlegmon n=1; Candida septicemia n=1). Four pts developed a malignancy (NSCLC [9 months after conversion]; Kaposi's sarcoma [8 months after conversion]; basal cell carcinoma [4 months after conversion]; CNS PTLD [17 months after conversion]).
Conclusion: Belatacept-based immunosuppression may be an alternative regimen for KTRs with CNI- or mTORi-induced toxicity.



350.4

Patterns of improvement in kidney function among patients converted to belatacept from calcineurin inhibitors for kidney allograft dysfunction

Dhiren Kumar[1], Anil Regmi[1], Hasan Fattah[1], Hitarth Dave[1], Justin Joseph[1], Pamela Kimball[2], Amit Sharma[2], Chandra S.Bhati[2], Hugh D.Massey[3], Anne L.King[1], Gaurav Gupta[1]. [1]Nephrology, Virginia Commonwealth University, Richmond, United States; [2]Transplant Surgery, Virginia Commonwealth University, Richmond, United States; [3]Pathology, Virginia Commonwealth University, Richmond, United States.

Background: Belatacept might be an alternative to Calcineurin Inhibitors (CNI) to avoid short and long-term nephrotoxicity. We have previously reported our initial experience on six patients suggesting improved renal function in high immunologic risk kidney transplant recipients (KTxp) switched from CNI to belatacept (Gupta et al, Am J Transplant. 2015 Oct). Here we present extended cumulative data on the use of a belatacept conversion regimen in patients with impaired graft function.
Methods: EBV seropositive patients were converted to belatacept from tacrolimus for biopsy proven acute CNI toxicity and/or interstitial fibrosis/tubular atrophy. Belatacept was initiated based upon prior published protocols. Mycophenolate mofetil dose was increased from baseline dose of 1-2g/d to a dose of 2-3g/d to minimize risk of rejection.
Results: Thirty one (mean age=46±11 years) patients were switched from tacrolimus to belatacept at a median of 7 months post-KTxp. A majority were African-American (23/31; 74%). Eleven patients (35%) were sensitized (mean PRA=27%; range=0-99%). Overall, renal function improved significantly from a peak mean GFR of 28±12 ml/min/1.73m2 to 36±15ml/min/1.73m2 (p=0.001) at a median follow-up of 14 (range=3-37) months post-conversion. Analysis of various metabolic parameters (lipid profile, hemoglobin A1c and hypertension) demonstrated a significant decline in a need for hypertension medications from a mean of 2.2 pre-conversion to 1.9 post-conversion (p=0.037). A comparison of patients who had an improvement in GFR (defined as >5ml/min/1.73m2; n=17) vs. those who did not (n=14) showed that increasing length of therapy with belatacept (21±10months vs 9±7months, p=0.001) was the only factor associated with improvement in graft function. Surveillance biopsies performed on 17 (out of 31) patients revealed 1 (6%) case of subclinical rejection. Cumulative chronicity scores pre- (3.6±1.6) and post-conversion (4.0±1.6) remained unchanged (p=0.49). In 28/31 (90%) patients there was no evidence of de-novo donor specific antibody (DSA). Two highly sensitized patients with stable creatinine had rising DSA after an infection. There were no new cases of BK viremia, CMV disease or malignancy.
Conclusions: In this extended experience on KTxp patients with significantly reduced GFR, we report remarkable improvement in renal function in patients converted from tacrolimus to belatacept with acute CNI toxicity and chronic allograft fibrosis without a significant concurrent increase in risk of rejection, worsening chronicity and DSA. Further follow-up and protocol biopsies are planned to ensure safety and wider applicability of this approach.
References:
[1] Gupta et al, Am J Transplant. 2015 Oct

350.5

Early conversion to belatacept in patients with poor or delayed graft function

Luz Liriano-Ward, Vinay Nair, Ron Shapiro, Sander S.Florman. Recanati/Miller Transplantation Institute, Icahn School of Medicine at Mount Sinai, New York, NY, United States.

Background: The lack of nephrotoxicity makes belatacept a useful agent for patients experiencing calcineurin inhibitor (CNI) nephrotoxicity. Limited data exist on early conversion from CNI to belatacept (<6 months). We present our experience with early conversion from CNI to belatacept in patients with presumed CNI nephrotoxicity manifested by either prolonged delayed graft function (DGF) or poor function. We hypothesized that conversion to belatacept would lead to improved graft function and facilitate recovery from DGF.
Methods: We performed a chart review between 2012 and 2014 to identify all patients converted from CNI to belatacept < 6months post transplantation.
Results: Ten patients were converted from CNI to belatacept. Three had DGF for 3, 30, and 46 days prior to conversion. Seven patients were converted for poor renal function (eGFR<30 ml/min/1.73m MDRD). Eight were converted to belatacept with a starting dose of 10mg/kg on days 1, 5, and every two weeks with a median duration of 130 days post-transplantation and then transitioned to 5mg/kg monthly. CNI was withdrawn 1 to 5 days after the initiation of belatacept. This approach is based on de novo dosing to facilitate early withdrawal of CNI. Conversion to 5mg/kg was also based on de novo dosing where 5mg/kg dosing starts 4 months post-transplant. For 2 patients, belatacept conversion was performed > 4 months post-transplant. In these patients, belatacept was dosed 5mg/kg every 2 weeks for 2 months, then monthly while CNI was withdrawn over 1 month. This is based on a published study including patients > 6 months post-transplant (Rostaing L. CJASN. 2011). All 3 patients with DGF had renal recovery after 6, 15, and 64 days post CNI discontinuation with a current creatinine of 1.4mg/dl, 1.3mg/dl, and 3.6 mg/dl respectively. Median creatinine for the 7 patients with poor function was 2.9mg/dl (average 4.2mg/dl) prior to conversion, 1.9mg/dl one year post conversion, and 1.6mg/dl at a median follow up of 591 days. One patient had a borderline rejection after conversion to belatacept. There were no opportunistic infections or malignancies.
Conclusion: We report safe and effective early conversion from CNI to belatacept in patients with DGF or poor graft function. In both groups we observed an improvement in renal function without an increase in rejection, infection or malignancy. Additional studies are needed to evaluate the safety of early conversion and whether early conversion can improve renal function.
References:
[1] Larsen CP, Grinyó J, Medina-Pestana J, Vanrenterghem Y, Vincenti F, Breshahan B, Campistol JM, Florman S, Rial Mdel C, Kamar N, Block A, Di Russo G, Lin CS, Garg P, Charpentier B. Belatacept-based regimens versus a cyclosporine A-based regimen in kidney transplant recipients: 2-year results from the BENEFIT and BENEFIT-EXT studies. Transplantation. 2010 Dec 27;90(12):1528-35.
[2] Pestana JO, Grinyo JM, Vanrenterghem Y, Becker T, Campistol JM, Florman S, Garcia VD, Kamar N, Lang P, Manfro RC, Massari P, Rial MD, Schnitzler MA, Vitko S, Duan T, Block A, Harler MB, Durrbach A. Three-year outcomes from BENEFIT-EXT: a phase III study of belatacept versus cyclosporine in recipients of extended criteria donor kidneys. Transplantation. 2010 Dec 27;90(12):1528-35
[3] Rostaing L, Massari P, Garcia VD, Mancilla-Urrea E, Nainan G, del Carmen Rial M, Steinberg S, Vincenti F, Shi R, Di Russo G, Thomas D, Grinyó J. Switching from calcineurin inhibitor-based regimens to a belatacept-based regimen in renal transplant recipients: a randomized phase II study. Clin J Am Soc Nephrol. 2011 Feb;6(2):430-9.

350.6

Tofacitinib in renal allograft recipients: long-term efficacy and safety in an active-comparator-controlled extension trial

Philip J. O'Connell[1], Stephan Busque[2], Flavio Vincenti[3], Helio Tedesco Silva[4], Atsushi Yoshida[5], John J.Friedewald[6], Steven J.Steinberg[7], Klemens Budde[8], Emine N.Broeders[9], Yon Su Kim[10], Carolyn Hahn[11], Huihua Li[11], Gary Chan[12].
[1]Westmead Hospital, Sydney, Australia; [2]Stanford University, Stanford, United States; [3]University of California, San Francisco, United States; [4]Hospital do Rim e Hipertensão, São Paulo, Brazil; [5]Henry Ford Hospital, Detroit, United States; [6]Northwestern University, Chicago, United States; [7]Sharp Memorial Hospital, San Diego, United States; [8]Charité Universitätsmedzin, Berlin, Germany; [9]Renal Transplantation Clinic, ULB Hospital Erasme, Brussels, Belgium; [10]Seoul National University College of Medicine, Seoul, Korea; [11]Pfizer Inc, Collegeville, United States; [12]Pfizer Inc, Groton, United States.

Introduction: Tofacitinib is an oral Janus kinase inhibitor. In a Phase 2b study, tofacitinib was effective in preventing acute renal allograft rejection in the first 12 months (mos) post-transplant but with an increased risk of serious infection events (SIEs) and three cases of post-transplant lymphoproliferative disease (PTLD)[1]. Here we evaluate the long-term efficacy and safety of tofacitinib in renal allograft patients (pts) over the next 60 mos.
Materials and Methods: Kidney transplant pts who completed 12 mos of randomised treatment with cyclosporine (CsA) or a Less Intensive (LI) or More Intensive (MI) tofacitinib regimen were enrolled in a Phase 2b, open-label extension study (NCT00658359). Pts continued their previous treatment of CsA or tofacitinib with mycophenolic acid products for 5 years, through Mo 72 post-transplant. Pts receiving tofacitinib MI/LI continued 10 mg twice daily (BID), decreasing to 5 mg BID by Mo 18. Primary outcomes were the incidence of first biopsy-proven acute rejection (BPAR), treated clinical acute rejection and adverse events (AEs). Secondary outcomes included glomerular filtration rate (GFR), chronic allograft nephropathy (CAN) and pt and allograft survival.

Table 1. Clinical outcomes through Month 72

	CsA (N=64)	Tofacitinib LI (N=60)	Tofacitinib MI (N=54)
Patients discontinued, n (%)	28 (43.8)	45 (75.0)	46 (85.2)
Patient survival, KM% (SE)	94.1 (3.4)	100 (0)	91.0 (4.5)
Graft survival (death censored), KM% (SE)	96.5 (2.4)	100 (0)	100 (0)
BPAR, KM% (SE)	13.3 (4.4)	10.0 (3.9)	7.4 (3.6)
Treated clinical acute rejection, KM% (SE)	29.7 (5.9)	11.7* (4.1)	11.1 (4.3)
CAN (IF/TA), KM% (SE)	69.3 (5.9)	60.2* (8.3)	62.2* (10.5)
Least square means eGFR by MDRD, mL/min (SE)	49.6 (3.5)	64.3 (3.6)†	59.2 (3.8)
Patients with AEs, n (%)	62 (96.9)	58 (96.7)	53 (98.1)
Patients with SAEs, n (%)	40 (62.5)	32 (53.3)	31 (57.4)
Patients with severe AEs, n (%)	31 (48.4)	20 (33.3)	22 (40.7)
TE SIEs, n (%)	18 (28.1)	21 (35.0)	14 (25.9)
TE malignancies, n (%)	6 (9.4)	6 (10.0)	8 (14.8)
Patients with PTLD, n	0	0	2
TE herpes zoster, (any) n (%)	5 (7.8)	14 (23.3)‡	6 (11.1)
TE serious herpes zoster, n (%)	2 (3.1)	2 (3.3)	1 (1.9)

Inferential statistical comparisons of tofacitinib LI, MI vs CsA were applied only to KM estimates, eGFR and % of patients with TE herpes zoster.
*p<0.05 vs CsA based on Log-Rank test comparing the overall survival curves through Mo 72.
†p<0.05 vs CsA using a linear mixed-effects model with repeated measures.
‡p<0.05 vs CsA based on exact test.

Treated clinical acute rejection was defined as an acute rejection episode that was diagnosed clinically and received anti-rejection treatment.
Serious herpes zoster infection was defined as herpes zoster infections that were serious AEs.
eGFR was calculated per the MDRD formula using LOCF and imputation (eGFR=0 for graft loss and death).
TE events do not include events in the first 12 months during the parent study.
CAN rate was based on all protocol-required and for-cause biopsies.

AE, adverse event; BPAR, biopsy-proven acute rejection; CAN, chronic allograft nephropathy; CsA, cyclosporine; eGFR, estimated glomerular filtration rate; IF, interstitial fibrosis; KM, Kaplan-Meier estimate; LI, Less Intensive; LOCF, last observation carried forward; MDRD, modification of diet in renal disease; Mo, month; MI, More Intensive; PTLD, post-transplant lymphoproliferative disease; serious adverse event, SAE; SE, standard error; SIE, serious infection event; TE, treatment emergent; TA, tubular atrophy.

Results: 178 pts were enrolled (CsA: n=64; tofacitinib LI: n=60; tofacitinib MI: n=54). Pt demographics were similar in all groups. Clinical outcomes are presented in Table 1. All groups had a similar BPAR rate but the treated clinical acute rejection rate was higher for CsA vs tofacitinib (CsA 29.7%, LI 11.7%, MI 11.1%). Estimated GFR was higher in tofacitinib pts than CsA pts by 10–15 mL/min/1.73m^2 but the rate of CAN was ≥60% in all groups. Pt and allograft survival rates, and AE and serious AE frequencies were comparable among groups. The SIE rates were: CsA 28.1%, LI 35.0% and MI 25.9%. The rates of malignancies were: CsA 9.4%, LI 10.0% and MI 14.8%. Two new cases of PTLD occurred in the tofacitinib MI group after Mo 12 post-transplant. A protocol amendment was implemented to discontinue 43 pts who had above-median tofacitinib exposure in the first 12 mos post-transplant, since all 5 cases of PTLD occurred in above-median exposure pts. No PTLD cases occurred in pts who continued after the protocol amendment.
Conclusion: Long-term tofacitinib treatment was effective in preventing acute allograft rejection. At Mo 72, tofacitinib pts had better renal function but similar cumulative CAN rates vs CsA. Long-term SIE risk remained but the magnitude of risk relative to CsA was reduced vs the first 12 mos post-transplant. Above-median tofacitinib exposure appears to be associated with increased PTLD risk. No new safety signals were reported.
This study was sponsored by Pfizer Inc. Editorial support, under the direction of the authors, was provided by Rebecca Bright of Complete Medical Communications, and funded by Pfizer Inc
References:
[1] Vincenti F et al. Am J Transplant 2012; 12: 2446-2455.

350.7

Efficacy of a chimeric anti-human ICAM-1 monoclonal antibody, MD-3, for kidney transplantation in Rhesus monkeys

Sang-il Min[1], Min-Ji Cho[1], Sungshin Cho[1], Chang Hwan Lee[1], Song-Yi Kim[1], Ahram Han[1], Chanjoong Choi[1], Sanghyun Ahn[1], Seung-Kee Min[1], Kyeong Cheon Jung[2], Seong Hoe Park[2], Sang Joon Kim[1], Jongwon Ha[1].
[1]Department of Surgery, Seoul National University Hospital, Seoul, Korea; [2]Department of Pathology, Seoul National University College of Medicine, Seoul, Korea.

Introduction: The MD-3 is a newly developed chimeric anti-human intracellular adhesion molecule 1 (ICAM-1) monoclonal antibody (mAb). The effects of this monoclonal antibody in solid organ transplantation have not been elucidated.
Methods: Five rhesus monkey renal allograft recipients treated with MD-3 induction and one recipient without MD-3 treatment were studied for the incidence of acute rejection and donor-specific antibody development. Cellular immune response was tested with ELISPOT assay. Development of anti-drug antibody was also tested. Maintenance immunosuppression included anti-CD154 mAb (hu5C8), mycophenolate mofetil and corticosteroid.
Results: No recipient monkey developed acute rejection as long as 8 months after transplantation. Donor-specific antibody was not developed in monkeys treated with MD-3. A monkey without MD-3 induction started to produce donor-specific antibody 3 weeks after cessation of anti-CD154mAb and persisted. No anti-drug antibody was detected during the study period.
Conclusions: MD-3 can be a highly promising agent for clinical use in human allotransplantation and MD-3 treatment can allow optimization of kidney transplant outcome. Further studies for refining dosages and dosing schedule of MD-3 in kidney transplantation are required.

350.8

A novel non-invasive blood transcriptional assay, kSORT, monitors alloimmune response in the SAILOR randomized multicenter EU trial

Per Lindner[1], Jana Ekberg[1], Bente Jespersen[2], Karin Skov[2], Tara Siegdel[3], Sue Hsieh[3], Minnie Sarwal[3].
[1]Transplant Institute, Sahlgrenska University Hospital, Gothenburg, Sweden; [2]Department of Renal Medicine, Skejby Hospital, Aarhus, Denmark; [3]Department of Surgery, University of California, San Francisco, San Francisco, CA, United States.

Background: Non-invasive diagnosis and prediction of acute rejection (AR) is a critical unmet need. A In a randomized multicenter trial of a target enrollment of 222 renal transplant recipients treated with steroid minimization and a tacrolimus, MMF based regimen, a novel blood transcriptional assay, kSORT (Roedder et al, PlosMedicine, 2014) was evaluated for its accuracy in diagnosing and predicting biopsy confirmed AR.
Methods: Serial peripheral blood samples were drawn at day 0, 10, months 3, 6, 12 at clinical graft dysfunction, prior to treatment intensification in PAXgene tubes to for blinded analysis of kSORT, a customized 17 gene assay (CFLAR, DUSP1, IFNGR1, ITGAX, MAPK9, NAMPT, NKTR, PSEN1,CEACAM4, EPOR, GZMK, RARA, RHEB, RXRA, SLC25A37, RNF130, RYBP) that provides AR high or low immune risk scores. The assay has a ~15% assay read out of intermediate (non-confirmatory) scores where repeat sampling is recommended. Serial DSAs were monitored. Biopsies were done on all study patients by protocol at engraftment and 12 months post-transplantation and when clinically indicated; central histology was read by Banff scores. The kSORT assay was run on 338 blood samples obtained from the first 79 enrolled patients, of which 98 blood samples were matched with protocol or indicated biopsies. 22 patients had clinically suspected acute rejection (AR) of which 18 were biopsy confirmed. RNA was extracted and QPCR for all 17 genes was normalized to 18S; data was profiled using a customized algorithm kSAS to produce an immune risk score for rejection.
Results: Of the 18 biopsy confirmed AR episodes, all were cellular and DSA negative; 15 had definite kSORT scores and 3 were intermediate; 14/15 AR had high-risk KSORT scores. 11 AR episodes had prior blood samples collected per protocol in the previous 4 months; 8/11 of the pre-AR samples had high kSORT scores, in the absence of clinical graft dysfunction. Of the 80 biopsy matched blood samples without histological AR, 73 had definite kSORT scores, and 7 had intermediate calls. 67/73 blood samples matched with biopsies without AR, had low-risk kSORT scores. There are no cases to date of BKVN, but 13 blood samples were positive by BK QPCR, and 12/13 of these blood samples had low-risk scores for kSORT and 1 was intermediate.
Conclusion: Interim results of the diagnostic accuracy of the kSORT assay in a randomized prospective multicenter trial in renal transplantation, confirms that the assay has 93.3% sensitivity and 90% specificity and 98.6% NPV for the non-invasive diagnosis of AR, and is not confounded by BK viremia. 73% of AR could have been diagnosed by the kSORT assay days-months prior their current time-line for diagnosis based on the serum creatinine alone, supporting the use of this assay for serial monitoring of rejection risk and proactive immunosuppression customization to alloimmune risk.
References:
[1] Ronder S, PLoS Med. 2014 Nov 11;11(11)

350.9

Immunologic basis for a novel organ-specific therapy: Immunocloaking

Lauren Brasile[1], Bart Stubenitsky[2], Nicholas Henry[1], Karina Kizjakina[1].
[1]Research and Development, BREONICS Inc., Watervliet, NY, United States; [2]Surgery, University of Maastrict, Maastrict, Netherlands.

Introduction: We have previously reported on an organ-specific immunomodifying therapy referred to as immunocloaking (IC) that provides protection from early allograft rejection without immunosuppressive drugs. The IC membrane is comprised of extracellular matrix proteins and is applied during ex vivo warm perfusion where it binds to the luminal surfaces of the vasculature within kidneys. The IC membrane functions by interrupting the interface between the donor vascular endothelium (VEC) and recipient immune cells; without adversely affecting renal function. The result is a newly engineered non-thrombogenic/non-immunogenic apical surface. We now report the underlying protective mechanisms involved with IC.
Materials: The IC membrane was applied to human VEC. The immunologic testing included: effect of IC on antigen presentation, T cell activation & diapedsis using standard methodology with flow cytometry, Luminex screening and chemotactic-driven diapedesis. The testing used responding mononuclear cells (MNC) stimulated by allogeneic VEC without IC (positive control) and the same VEC that were IC (test). Transendothelial migration involved VEC grown on filters in Transwell™ plates. MNC were added to the upper chambers and the chemoattractant SDF-1, a potent chemotactic factor for lymphocytes, was added to the lower chambers to form chemokine gradients.
Results: The immunocloaking membrane prevents antigen presentation and early T cell activation, thereby eliminating T cell proliferation. The results from mixed lymphocyte/VEC studies demonstrate that VEC strongly stimulated MNC. Immunocloaking the same VEC inhibited the responses by >99% (Figure 1).

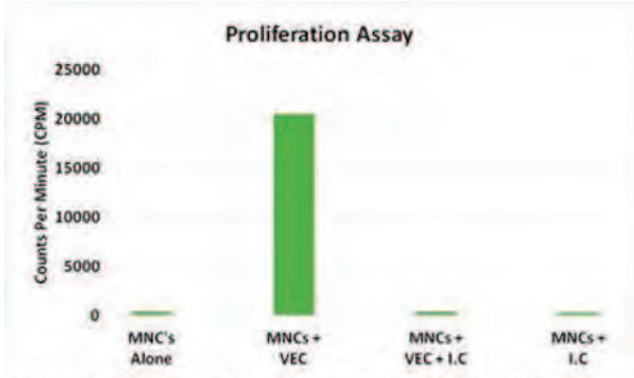


Figure 1 : A mixed lymphocyte-endothelial reaction was performed in a standard 96 well plate. Mononuclear Cells (MNC) were plated alone, with allogeneic vascular endothelial cells (VEC) or on Immunocloaked (IC) VEC. Cells were allowed to proliferate for 4 days prior to pulsing with [3H] thymidine. The cells were then allowed to incubate for 18H and read on a scintillation counter.

Transmigration: In negative controls without SDF-1 few MNC migrated through VEC layers into the lower chambers. In positive controls >65% of the cells migrated through the VEC into the lower chambers containing the SDF-1. When VEC monolayers were IC, the MNC migration into lower chambers with the SDF-1 was inhibited by a mean of 86.7% (Figure 2).
Conclusions: We previously demonstrated that IC prevents renal allograft rejection for a mean of 30 days in the absence of systemic immunosuppression. How the IC prevented rejection was previously

unknown. We now show that IC temporarily prevents alloresponses. Significantly, IC prevents chemokine-stimulated transmigration. Eliminating the need for nephrotoxic drugs during the early posttransplant period could help to ameliorate the severity of delayed graft function.

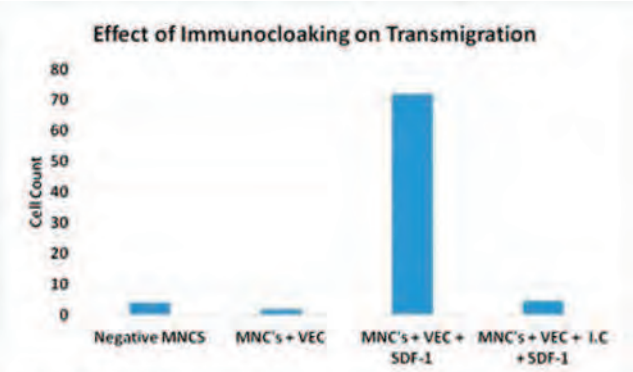


Figure 2: Chemotactic driven migration of mononuclear cells through Transwell. A concentration gradient was created with SDF-1 (10ug/ml). N=3. Cells were incubated for 3 hours and final counts were performed using a Nexcelom Cellometer and the mean of graphed. Immunocloaking provided an 86.7% reduction in migration of MNC.

351.1

Composite pancreatic islet grafts with adipose tissue-derived stem cells enhance the survival and insulin function of islet grafts in diabetic mice

Masahiro Tanemura[1,2], Yoshiaki Ohmura[2], Hiroaki Nagano[2], Hidetoshi Eguchi[2], Hideshi Ishii[2], Yuichiro Doki[2], Masaki Mori[2].
[1]Department of Surgery, Osaka Police Hospital, Osaka, Japan; [2]Department of Gastroenterological Surgery, Osaka University Graduate School of Medicine, Suita, Japan.

Introduction: Combined transplantation of allogeneic islets and adipose tissue-derived stem cells (ADSCs) under the kidney capsule promoted survival and reduced the islet mass required for reversal of diabetes in our previous study. [Aim] The aims of this study are to determine similar outcome can be obtained by islet transplantation (ITx) combined with ADSCs into the portal vein.
Materials and Methods: Isolation of islets and ADSCs: Anesthetized male BALB/cA mice underwent pancreatic inflation. The inflated pancreas was digested by collagenase (VIII), followed by purification using a Ficoll gradient. The adipose tissues from male EGFP-C57BL/6 mice were digested by collagenase (II). Generation of composite graft: Isolated islets were co-cultured with ADSCs in RPMI1640 medium for 24 hr. All islets were entirely covered with GFP-ADSCs. ITx into the liver: Streptozotocin(STZ)-induced diabetic male C57BL/6J mice were divided into five experimental groups (n=5/group) to receive allogeneic islets into the liver. ITx groups included group A: islet alone (600 IEQ) and group B: islet alone (400 IEQ). Hybrid group included group C: injection with islet (400 IEQ) + syngeneic GFP-ADSCs (4x105cells). Composite group included group D: composite graft with islets (400 IEQ) + syngeneic GFP-ADSCs (4x105 cells). 4x105cells of GFP-ADSCs were implanted as group E. After ITx, the non-fasting blood glucose level was monitored. Islet graft rejection was defined when two consecutive blood glucose levels exceeded 250 mg/dl. IPGTT was performed at day 7.
Results: The data were summarized in table 1.

Table 1

Group	Graft survival (n=5, days)	Mean graft survival (n=5, mean ± SD days)	Glucose pattern of IPGTT (n=5)	Blood glucose at 120 min during IPGTT (n=5, mg/dl)
A: Islets alone (600 IEQ)	20, 20, 19, 19, 18	19.2 ± 0.8	Normal pattern	130 ± 43*
B: Islets alone (400 IEQ)	0, 0, 0, 0, 0	0	DM pattern	347 ± 54
C: Hybrid ITx (: mixture) (islets 400 IEQ + 4 × 10^6 ADSCs)	3, 0, 0, 0, 0	0.6 ± 1.2	DM pattern	435 ± 48
D: Composite graft ITx (islets 400 IEQ + 4 × 10^6 ADSCs)	22, 17, 16, 13, 12	16.0 ± 3.5	Normal pattern	129 ± 19*
E: ADSCs alone (4 × 10^6 ADSCs)	0, 0, 0, 0, 0	0	Not done	Not done

*: blood glucose of non-diabetic mice: 110 ± 26 mg/dl

- Transplantation of 600 IEQ islets only achieved normoglycemia, whereas that of 400 IEQ allogeneic islets never reversed diabetes. Composite graft transplantation with 400 islets and ADSCs reversed diabetes and significantly prolonged graft survival.
- Few number of fragmented islet grafts were barely detected in 400 IEQ islets-ADSCs hybrid transplant, whereas insulin positive islets covered with GFP-ADSCs, which were well-preserved islets structure were found in islets-ADSCs composite transplant.

Conclusions: Our innovative methods may improve the efficiency of islet transplantation and could have a significant clinical impact on the patients with type 1 diabetes.

351.2

What are factors affecting insulin independence in patients after total pancreatectomy and islet autotransplantation (TPIAT)?

Julia Solomina[1], Sabrina Matosz[1], Celeste Thomas[2], Andres Gelrud[2], Zehra Tekin[1], Justyna Golebiewska[3], Maciej Sledzinski[4], Karolina Golab[1], Lindsay Schenck[1], W. James Chon[2], Sabarinathan Ramachandran[1], Martin Tibudan[1], Jeffrey B.Matthews[1], Piotr Witkowski[1].
[1]Surgery, University of Chicago, Chicago, United States; [2]Medicine, University of Chicago, Chicago, United States; [3]Nephrology, Transplantology and Internal Medicine, Medical University of Gdańsk, Gdańsk, Poland; [4]General Surgery, Endocrinology and Transplantology, Medical University of Gdańsk, Gdańsk, Poland.

Background: Islet autotransplantation allows preserving glucose control with or without insulin supplementation after total pancreatectomy. Insulin independence has been correlated with higher islet beta cell mass transplanted[1]. However, there are still patients who require insulin treatment despite receiving high islet mass. In this study, we looked for other factors influencing insulin independence after islet autotransplantation.
Material and Method: Twelve recent patients after TPIAT were submitted to the Mixed Meal Tolerance Test (MMTT) on day 75 after pancreatectomy and islet transplantation. Patients with peak blood glucose below 180mg/ml on MMTT were asked to stop insulin supplementation. Patients with subsequent hyperglycemia and rising A1c over 6.5 mg/ml had insulin reinstalled or increased, and those with episodes of hypoglycemia- insulin reduced or stopped.
Results: Six patients became insulin free and remaining 6 required insulin support. Five patients, who were off insulin, received at least 200,000 islet equivalents (IEQ). Although our last patient from this group was infused with only 175,000 IEQ, it constituted over 2,500 IEQ/kg body mass due to his low body weight. His glucose level oscillated between 90mg/ml and 120mg/ml with relatively low peak serum c-peptide concentration- 0.72pmol/ml but high- 4.4 fold increase in MMTT, indicating his high insulin sensitivity.
On the other hand, 4 patients who received less than 100,000 IEQ (<=1,000 IEQ/kg) required chronic insulin support, as expected. However, 2 remaining patients required insulin treatment despite receiving islet infusion in relatively high doses of 3,6000 IEQ and 4,100 IEQ/kg. First patient had peak of glucose at 200mg/ml with relatively low peak c-peptide of 0.8pmol/ml in MMTT, indicating low islet engraftment. In the next 6 months, the same patient gradually intensified physical exercise and required only 2 units of insulin daily. She lowered her daily blood glucose excursion from 300mg/ml to below 180ml/mg, most likely due to increased insulin sensitivity, with serum A1c declining from 6 to 5.4.
Second patient presented normal MMTT results with peak blood glucose of 154mg/ml and c-peptide of 1.75pmol/ml. Patient still requires at least 10 units of insulin per day to prevent hyperglycemia due to high sugar diet (>50 carbs per meal).
Conclusion: Insulin independence strongly correlates with higher infused islet mass but it can be compromised by poor islet engraftment, high glucose intake with meals, and with increased insulin resistance. Luckily, physical exercise and low sugar diet can improve glucose control and insulin sensitivity as well as lower insulin requirements. Such observations are consistent with borderline beta cell islet mass and function in patients after TPIAT.
References:
[1] Savari O, Gołąb K, Wang LJ, Ramachandran S, Schenck L, Grose R, Tibudan M, Chon JW, Millis MJ, Matthews JB, Gelrud A, Witkowski P. Preservation of Beta Cell Function Following Pancreatic Islet Autotransplantation. University of Chicago Experience. The Am Surg. 2015. Apr; 81(4): 421-7.

351.3

Cyanidin-3-O-glucoside (C3G) improves the cell viability of human islets treated with human amylin or Abeta1-42 in vitro

Josue Rodrigues Silva[1], Wen Fu[2], Jack H.Jhamandas[2], Gina R.Rayat[1].
[1]Department of Surgery, University of Alberta, Edmonton, Canada; [2]Department of Medicine, University of Alberta, Edmonton, Canada.

Background: Amyloid deposition is linked to different diseases such as type 2 diabetes and Alzheimer's disease in which amyloid is formed by islet amyloid polypeptide (IAPP, also called amylin) and amyloid β (Aβ), respectively. Amyloid aggregation has been thought to induce processes that lead to a perturbation in the structure of lipid membranes, which subsequently disturb cellular ion homeostasis and trigger a cascade of events, including the formation of reactive oxygen species (ROS). Flavonoids are known to have antioxidant property and are capable of protecting islets from the harmful effect of ROS. Previously we showed that C3G a flavonoid found in Chinese bayberry (Myrica rubra) enhanced the function of mouse and pig islets after transplantation into diabetic mice[1],[2]. In this study, we tested the effect of C3G on human islets *in vitro*.
Methods: Human islets were cultured with or without C3G for 24 hours, and then treated with human amylin or $A\beta_{1-42}$ at physiological conditions. Samples were stained with Thioflavin-S, Calcein/Ethidium and dichlorodihydrofluorescein diacetate to evaluate the amyloid aggregation, cell viability and ROS by ImageJ and stereological analysis. The ultrastructure of islets was also assessed using transmission electron microscope (TEM).
Results: Treatment of human islets with C3G reduced the amyloid-like deposition compared to untreated islets. C3G treatment also improved the survival of islet cells and reduced the presence of ROS in the islets that were exposed to human amylin or $A\beta_{1-42}$. TEM images of islet cells treated with C3G showed an increased autophagic activity in contrast with other conditions.
Discussion: The natural electron deficiency of anthocyanins such as C3G, endows these compounds with high reactivity toward ROS, and they are therefore among the most powerful natural antioxidants in dietary plants. In this study, we found that human amylin and $A\beta_{1-42}$ are toxic to human islets similar to what was reported in neurons[3],[4]. However, C3G attenuated this effect and enhanced the viability of human islets as well as decreased the levels of ROS in the presence of human amylin and $A\beta_{1-42}$. Further analysis of TEM images of human islets showed that selective degradation of organelles such as mitochondria and endoplasmic reticulum as well secretory granules was prominent in islets treated with C3G. It is possible that this selective autophagy plays an important role in protecting islets against cell death in the presence of C3G.
Conclusion: Our preliminary results indicate that C3G could enhance the survival of human islets *in vitro* by reducing amyloid-like aggregates and ROS and by increasing autophagy as a survival mechanism under oxidative stress. It remains to be determined whether C3G could also enhance insulin secretion of human islet beta cells.
Alberta Diabetes Institute
References:
[1] 1. Cai H, et al. Cyanidin-3-O-Glucoside enhanced the function and survival of mouse islets transplanted under the kidney capsule or into the portal vein. *Transplantation*. 2015; 99: 508-514.
[2] 2. Li C, et al. Protective effect of Cyanidin-3-O-Glucoside on xenotransplanted neonatal porcine islets through ERK1/2 and PI3K/Akt-induced Nrf2-mediated HO-1 induction. 2015 IPITA, CTS, and IXA Joint Congress (Melbourne, Australia, November 15-19, 2015).
[3] 3. Lim YA, et al. Aβ and human amylin share a common toxicity pathway via mitochondrial dysfunction. *Proteomics* 2010; 10: 1621-1633.
[4] 4. Jhamandas JH, Mactavish D. Antagonist of the amylin receptor blocks β-amyloid toxicity in rat cholinergic basal forebrain neurons. *J Neurosci*.2004; 24: 5579-5584.

351.4

The protective effect of dimethyl fumarate on human pancreatic islets

Shiri Li[1], Nosratola D. Vaziri[2], Chie Takasu[3], Lourdes Robles[1], Akihiro Shimomura[1], Clarence E. Foster[1], Michael J Stamos[1], Hirohito Ichii[1].
[1]Department of Surgery, University of California, Irvine, Orange, CA, United States; [2]Department of Medicine, University of California, Irvine, Orange, CA, United States; [3]Department of Digestive and Pediatric Surgery, Tokushima University Graduate School, Tokushima, Japan.

Background: It is well known that pancreatic islets contain low level of antioxidants which renders them vulnerable to oxidative stress. Activation of Nuclear factor erythroid 2-related factor 2 (Nrf2) pathway induces up-regulation of numerous genes encoding antioxidant and phase II detoxifying enzymes and related proteins. Our previous study showed that treatment of human pancreatic islets with Nrf2 activator, dh404, protected beta cells against oxidative stress by significantly increasing expression of anti-oxidant enzymes. Dimethyl fumarate (DMF), a novel anti-inflammatory drug, was recently approved by the FDA for the treatment of multiple sclerosis. DMF has been shown to activate the cytoprotective Nrf2 pathway, inhibit production of pro-inflammatory mediators, and modulate the immune system. In this study, we tested the hypothesis that treatment with DMF in vitro may protect human pancreatic islets and improve islet function in vivo.
Methods: Human islets obtained from cadaveric donors were cultured in media containing DMF or vehicle. After culture, Nrf2 translocation of islet was examined by immunofluorescence and confocal microscopy. The expressions of key antioxidant proteins in islet were also assessed by western blot. To evaluate the potency of islets in vivo, 2000 IEQ of human islets were transplanted to diabetic nude mice. Glucose tolerance test was performed four weeks after transplant.
Results: Nrf2 nuclear translocation confirmed by confocal microscopy 6 hours after DMF treatment, revealed a concentration-dependent increase in expression of anti-oxidant enzyme, including NQO-1, GCLC and GCLM. Transplantation with 2000 IEQ islets in both DMF and vehicle groups reversed diabetes in all diabetic nude mice. Nevertheless, DMF treatment group showed significantly lower blood glucose value at 10, 30 min and the area under the curve of the IPGTT graph.
Conclusion: Treatment of human pancreatic islets with DMF significantly up-regulated expression of the key anti-oxidant enzymes and improved islet function in vivo.

351.5

Optimization of pre-vascularization procedures for improving islet engraftment under the skin

Satomi Uematsu[1], Akiko Inagaki[2], Kozue Imura[2], Yasuhiro Igarashi[2], Noriaki Ohuchi[1], Susumu Satomi[1], Masafumi Goto[1,2].
[1]Division of Advanced Surgical Science and Technology, Tohoku University School of Medicine, Sendai, Japan; [2]Division of Transplantation and Regenerative Medicine, Tohoku University School of Medicine, Sendai, Japan.

Introduction: The current standard procedure of islet transplantation, intraportal injection, has several issues to be solved including strong innate immune reactions, risk of portal vein embolism, and limitation of repeated injections. By contrast, in subcutaneous site, the procedure of islet injection is minimally invasive, thus it is possible to repeat islet transplantation. However, it is well known that subcutaneous site has poor vascularization. Therefore, optimization of pre-vascularization procedures prior to islet transplantation is crucial for improving the outcome of subcutaneous islet transplantation. Although the effectiveness of basic fibroblast growth factor (bFGF) was previously reported, the detailed procedures including heparin necessity remain uncertain. Here, we sought to optimize pre-vascularization procedures to subcutaneous transplant site.
Materials and Methods: The releasing devices including bFGF (0, 10, 20, or 30 ug) with or without heparin were implanted into the subcutaneous space of diabetic C57BL/6 mice 5-7 days after Streptozotocin injection. Vascularized area was assessed using contrast enhanced computed tomography (CECT). Marginal amount of syngeneic islets were transplanted into the pre-vascularized space 9-11 days after removal of the devices. Blood glucose, body weight, intra-peritoneal glucose tolerance test of the recipients, and immunohistochemistry of the grafts were evaluated.
Results and Discussion: The curative rates of diabetic recipients in both the bFGF 30 ug without heparin group and 20 ug with heparin group were significantly higher than that in the control group ($P<0.05$). According to the CECT, irrespective of bFGF dosage, pre-vascularized area in the heparin-supplemented groups was apparently expanded comparing with bFGF alone groups. In contrast, no beneficial effects on graft survival were observed by heparin addition in any dosages of bFGF groups. The curative rate in the highest dosage of bFGF combined with heparin group was even worse compared to the bFGF(-) group. Most likely, this is due to effusion and bleeding caused by synergistic effects of bFGF and heparin. Of particular interest, the curative rate in the bFGF(-) group was considerably higher than that in the control group (66.7% vs 16.7%), and was comparable to those in the bFGF groups. Especially in severe diabetic models, the duration to cure was significantly higher in the bFGF 20 ug and 30 ug groups ($P=0.03$, $P=0.01$, respectively), and also glucose tolerance was significantly ameliorated in the bFGF 10 ug group ($P=0.04$), suggesting that bFGF could additionally contribute to raising the graft function by promoting pre-vascularization to the subcutaneous site.
Conclusion: These data suggest that not only chemical effects by bFGF, but also mechanical stimulation based on foreign-body reactions might be crucial for pre-vascularization to the subcutaneous transplant site. Material compatibility for efficient pre-vascularization would therefore be a novel strategic target towards improving islet engraftment under the skin.
Megumi Goto; Takehiro Imura

351.6

Y1 receptor antagonism enhances insulin secretion and improves islet transplantation efficiency

Stacey Walters[1], Kim Loh[2], Yan-Chuan Shi[2], Kailin Lee[2], Herbert Herzog[2], Shane Grey[1].
[1]Immunology, Garvan Institute, Darlinghurst (Sydney), Australia; [2]Neuroscience, Garvan Institute, Darlinghurst (Sydney), Australia.

Introduction: Islet transplantation for the treatment of type-1 diabetes is hampered due to insufficient availability and quality of donor islets. One option to improve this is to enhance β-cell function through pharmacological modulation therefore reducing the number of donor islets required. Efforts have previously focused on pathways that stimulate insulin secretion with limited success; here we investigate a novel role for the neuropeptide YY (PYY) in the termination of insulin secretion. PYY can act upon the Y1 receptor which is widely expressed in pancreatic β-cells. Y1R is a G-protein coupled receptor that preferentially associates with Gi/o G-protein and can act as an inhibitor for intracellular cyclic AMP (cAMP) levels. It has been demonstrated cAMP reduction can reduce insulin release, indicating potential for an inhibitory effect of Y1 receptor signalling induced by locally produced PYY in regulating insulin release.
Materials and Methods: $Y1^{lox/lox}/INS2^{cre/+}$ mice were generated. Isolation & transplantation of islets, glucose stimulated insulin release (GSIS) & intravenous glucose tolerance test (IVGTT) were conducted as per standard protocols.
Results: GSIS on C57/BL6 mouse islets in the presence of PYY exhibited reduced insulin release; however this reduction was abolished in the presence of a Y1 selective antagonist, highlighting the critical role of Y1 signaling as a negative regulator of insulin release. Moreover islets from β-cell-specific $Y1R^{-/-}$ mice exhibited increased cAMP and insulin secretion (GSIS). To further explore these findings islet transplantation was performed. Using a minimal mass transplant model, $Y1R^{-/-}$ islets showed significantly better glucose control compared to controls and restored normoglycaemia in 100% of cases. Mice harboring $Y1R^{-/-}$ islets had improved glycemic control and increased insulin secretion compared to controls (IVGTT), demonstrating that lack of Y1 receptor signaling is advantageous for islet function especially where islet numbers are of limited supply. To see whether these effects could be induced pharmacologically transplant experiments were repeated using C57/BL6 islets +/- Y1R antagonist administered orally. Compared to controls treated mice rapidly regained normoglycaemia and were able to maintain this after the treatment regime had ceased (10 doses). This suggests that even short-term blockage of Y1 signaling in islet transplantation is sufficient to improve insulin secretion and normalize glucose homeostasis. Furthermore these experiments were repeated using human islets and the results were duplicated.
Conclusion: Together these data demonstrate that manipulation of Y1R signaling can enhance β-cell function in situations where islet mass is limited. Importantly utilizing this finding via pharmacological intervention has the potential to improve clinical islet transplant efficiency and help overcome the limited availability of transplant material.

351.7

Human mesenchymal stromal cells improve survival and function of pancreatic islets by cell-to-cell contact through N-cadherin

Elisa Montanari[1], Redouan Mahou[1,2], Solene Passemard[2], Francois Noverraz[2], Philippe Morel[1], Raphael Meier[1], Domenico Bosco[1], Jorg D.Seebach[3], Christine Wandrey[2], Sandrine Gerber-Lemaire[2], Carmen Gonelle-Gispert[1], Leo H.Buhler[1].
[1]Department of Surgery, Cell Isolation and Transplantation Center, Geneva, Switzerland; [2]Ecole Polytechnique Fédérale de Lausanne, Institut d'Ingénierie Biologique et Institut des Sciences et Ingénierie Chimiques, Lausanne, Switzerland; [3]Division of Immunology and Allergy, Division of Immunology and Allergy, Geneva, Switzerland.

Background and Aim: The aim of this study was to evaluate the survival and function of human pancreatic islets co-encapsulated with human Mesenchymal Stromal Cells (MSC) both in vitro and in vivo after transplantation in diabetic mice.
Methods: Human MSC and islets (or pseudo-islets, obtained after digestion and reaggregation of islet cells) were coencapsulated in new hydrogel microspheres composed of calcium alginate and covalently crosslinked polyethylene glycol. Encapsulated cells were transplanted intraperitoneally in streptozotocin-induced diabetic mice. Islet function was evaluated by intraperitoneal glucose tolerance test (IPGTT). Grafts were retrieved after 15 days for morphological analysis. Cell function was tested in vitro by static incubation for islets or pseudo-islets alone and together with MSC. Anti-N-cadherin antibody was added on islets alone or together with MSC for 24h and in vitro insulin secretion was tested by static incubation.
Results: Encapsulated islets alone reversed diabetes in mice after intraperitoneal transplantation after 2 days and allowed to maintain normoglycemia up to 70 days, compared to free islets, that were rejected in 6±1 days ($p<0.0001$, Mantel Cox). Transplantation of co-encapsulated islets and MSC maintained normoglycemia in mice up to 90 days ($p<0.05$, Mantel Cox). IPGTT was performed at day 15 and mice transplanted with combined MSC-islets showed an improved glycemic response compared to mice with islets alone ($p<0.001$). Graft histology showed MSC located within and around the islets (or pseudo-islets), serving as stromal structure. In vitro, insulin secretion was significantly improved when MSC were in cell-cell contact with islets (or pseudo-islets) compared to islets that were only in paracrine exchange with MSC (co-culture in dual chambers, $p<0.05$). When N-cadherin was blocked, glucose-induced insulin secretion was decreased in islets cocultured with MSC and not affected in islets incubated alone.
Conclusions: The adhesion molecule N-cadherin is essential for MSC and islets cell-to-cell contact to improve function and survival of islets of Langerhans.

351.8

Protection of stem-cell-derived insulin-producing cells from ischemic injury

Gaetano Faleo[1], Holger Russ[2], Vinh Nguyen[1], Jonathan Freise[1], Annie Albright[1], Steven Wisel[1], Karina Villanueva[2], Matthias Hebrok[2], Qizhi Tang[1,2].
[1]Department of Surgery, Transplantation Research Laboratory, UCSF, San Francisco, United States; [2]Diabetes Center, UCSF, San Francisco, United States.

Introduction: Insulin-producing cells can now be reproducibly generated in large numbers from human embryonic stem cells, providing a renewable source of beta cells for the treatment of type 1 diabetes. Past experiences in experimental and clinical islet transplantation show that a large proportion of islets die shortly after transplant due to ischemia, contributing to poor long-term outcomes. In this study, we investigated the impact of ischemia to human stem-cell-derived insulin-producing cells (SCIPC). Ischemia leads to both hypoxia and nutrient deprivation and each can independently kill islet cells.
Materials and Methods: We exposed SCIPC and mature islets to hypoxia, nutrient deprivation, or both *in vitro* and measured cell viability using flow cytometry.
Results and Discussion: Our results show that SCIPC are more resistant to hypoxia or nutrient deprivation alone than mature islets. However, combined hypoxia and nutrient deprivation synergistically killed SCIPC (Figure 1).

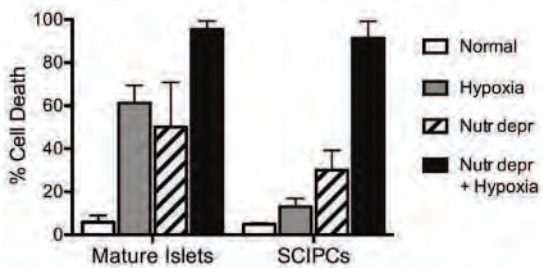


Figure 1. Impact of hypoxia and nutrient deprivation on islet cell death. Mature mouse islets and SCIPC were cultured in normal, hypoxic, nutrient deprived, or both hypoxic and nutrient deprived conditions for 24 hours. Cell viability was measured using flow cytometry after staining the cells with propidium iodide.

Similarly, using luciferase-expressing SCIPC, we found that more than 50% of SCIPC die within 3 days after transplantation into the subcutaneous space of immunodeficient mice. Grafts that survived ischemia contained lower percentages of insulin-producing cells than before transplant suggesting more differentiated cells were more susceptible to ischemia. We next investigated approaches to mitigate ischemia damage to SCIPC. We intentionally exposed SCIPC to 5% oxygen instead of the normal 21% oxygen during their *in vitro* differentiation culture. This did not affect the efficiency of SCIPC differentiation, but made the cells resistant to later challenge to extreme hypoxia (Figure 2A).
We explored nutrient supplementation as a strategy to protect against nutrient deprivation. We found that addition of glucose to base medium was ineffective in protecting SCIPC in nutrient-limiting cultures. In contrast, single amino acid supplementation with alanine or glutamine, but not phenylalanine, could rescue SCIPC from nutrient deprivation (Figure 2B). Protection by amino acids was intact in the presence of rapamycin suggesting that it was independent of amino-acid-induced mTOR activation. Instead, amino acids likely served as cellular fuels by being substrates of the tricarboxylic acid cycle because pyruvate was similarly effective at protecting SCIPC. Lastly, we found hypoxia preconditioning along with local supplementation of alanine and glutamine improved SCPIC survival after transplantation *in vivo*.

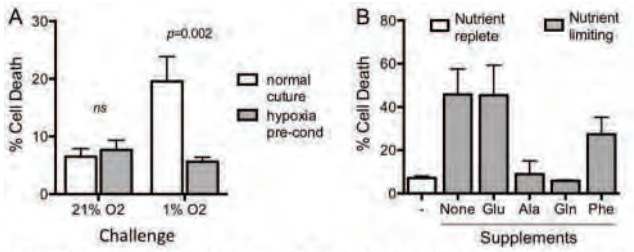


Figure 2. Protecting SCIPC against hypoxia and nutrient deprivation. (A) SCIPC were differentiated under normal culture condition or at 5% oxygen (hypoxia pre-cond). The cells were then challenged with exposure to 1% oxygen and cell viability was measured after 24 hours. (B) SCIPC were cultured in nutrient-replete or nutrient-limiting condition without supplements or with glucose (Glu), alanine (Ala), glutamine (Gln), or phenylalanine (Phe). Cell viability was measured after 5 hrs.

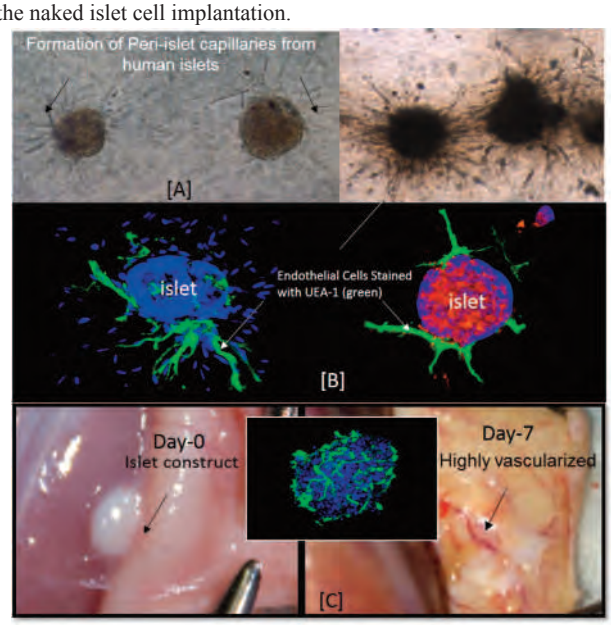
Conclusion: Taken together, our results demonstrate that SCIPC are susceptible to ischemic injury and rationalized interventions can effectively protect them from ischemia after transplantation.
The Larry L. Hillblom Foundation; American Diabetes Association

351.9

Sprouting of Intra-islet endothelial cells to form peri-islet vessels in human islets

Balamurugan Appakalai, Gopalakrishnan Loganathan, Venkat M.Ramakrishnan, Nolan L. Boyd, Jason E.Beare, Michael J. Hughes, James B.Hoying, Stuart K.Williams .
Cardiovascular Innovation Institute, Department of Surgery, University of Louisville, Louisville, United States.

The current islet isolation technique completely severs the vasculature to islets. As such, these islets become avascular and are susceptible to ischemic damage. Stimulation of intraislet endothelial cells (IIEC) to form peri-islet capillaries (PIC) in vitro prior to transplantation may accelerate post-implant islet revascularization. In this study we accelerated the formation of a functional microcirculation to human islets by stimulating the intra-islet endothelial cells with the addition of ECGS (a mixture of endothelial growth factors) in a 3D-culture system.
Human islets cells were isolated from brain-dead cadaveric donor pancreas (n=10). In in vitro experiments, human islets (200 per well) were cultured with ECGS in a 3D collagen-I gel for 14 days. Confocal microscopy was used to assess islet-derived PIC growth. Human endothelium was identified via labeling with UEA-1 staining. To study inosculation potential, the aforementioned collagen gel construct were be implanted in nude mice at subcutaneous site.
Naked islets cultured in a 2D environment did not form any sprout. However, the presence of ECGS and 3D-culture system induced cellular sprouting from human islets within 7 days (Figure). Many of these sprouts appeared to be PIC since they resembled a vasculature that arose from the islets. UEA-1 FITC Green and RedDot 2 (nuclear label) labeling indicated that several of these sprouts were endothelial. We speculate that our isolated islets gave rise to peri-islet sprouts. 3D-cultured islets maintained >90% cellular viability after 14 days, whereas 2D-cultured islets displayed disintegration of their borders as well as reduced viability. And also, we have confirmed, the inosculation ability of islet construct with the host blood circulation was better than that the naked islet cell implantation.



Here, we demonstrate that 3D matrix support and ECGS are required to elicit successful, spontaneous PIC formation in human islets. This may provide unique avenues to accelerate islet neovascularization following implantation.

352.1

Kidney transplantation from uncontrolled donors after circulatory death: the Spanish experience

Cristina Vidal[1], Francisco Del Río[2], Amado Andrés[3], Angel Ruiz[4], Teresa Aldabó[5], José M.Pérez-Villares[6], Lorena Z.Peiró[7], Antón Fernández-García [8], Eduardo Miñambres[9], Juan Galán[10], Pablo Castro[11], Marga Sanromá[12], Elisabeth Coll[1], Beatriz Domínguez-Gil[1], Rafael Matesanz[1].
[1]Organización Nacional de Trasplantes, Madrid, Spain; [2]Hospital Clínico San Carlos, Madrid, Spain; [3]Hospital Doce de Octubre, Madrid, Spain; [4]Hospital Clínic, Barcelona, Spain; [5]Hospital Virgen del Rocío, Sevilla, Spain; [6]Hospital Virgen de las Nieves, Granada, Spain; [7]Hospital de San Juan, Alicante, Spain; [8]Hospital Universitario A Coruña, A Coruña, Spain; [9]Hospital Marqués de Valdecilla, Santander, Spain; [10]Hospital Universitario la Fe, Valencia, Spain; [11]Coordinación Autonómica de Trasplantes de Andalucía, Sevilla, Spain; [12]Coordinación Autonómica de Trasplantes de Cataluña, Barcelona, Spain.

Background: Uncontrolled donation after circulatory death (uDCD) or Maastricht II DCD refers to organ donation from persons who have died following an unsuccessfully resuscitated cardiac arrest (CA). In Spain, national recommendations establish, *inter alia*, a maximum donor age of 60 years, maximum warm ischemia time (WIT) of 150 min and maximum time under abdominal preservation strategies of 180 min for in situ cooling (ISC) and 240 min for normothermic (nRP) or hypothermic regional perfusion (hRP). Single-centre studies have provided information on successful outcomes following the transplantation of kidneys from uDCD donors, but there is limited information on the factors that have an impact upon kidney graft survival.
Objective: To report the Spanish experience with kidney transplantation from uDCD donors, and to identify factors related to graft loss during the first year after transplantation.
Patients and Methods: A dedicated data collection on DCD donors and recipients is being undertaken at a national level since 2012. Information has been analysed for kidney recipients transplanted from uDCD donors during 2012 and 2013. Univariate (Kaplan-Meier) and multivariate survival analysis (Cox-model) have been performed to identify factors related to graft loss during the first year.
Results: During 2012-2013, 275 kidney transplants from 152 uDCD donors were performed in Spain.
Demographics and clinical characteristics of donors and key aspects of the uDCD process are depicted in **table 1**.

Table 1: Demographics and clinical characteristics of uDCD kidney donors and key aspects of the uDCD process. Spain 2012-2013.

	N=152
Age (years)	
Mean (SD)	45.2 (10.6)
Min-Max	15-79
Gender, n (%)	
Male	137 (90.1%)
Female	15 (9.9%)
Site of Cardiac Arrest, n (%)	
Out-of-Hospital (Maastricht IIa*)	146 (96.1%)
In-Hospital (Maastricht IIb*)	6 (3.9%)
Time from Cardiac Arrest to advanced Cardio Pulmonary Resuscitation (min)	
Median (IQR)	10 (6-13)
Min-Max	0-34
Warm Ischemia Time (from Cardiac Arrest to abdominal preservation) (min)	
Median (IQR)	126 (112-137)
Min-Max	67-185
Type of abdominal preservation method, n (%)	
Cardiac Compression, followed by *in situ* cooling	17 (13.5%)
Cardiac Compression, followed by hypothermic Regional Perfusion	64 (50.8%)
Cardiac Compression, followed by normothermic Regional Perfusion	42 (33.3%)
Cardiac Compression alone	3 (2.4%)
Time under abdominal preservation (min)	
Median (IQR)	165 (135-220)
Min-Max	60-323

* Modified Maastricht Classification for Donors after Circulatory Death (Madrid, Spain, 2012)

Mean recipient age was 49.5 (SD=11.7) years, 68.6% were male and 7% had a previous history of kidney transplants. *Ex situ* preservation of kidneys was performed with machine perfusion in 43.4% and cold storage in 56.6%.
The incidence of primary non function was 3% and that of delayed graft function (dialysis during the first week) was 78.8%. 1 year death-censored graft survival was 87%, with no statistically significant differences between those recipients with (85.5%) or without (92.6%) delayed graft function (p=0.162).
In the univariate analysis, 1 year death-censored graft survival was lower when the donor CA had occurred in the hospital (p=0.044), ISC of kidneys was used instead of hRP or nRP (p=0.004), and the recipient had a history of previous transplants (p=0.001). No statistically significant differences were observed by donor age (p=0.211), WIT (p=0.144), recipient age (p=0.404) or type of *ex situ* preservation of kidneys (p=0.973).
In the multivariate analysis (**table 2**), in-hospital donor CA, ISC of kidneys (*versus* hRP or nRP) and recipient´s previous history of kidney transplants were identified as independent risk factors for 1-year graft loss.

Table 2: Factors related with death-censored graft loss during the first year after transplantation among kidney recipients transplanted from uDCD donors (Cox-Model). Spain 2012-2013.

	OR	CI 95%		p
Donor in-hospital CA *(ref. out-of-hospital CA)*	**6.5**	**1.6**	**27.0**	**0.010**
Donor age > 55 years *(ref. donor age ≤ 55 years)*	1.7	0.7	4.0	0.219
WIT > 120 min (ref ≤ 120 min)	2.0	0.8	5.0	0.121
Type of abdominal preservation *(ref. normothermic Regional Perfusion)*				0.005
In situ cooling	**3.6**	**1.4**	**9.0**	**0.006**
hypothermic Regional Perfusion	1.0	0.4	2.2	0.927
Recipient age > 55 years *(ref. recipient age ≤ 55 years)*	0.6	0.26	1.4	0.233
Recipient with a history of previous transplants *(ref. no previous transplants)*	**5.2**	**2.1**	**12.7**	**<0.001**

Conclusion: Kidney transplantation from uDCD donors in Spain provides good outcomes during the first year. Abdominal hRP or nRP is preferred over ISC of kidneys. When the first technique is not available, more stringent donor and recipient selection criteria should be used.

References:

[1] Sánchez-Fructuoso AI. Ann Intern Med 2006; 145(3): 157-164.
[2] De Gracia MC. Transplant Proc 2012;44(9):2518-2520.
[3] Miranda-Utrera N. Clin Transplant 2013;27(2):283-288.
[4] Hoogland ER. Transplant Int 2013; 26(5):477-484.
[5] Abboud I. Nephrol Dial Transplant 2012;27(6):2583-2587.
[6] Domínguez-Gil B. Transpl Int 2015 Dec 26. doi: 10.1111/tri.12734.

352.2

Largest US OPO DCD Experience: 1014 DCD Donors over two decades

Howard Nathan[1], Richard Hasz[1], Sharon West[1].
[1]Gift of Life Donor Program, Philadelphia, PA, United States.

Purpose: To demonstrate that an effective DCD program can lead to an increase in the procurement of transplantable organs while simultaneously increasing DBD.
Methods: This was a single OPO, multi-center study evaluating the procurement and utilization of DCD donor organs. Ongoing hospital education on early referral and DCD protocols was initiated prior to DCD donor recoveries.
Results: Since the implementation of its DCD program in June 1995 through December 2015, this OPO has procured 1014 DCD organ donors. DCD donor procurement increased the donor pool by 13% and resulted in the transplantation of 1832 organs. This increase was achieved while simultaneously achieving a 83% increase in annualized DBD donor procurement for 2015 versus 1995. Mean donor age was 39 years (r = 0.5 - 76). The mean time from donor extubation to cross-clamp, or warm ischemic time (WIT), for kidneys transplanted (N=1597) was 32 minutes (r = 2 - 214). The mean WIT for livers transplanted (N=203) was 23 minutes (r = 2 - 69). The kidney ATN rate was 46% and kidney graft survival was 86% at 1 year, 75% at 3 years and 64% at 5 years. Liver graft survival was 72% at 1 year, 63% at 3 years and 56% at 5 years.

Year	Total Donors	DBD Donors Procured (% of Total Donors)	DCD Donors Procured (% of Total Donors)	DCD Kidney Utilization (Transplanted/ Procured)	DCD Liver Utilization (Transplanted/ Procured)	DCD Pancreas Utilization (Transplanted/ Procured)	DCD Lung Utilization (Transplanted/ Procured)	Total DCD Organs Transplanted
1995	222	220 (99%)	2 (01%)	4/4 (100%)	1/1 (100%)	-	-	5
1996	262	250 (95%)	12 (05%)	17/24 (71%)	2/4 (50%)	-	-	19
1997	292	278 (95%)	14 (05%)	21/28 (75%)	3/6 (50%)	-	-	24
1998	298	273 (92%)	25 (08%)	37/48 (77%)	9/14 (64%)	-	-	46
1999	331	307 (93%)	24 (07%)	43/48 (90%)	9/12 (75%)	-	-	52
2000	298	275 (92%)	23 (08%)	42/44 (95%)	9/12 (75%)	-	-	51
2001	315	283 (90%)	32 (10%)	50/64 (78%)	13/18 (72%)	2/3 (67%)	-	65
2002	354	318 (90%)	36 (10%)	65/72 (90%)	12/19 (63%)	0/1 (00%)	-	77
2003	344	293 (85%)	51 (15%)	77/96 (80%)	12/21 (57%)	1/1 (100%)	-	90
2004	387	340 (88%)	47 (12%)	72/94 (77%)	9/14 (64%)	4/4 (100%)	-	85
2005	382	325 (85%)	57 (15%)	86/108 (81%)	22/28 (79%)	4/6 (67%)	-	114
2006	401	334 (83%)	67 (17%)	122/132 (92%)	16/28 (57%)	2/5 (40%)	-	140
2007	389	342 (88%)	47 (12%)	83/94 (88%)	11/21 (52%)	-	-	94
2008	428	358 (84%)	70 (16%)	100/136 (74%)	14/23 (61%)	-	4/4 (100%)	118
2009	439	374 (85%)	65 (15%)	89/129 (69%)	8/22 (36%)	0/1 (0%)	2/2 (100%)	99
2010	392	307 (78%)	86 (22%)	142/165 (86%)	8/25 (32%)	0/2 (0%)	-	150
2011	441	357 (81%)	84 (19%)	130/167 (78%)	10/26 (38%)	-	0/2 (0%)	140
2012	417	352 (84%)	65 (16%)	90/130 (69%)	7/21 (33%)	0/3 (0%)	2/2 (100%)	99
2013	447	386 (86%)	61 (14%)	99/119 (83%)	6/17 (35%)	0/1 (0%)	7/16 (44%)	112
2014	447	371 (85%)	66 (15%)	102/132 (77%)	12/26 (46%)	0/1 (0%)	0/4 (0%)	114
2015	483	402 (83%)	81 (17%)	124/161 (77%)	10/24(42%)	1/1 (100%)	3/11 (27%)	138
Total	7,769	6,745 (87%)	1,014 (13%)	1597/1995 (80%)	203/382 (53%)	14/30 (47%)	18/41 (44%)	1,832

Conclusion: An effective DCD program increased the donor pool by 13%. DCD donor procurement was achieved while simultaneously achieving a 83% increase in DBD donor procurement over the two decade evaluation period.

352.3

Experience with donation after circulatory death (DCD) in donors aged 56-65: A preliminary report on kidney utilization and initial outcomes

Jeffrey P. Orlowski[1], Chad C. Muse[1], Janice F. Whaley[1], Jan Foutch[1], Ronald A. Squires[1,2].
[1]LifeShare Transplant Donor Services of Oklahoma, Oklahoma City, OK, United States; [2]Department of Surgery, University of Oklahoma Health Science Center, Oklahoma City, OK, United States.

Introduction: One U.S. organ procurement organization (OPO) achieved 96% growth in total deceased organ donors and 66% growth in organs transplanted in 24 months (comparing year-end 2013 to year-end 2015 data). One contributing factor has been aggressive pursuit of DCD initiated in 2012 including use of DCD donors aged 56-65 years. The following is a preliminary report on our experience utilizing these older DCD donors.
Methods: A retrospective review of DCD donors recovered between 2012 and 2015 was conducted. DCD donors 0-55 years of age (DCD 0-55) were compared to those aged 56-65 years (DCD 56-65). Time from extubation to arrest, time from extubation to organ perfusion, duration of agonal period, kidney transplantation rates, initial function, and function at time of data analysis were compared.
Results and Discussion: Between 2012 and 2015, 93 total DCDs were recovered (18.7% of 498 total deceased donors); in 2012 DCDs were 10 of 94 total deceased donors (10.6%) which increased to 42 of 174 total deceased donors (24.1%) in 2015. Overall, 14 of 93 DCD's during the 4 years (15.1%) were age 56-65. For DCD 0-55, 83% (131/158) of kidneys were transplanted; for DCD 56-65 utilization was 68% (19/28). Extubation to arrest and perfusion averaged 21 and 38 minutes respectively in DCD 0-55 vs 14 and 32 minutes in DCD 56-65; average agonal time was 3 minutes in both groups. Delayed graft function (DGF) was 34% for DCD 0-55 and 21% for DCD 56-65. Current function at time of data analysis was 95% for DCD 0-55 and 79% for DCD 56-65. With the exception of 6 DCD 0-55 kidneys, all kidneys underwent pulsatile perfusion.
Conclusions: Our data suggest DCD donors aged 56-65 years can be recovered with careful selection. While kidney utilization was higher in the DCD 0-55 group, the DCD 56-65 group's kidney utilization was 68%, which we accept as a very reasonable rate of transplant for this population that many U.S. OPOs do not pursue. The fact that DGF in DCD 56-65 was 21% compared to 34% for DCD 0-55 kidneys was unexpected and may be related to shorter time from withdrawal to arrest or may also indicate we are overly selective in DCD 56-65. While current kidney graft function in the DCD 56-65 group is lower (79%) than the 0-55 group (95%), not enough data is known about the recipients' characteristics or reasons for graft failure to draw any conclusions. We are committed to continue selective use of these donors and plan a thorough multivariate analysis of donors and recipients with a larger sample size; at our current DCD recovery rate, we expect to approximately double our DCD experience in the next 24 months which will allow for a statistically more powerful analysis.

352.4

World first report of liver transplantation from an HIV-positive donor to an HIV-positive recipient

Christian van Delden[1,3], Alexandra Calmy[3], Emiliano Giostra[1], Christian Junet[3], Laura Rubbia Brandt[2], Sabine Yerly[3], Jean-Pierre Chave[3], Thierry Berney[1].
[1]Service of Transplantation, University Hospitals Geneva, Geneva, Switzerland; [2]Department of Pathology, University Hospitals Geneva, Geneva, Switzerland; [3]Service of Infectious Diseases, University Hospitals Geneva, Geneva, Switzerland.

Introduction: HIV-positive candidates for solid organ, and especially liver, transplantation remain disadvantaged on waiting lists because of faster evolution towards organ failure. Transplantation of organs from HIV-positive donors is illegal in most countries due to concern of HIV donor-recipient transmission. Only transplantation of kidneys restricted to treatment-naive or first-line treated HIV-positive donors to HIV-positive recipients have been reported from South Africa so far. This discriminates HIV-positive patients on successful Anti Retroviral Therapy (ART) from organ donation, and reduces the donor pool for HIV-positive potential recipients. In 2007 the Swiss Transplantation Law included the opportunity to transplant organs from HIV-positive donors to HIV-positive recipients, independently of previous ART and resulting potential viral resistance phenotypes. We report the first liver transplantation from an ART-experienced HIV-positive deceased donor to an ART-experienced HIV-infected recipient in Switzerland.
Methods: A patient infected by HIV since 1987, treated with dolutegravir, tenofovir and emtricitabine with undetectable viremia despite multiple nucleoside retrotranscriptase inhibitor resistance mutations died of a cerebellar haemorrhage. He had previously provided written explicit consent for organ donation. We therefore identified a 53-year-old Caucasian male, HIV-infected since 1987, successfully treated with a combination regimen of rilpivirine/tenofovir/emtricitabine for an HIV and HBVco-infection, awaiting a liver donation.
Results: The transplantation candidate was informed about potential virus transmission and the requirements to adapt his anti-HIV therapy. He accepted the risk and signed an informed consent for liver transplantation. Post-transplant the liver graft showed immediate function, and no medical or surgical complications occurred. The immunosuppressive regimen was standard associating tacrolimus and mycophenolate mofetil. The recipient's antiretroviral therapy was modified by the addition of raltegravir and enfuvirtide to his ongoing regimen. This regimen covered the HIV-resistance mutation patterns of both donor and recipient. We documented no interactions between the anti-HIV therapy and the immunosuppressive drugs based on normal target tacrolimus plasma levels. No rejection episodes occurred. The patient could be discharged on day 22 post-transplant. Plasma HIV-RNA remained undetectable throughout the four-month follow-up.
Conclusion: We report here the world first successful liver transplantation from a non-treatment naive HIV+ donor to an HIV-positive recipient. Despite numerous resistance mutations in both the donor and recipient's viruses the HIV-infection remained controlled at 4 months follow-up by modifying the ART. This encouraging observation should lead policy makers to lift the restriction limiting the transplantation of organs from non-treatment naive HIV-positive donors towards HIV-positive recipients, increasing thereby the pool of potential donors to this disadvantaged transplant candidates.

352.5

Initial experience of en-bloc kidney transplantation from donors less than two months of age in the United Kingdom: A potential new donor pool

Imeshi Wijetunga[1], Rupaly Pande[1], Sonsoles Martinez-Lopez[1], Shahid Farid[1], Muhammed Jameel[1], Vivek Upasani[1], Lutz Hostert[1], Matthew Wellberry-Smith[1], Chas Newstead[1], Andrew Lewington[1], Clare Ecuyer[1], Richard Baker[1], Niaz Ahmad[1].
[1]Department of Transplantation, Leeds Teaching Hospitals NHS Trust, Leeds, United Kingdom.

Introduction: Kidney transplantation from donors less than two years of age have been rare in the United Kingdom (UK) at the rate of one transplant per year over a 15-year period[1]. A kidney transplant from a donor less than two-months of age was first performed in the UK in 2013. Until recently (2015), UK legislation did not permit the diagnosis of brain-stem death (BSD) in infants under two-months of age and only DCD donations have been possible. We describe our initial experience of kidney transplantation from donors less than two-months, including those from neonatal donors.
Methods: Kidney transplants performed at our centre from donors less than two-months of age were identified from a prospective database. All kidneys were retrieved and transplanted en bloc with abdominal aorta and inferior vena cava. Hypothermic machine perfusion was used in most cases. Recipients were young to middle-age adults with body mass index <30 and no cardiovascular risk factors. Donor & recipient characteristics as well as short-term graft & recipient outcomes are described.
Results: Seven En bloc Kidney Transplants (EKT) were performed from donors less than two-months of age from March 2013 to October 2015, six from DCD (86%) and one from DBD donor. Median follow up was 319 days (96-1,051). Median donor age and weight were 23days (range 0-58) & 3.4kg (1.9-5.0) respectively. Median recipient age and weight were 33yrs & 49.5kg, respectively. One primary non-function was observed and the recipient was excluded from subsequent analysis of graft function. The graft was from a 0day old 1.9kg DCD donor. Primary function was observed in 6 (86%) recipients with no early post-operative complications. Median GFR (Cockcroft-Gault, in ml/min/1.73m^2) at 1-month and 6-months post-transplant was 25 and 54 respectively. Graft function continues to improve over the first year with those completing one year (three) achieving a GFR>60.
Discussion: This is the first reported series of kidney transplants from donors less than two-months of age including neonatal donors in the UK. The recipient and graft outcomes are encouraging. Our study identifies small infants and neonates as a potential 'new' donor pool for kidney transplant. We suggest further consolidation of experience in this area in designated centres.
References:
[1] Dave R. Renal transplantation from paediatric donors in the United Kingdom. *Transplantation*. 2015 Sep; 99(9): 1968-75

352.6

Organ donation after euthanasia leads to good transplant results

Jan Bollen[1,6], Tim van Smaalen[4], Wim de Jongh[4], Hanneke Hagenaars[5], Rankie ten Hoopen[6], Dirk Ysebaert[3], Ernst van Heurn[2], Walther van Mook[1].
[1]Intensive Care, Maastricht University Medical Center, Maastricht, Netherlands; [2]Pediatric Surgery, Academic Medical Center, Amsterdam, Netherlands; [3]Hepatobiliary, Transplant and Endocrine Surgery, University Hospital Antwerp, Antwerp, Belgium; [4]Transplant Coordination, Maastricht University Medical Center, Maastricht, Netherlands; [5]Transplant Coordination, Erasmus University Medical Hospital, Rotterdam, Netherlands; [6]Medical Law, Maastricht University, Maastricht, Netherlands.

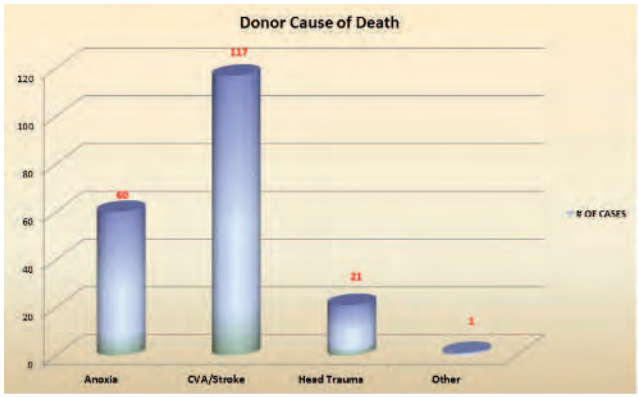
Introduction: Organ donors after euthanasia are considered Maastricht category 3 donation after circulatory death (DCD) donors. From these donors, kidneys, lungs, liver and pancreas can be procured and transplanted. Because this combined procedure has only rarely been performed, it is currently unclear if transplantation of these organs may result in acceptable transplant outcome. In this abstract we present the outcome of kidneys transplantations for all 15 first organ donations after euthanasia in the Netherlands. In August, we aim to present outcome of all donations until 2015 for lung, liver, pancreas and kidney transplantation in both Belgium as the Netherlands (±n=40).
Methods: All cases of donation after euthanasia in the Netherlands until 2015 were studied. Donor, graft and recipient characteristics were anonymized and registered. Primary outcome parameter was short term graft function. For kidney transplantation, this was defined as immediate graft function, delayed graft function or primary non-function.
Results: Fifteen patients donated organs after euthanasia. One donor had already donated one kidney to a relative during life. 29 kidneys were eligible for donation, of which all were procured. Three kidneys were rejected by the transplantation team. 26 kidneys were successfully transplanted, of which 14 (53,8 %) had immediate graft function, 11 (42,3 %) had delayed graft function, and 1 (3,8 %) suffered from primary non-function. The mean donor age was 52,1 (SD ± 13,0) years and mean recipients age was 52,8 (SD ± 12,3) years.
Discussion: Donation after euthanasia has only been performed a dozen times in the Netherlands. These results show that the outcome of kidney transplantation, with a majority of immediate function, is at least equal to regular DCD-3 donors[1]. The good outcome may be explained by a multiple factors. Firstly, the process of death differs for donors after euthanasia compared to donors who normally sustained severe head injury after trauma or a cerebrovascular accident. Furthermore, the agonal phase during donation after euthanasia is mostly short and controlled by the short time to death by euthanasia. Euthanasia donors are also, on average, younger than 'regular' DCD-3 donors[2].
Conclusion: We conclude that donation after euthanasia leads to acceptable short-term transplant results. Organ donation after euthanasia may be a valuable way to increase the number of organs available for the limited donor pool. Further studies need to evaluate long term graft function and graft and patient survival.
References:
[1] Summers DM, Watson CJ, Pettigrew GJ, et al. Kidney donation after circulatory death (DCD): state of the art. Kidney international 2015;88(2):241-9 doi: 10.1038/ki.2015.88
[2] Huynh MJ, Violette PD, Rowe NE, et al. Donation after Circulatory Death Renal Allografts—Does Donor Age Greater than 50 Years Affect Recipient Outcomes? The Journal of urology 2015;194(4):1057-61 doi: 10.1016/j.juro.2015.04.110

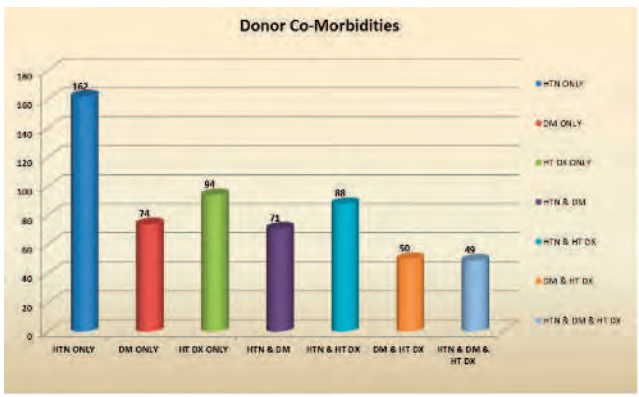
352.7

Saving lives one organ at a time: One OPO's experience in a service area with a high incidence of cerebrovascular accidents

Vernon Langley[1], Kathleen Lilly[1], Jean A.Davis[2], Bryan McDonald[3], Thomas Pearson[4], Nicole Turgeon[4], Marty Sellers[1].
[1]LifeLink of Georgia, LifeLink, Norcross, GA, United States; [2]Foundation, LifeLink, Tampa, FL, United States; [3]Accounting, LifeLink, Tampa, FL, United States; [4]Department of Surgery, Emory University, Decatur, GA, United States.

Introduction: From 2010-2014, LifeLink® of Georgia (GALL) noted a significant increase in single-organ donors (SODs). Review of national and Georgia-specific data[1] for age-adjusted mortality rates between 1994-2011 show our service area is higher for strokes (42.2 vs 38.9), diabetes (23.4 vs 20.9), and cardiovascular disease (242.5 vs 234.8) compared to the national average. GALL's donor service area, part of the "stroke belt" due to a high concentration of cerebrovascular accident (CVA) mortality rates in the southeastern United States, has large pockets of medically underserved areas, increasing the likelihood of undiagnosed diabetes and hypertension, which may lead to end-organ damage before death and donation. We hypothesize these factors contribute to our single organ yields.
Methods: Organ recovery data were reviewed for calendar years 2010-2014; donor demographics, cause of death, presence and duration of diabetes (DM), insulin therapy and hypertension (HTN) were recorded for SODs. These data were recorded for all donors from July 1, 2013 through December 31, 2014. Univariate and multivariate analyses (logistic regression) were performed.





Results: From 2010-2014 there were 1104 organ donors; causes of death were CVA/stroke (59%), anoxia (30%), and head trauma (11%). 199 (18%) were SODs; 196 (98.5%) were liver-only donors; 162 (81%) had hypertension; 74 (37%) had diabetes; 34 (17%) had chronic or end-stage renal disease. Single-organ donors increased from 27 (10%) in 2010 to 68 (25%) in 2014.

From July 2013-December 2014, there were 399 donors; 93 (23%) were SODs. CVA as the cause of death was more common in SODs (60.2 vs. 36.0%; $p<0.001$). DM was more prevalent in SODs (36.6% vs. 6.2%; $p<0.001$), as was HTN (85% vs. 29.7%; $p<0.001$). SODs were older (55.8 36.3
Conclusion: From 2010-2014 18% of our donors were SODs, and they have increased significantly during that time. Almost all SODs were liver-only donors. Independent predictors of SOD included DM, HTN and age. GALL positively impacted 199 recipients through aggressive donor evaluation protocols during the past 5 years. As a result of our review, we continue pursuing SODs to address the ever-increasing need for transplantable organs. We believe an OPO's responsibility is to pursue all potential organ donors and allow the transplant center to determine if an organ is suitable for one of their patients, regardless of performance measures (e.g., organs transplanted per donor) evaluated by various regulatory agencies.

References:

[1] Age-Adjusted to the 2000 Standard Million; Age-Adjusted to the 2000 Standard Million, Stroke = ICD I60-I69 (ICD9 430-434, 436-438); Age-Adjusted to the 2000 Standard Million, Cardiovascular=ICD10 I00-I78 (ICD9 390-434, 436-448). Sources: Centers for Disease Control & Prevention, CDC WONDER, http://wonder.cdc.gov/ Online Analytical Statistical Information System (OASIS). Georgia Department of Public Health, Office of Health Indicators for Planning (OHIP). http://oasis.state.ga.us/

352.8

Identifying neonatal potential for solid organ and tissue donation in the Newcastle upon Tyne Hospitals (UK)

Aranzazu Baglietto[1], Rashmi Kuttysankaran[2].
[1]ODT, NHSBT, Newcastle upon Tyne , United Kingdom; [2]GNCH, NUTH, Newcastle upon Tyne , United Kingdom.

Introduction: Organ donation has transformed the lives of many children. Unfortunately infants still die while awaiting transplant. The shortage of donor organs of suitable size is without question the major impediment to extending the lives of more patients awaiting solid organ transplantation. Currently organ donation is rarely considered in neonatal units; however there is increasing awareness about untapped potential of neonatal organ donation. Recently in the UK neonatal organ donation after circulatory determination of death has been facilitated. Since then guidelines have been published for diagnosis of death by neurological criteria (DNC) in infants from 37 weeks corrected gestation .Donation after neurological death is now possible in neonates. We studied the potential for solid organ and tissue donation in our Neonatal intensive care unit in the Newcastle upon Tyne Hospitals (NUTH) for last 6 years. The NUTH neonatal intensive care (NICU) is the centre of excellence for neonatal care in the North of England.
Methodology: *Design:* Retrospective review of the patient medical notes.
Setting: Neonatal Intensive Care Unit, NUTH.
Patients: Infants dying in NICU between 1st April 2010 and 31st December 2015 meeting the following criteria:
Potential tissue donation: Infants dying from 32 gestational age and over 2.5kg.
Potential Organ donation: Infants dying from 37 weeks gestational age.
Results: There were 44 deaths fulfilling the above criteria.
-19 (45%) infants died following an unplanned/uncontrolled death (UD).
-25 (55%) infants died following withdrawal of life sustaining treatment (WLST).
- 20 WLST (43%) infants were potential organ donors.
- 20 (15 UD + 5 WLST) (45%) infants had only tissue donation potential.
Donation type:
- 9 (45%) fulfil the criteria for donation after circulatory death (DCD).
- 11(55%) could theoretically fulfil the criteria for donation after brain stem death (DBD), however complete neurological assessment, including brain stem reflexes not documented. Evidence of brain stem death is documented in 4 infants.

Length of time from WLST to cardiorespiratory death is:
- Less than 3 hours in 18 (72%) infants.
- More than 3 hours in 6 (24%) infants.
- Not documented time of WLST in one patient.

Discussion: Excellent guidelines, standardised pathways and contemporary governance processes to structure organ donation in the Neonatal ICU are required.
Complete neurological assessment and detailed documentation is essential when assessing infants.
Changes in attitude and practice are necessary to include consideration for organ donation as part of the assessment once decisions of withhold or withdraw treatment have been made.
Meeting educational needs of the health care professionals regarding training in brain stem testing should be considered.
Determination of accurate time of cardiorespiratory death following WLST will be necessary where organ donation is offered.
Conclusion: The analysis of the data obtained from the audit confirms the significant potential for organ and tissue donation that the Neonatal population holds.
Dr Sundeep Harigopal

352.9

Evaluate quality of kidneys from DCD/ECD donors by parameters of machine perfusion

Guodong Chen, Chang Wang, Changxi Wang, Xiaopeng Yuan, Jiang Qiu, Lizhong Chen.
Organ Transplant Center, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, People's Republic of China.

Background: Donors from donation after cardiac death(DCD) and expanded criteria donors(ECD) have poorer outcomes compared to standard deceased donors(SCD). It is important to evaluate quality of kidney from DCD/ECD before transplantation. In this study, machine perfusion was used to preserve the DCD/ECD kidneys with the aim to investigating whether the parameters of machine perfusion could predict the quality of kidneys from DCD/ECD donors.
Methods: 72 kidneys from DCD/ECD donors were harvested in our hospital from July 2011 to August 2014. All kidneys were preserved with machine perfusion(Life Port), and parameters of machine perfusion were collected. All kidneys were biopsied before transplantation. The kidneys were discarded if histology results showed glomerulosclerosis or interstitial fibrosis were more than 20%. The primary endpoints were delayed graft function(DGF), graft loss and patient death. Postoperative complications and 1-year serum creatinine levels were also recorded.
Results: 14 kidneys(19.4%) were discarded before transplantation. During machine perfusion, resistant index(RI) were significantly higher and flow rate were significantly lower in discarded kidneys compared to the kidneys that were transplanted($p<0.05$). 58 recipients with kidneys transplanted were divided into two groups according to 1-hour RI of machine perfusion. 22 cases in high RI group (RI>0.4) and 36 cases in low RI group(RI $\leq$0.4). DGF rate was significantly higher in the high RI group(72.7% vs. 27.8%). 1-year serum creatinine levels were also significantly higher in the high RI group($p<0.05$). Acute rejection rate and 1-year graft survival were comparable between the two groups. Logistic multivariate regression showed that high RI was an independent risk factor for DGF after DCD/ECD kidney transplant(OR value=6.635, p=0.004).
Conclusion: Parameters of machine perfusion are good tools for evaluating quality of kidneys from DCD/ECD donors, and may predict DGF and 1-year graft function after transplantation.

353.1

Stem cell-mediated regeneration of ischemically damaged human renal allografts during ex vivo warm perfusion

Lauren Brasile[1], Nicholas Henry[1], Bart Stubenitsky[2].
[1]Research and Development, BREONICS Inc., Watervliet, NY, United States; [2]Plastic Surgery, University of Maastrict, Maastrict, Netherlands.

Introduction: Our goal is to address a large unmet medical need by providing more kidneys for transplantation. We evaluated if a mesenchymal stem cell (MSC) therapy could accelerate the repair of ischemically damaged human kidneys during 24 hours of ex vivo warm perfusion. We targeted the delivery of mesenchymal stem cells (MSC) directly to human kidneys to increase the intrarenal concentrations of paracrine/endocrine effects over what can be achieved with systemic administration of MSC. We hypothesized that by infusing MSC directly to the renal tissue during the ex vivo, warm perfusion, there would be an improved opportunity for repair relative to EMS perfusion alone.
Methods: An Exsanguinous Metabolic Support (EMS) perfusion technology (32°C), that supports ex vivo metabolism in isolated organs for several days, was used to evaluate the potential of repairing ischemically damaged renal allografts. Human MSC (1X108) were infused into the renal artery during the EMS perfusion at an individualized rate that did not adversely affect perfusion pressures and vascular flow rates. One kidney served as the control that was warm perfused, while the paired kidney was treated with MSC during the 24 hours of warm perfusion. Evaluation of the repair potential included: DNA synthesis, cytoskeletal regeneration, chemokine/cytokine synthesis, histologic evaluations and nuclear staining using Toluidine Blue.
Results: Treatment with MSC resulted in a significant reduction of inflammatory cytokines / chemokines synthesized by the kidneys (Figure 1).

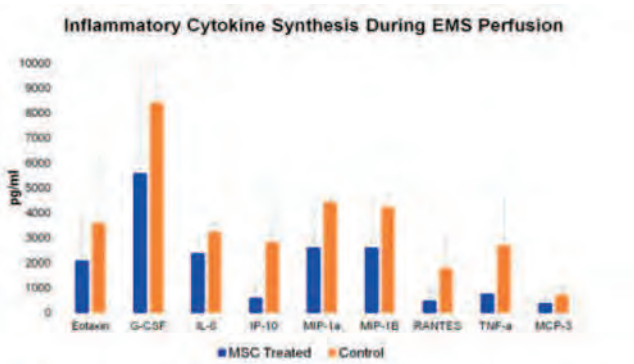


Figure 1: Human kidneys were EMS perfused with or without infusion of Mesenchymal Stem Cells (MSC). After 24 hours of EMS perfusion, perfusate samples were taken and analyzed on the Luminex Multiplex Platform.

MSC treatment lead to a significant increase in the synthesis of ATP and growth factors resulting in normalization of the cytoskeleton during 24 hours of perfusion. Toluidine Blue staining of MSC treated kidneys showed a significant increase in mitotic figures (23%) compared to EMS alone (Figure 2).
PCNA and Clusterin staining also detected the same positive MSC mediated effect.
Conclusions: Our work is the first to have achieved actual cellular regeneration while ischemically damaged human kidneys are perfused ex vivo for 24 hours. The observed regeneration entails: increased synthesis of ATP, a reduced inflammatory response, increased synthesis of growth factors, normalization of the cytoskeleton and mitosis. The ability to regenerate renal tissue ex vivo sufficiently enough to result in immediate function could revolutionize transplantation by solving the chronic organ shortage.

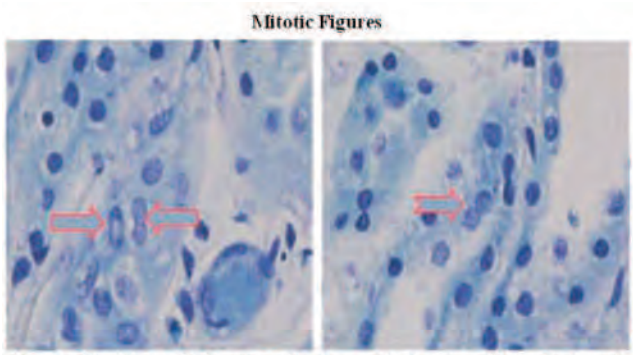


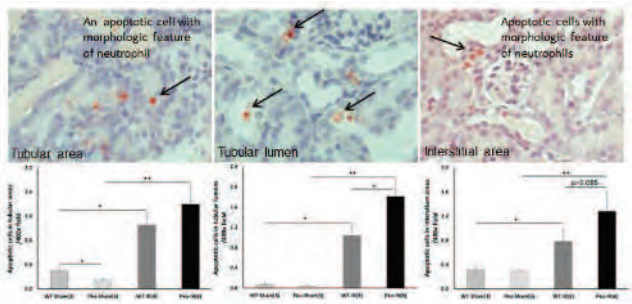
Figure 2: After 24 hours of EMS perfusion, biopsies were taken from stem cell infused and control kidneys, samples were processed and stained with a 1% Toluidine Blue solution. The left image represents an EMS perfused kidney w/ the addition of MSC. The right is the EMS control. The addition of MSC lead to a 23% increase in mitotic figures.

353.2

Properdin deficiency aggrevates the renal injury of ischemia reperfusion

Zinah D.Zwaini[1,3], Nigel J.Brunskill[1,2], Wilhelm Schwaeble[1], Cordula M. Stover[1], Bin Yang[1,2,4,5].
[1]Infection, Immunity and Inflammation, University of Leicester, Leicester, United Kingdom; [2]Renal Group, University Hospitals of Leicester, Leicester, United Kingdom; [3]College of Medicine, University of Kufa, Kufa, Iraq; [4]Nephrology, Affiliated Hospital of Nantong University, Nantong, People's Republic of China; [5]Basic Medical Research Centre, Medical School of Nantong University, Nantong, People's Republic of China.

Introduction: Ischemia reperfusion injury (IRI) is a major cause of acute renal injury, affecting native and transplant kidneys alike. Properdin stabilizes C3bBb/(C3b)nBb and positively regulates the alternative pathway (AP) of complement activation, which may play key roles in renal IRI. The aim of this work was to investigate the effect and mechanism of sole properdin knockout (PKO) on renal IRI.
Methods: Male C57/BL6 PKO (n=8) and their age and sex matched wild type (WT, n=9) mice were subjected to 30 min bilateral clamping of renal pedicles followed by 72 h reperfusion, in addition to their sham controls (n=3-5). The changes in renal function, histology, apoptosis, inflammation (HMGB1) and proliferation (PCNA), as well as complement activation, were measured.
Results: An IRI model was successfully established using serum creatinine (SCr), blood urea nitrogen (BUN) and tubulointerstitial damage (TID) as measurements, when compared to the respective sham groups. In PKO mice, IRI injury was clearly increased compared to the WT group on all levels showing SCr (1.78±0.29 vs. 1.19±0.18 mg/dl, P=0.046), BUN (481.59±109.92 vs. 258.42±51.38 mg/dl, P=0.034) and TID (2.67±0.26 vs. 1.94±0.22, P=0.025). Moreover, PKO presented with a higher number of apoptotic cells in the tubular lumen than the WT control (1.81±0.34 vs. 1.04±0.26, P=0.039). Likewise, the expression of inflammatory marker HMGB1 was significantly higher in Pko mice (1103000±316202 vs. 361636±134820, P=0.034) at time point 72 h after reperfusion. There was no statistically significant increase in caspase-3 activation, PCNA expression and mitosis (3.43±1.06 vs 1.72±0.69, P=0.18). There was also no significant change in the degree of complement C5 activation by measuring serum C5a (762.8±172.6 vs 850.8±170.5 ng/ml, P=0.7) and C5b-9 (2713±498.3 vs 2983±618.3 ng/ml, P=0.9) between two genotypic groups post IRI.



Conclusion: In PKO mice renal IRI was significantly aggregated, associated with increased apoptosis and inflammation compared to the WT control. This unexpected outcome is similar to that in mice with the deficiency of membrane bound negative regulators in complement activation, CD55 and CD59, and Crry. The underlying mechanisms are worthy to be further investigated.
University of Leicester; University Hospitals of Leicester; University of Kufa; Affiliated Hospital of Nantong University; Medical School of Nantong University; The National Natural Science Foundation of China

References:
[1] Ruseva MM. J Am Soc Nephrol. 2013; 24: 43-52.
[2] Yang C. Exp Biol Med. 2013;238:111-9.
[3] Wu Y. Clin Dev Immunol. 2013;2013:758159.
[4] Miwa T. J Immunol 2013;190:3552-3559.
[5] Miao J. Kidney Int. 2014;86:726-737.

353.3

Fractional excretion of NGAL instead of 99mTcMAG3 renography to monitor resolution of delayed graft function

J.R. Bank, M. E.J.Reinders, L. Noordermeer, M. J.K.Mallat, S. W.van der Kooij, C. van Kooten, J. W.de Fijter.
Nephrology, Leiden University Medical Center, Leiden, Netherlands.

Introduction: Kidneys transplanted from extended criteria donors have a higher prevalence of delayed graft function (DGF). Early identification of factors that prolong duration of DGF may improve clinical outcome and prevent invasive procedures. The current standard in our center to monitor resolution of DGF is a renography with ^{99m}Tc labelled mercaptoacetyltriglycine (MAG3). Since ischemia/reperfusion injury results in impaired extraction of ^{99m}TcMAG3 via the organic anion transporter, but also in impaired reabsorption of NGAL by proximal tubule epithelial cells (luminal side), we hypothesize that the fractional excretion of NGAL (FE-NGAL) can replace MAG3 scans to follow resolution of functionally defined DGF[1] (fDGF).
Methods: Ninety-two consecutive DCD transplant recipients were included, all received IL2-RB induction and maintenance therapy with steroids, MMF and delayed-CsA introduction at day 4. Before introduction of CsA on day-4 and at day-10, MAG3 scans were performed and tubular function slope (TFS) score[2] was calculated. Daily FE-NGAL was calculated using urinary and serum NGAL. Statistical analysis included positive and negative predictive values (PPV and NPV).
Results: In DCD recipients a day-4 TFS score >1.5 could distinguish patients with mild fDGF (≤7 days) from patients with moderate/severe fDGF (≥8 days), with PPV of 93.4% and NPV of 63.3%. Day-4 FE-NGAL <2.5% could even better distinguish patients with mild fDGF from those with moderate/severe fDGF (PPV=92.6%, NPV=100%). Also in patients with residual native kidney diuresis (>1L) FE-NGAL was highly distinctive (PPV=85.7%, NPV=100%). However, for the prediction of moderate (8-20 days) versus severe fDGF (≥21 days), both TFS and FE-NGAL had limited value (PPV=45.6% and 40.5%, NPV=100% and 87.5%, respectively). Furthermore, preliminary results suggest that sequential FE-NGAL may help to identify patients with additional CNI-toxicity and/or rejection.
Conclusion: In this cohort of DCD recipients FE-NGAL better distinguished patients with mild fDGF from those with moderate/severe fDGF compared to standard follow-up using ^{99m}TcMAG3 renography, also in patients with preserved residual diuresis. Sequential FE-NGAL may prove to be a useful non-invasive biomarker to guide optimal timing of a renal allograft biopsy in recipients with DGF and additional CNI toxicity and/or acute rejection.
References:
[1] Boom H, Mallat MJ, de Fijter JW, Zwinderman AH, Paul LC. Delayed graft function influences renal function, but not survival. *Kidney Int*. 2000 Aug;58(2):859-66.
[2] El-Maghraby TA, Boom H, Camps JA, Blokland KA, Zwinderman AH, Paul LC, Pauwels EK, De Fijter JW. Delayed graft function is characterized by reduced functional mass measured by (99m)Technetium-mercaptoacetyltriglycine renography. *Transplantation*. 2002 Jul 27;74(2):203-8

353.4

Hypothermic pulsatile preservation of kidneys from uncontrolled deceased donors after cardial arrest

Palmina Petruzzo, Xavier Matillon, Ricardo Codas, Emmanuel Morelon, Lionel Badet.
Department of Transplantation, Edouard Herriot Hospital, Lyon, France.

Background: Kidneys from uncontrolled donors after cardiac arrest (uDCD) suffer from a period of warm ischemia between cardiac arrest and cold flushing. Aim of the study was to evaluate renal outcomes in pump-perfused uDCD kidneys and the influence of resistive index (RI) on graft function and survival.
Materials: Forty-four kidneys from uDCD were included. The potential donors (Maastricht category I and II) underwent cardiopulmonary resuscitation by assisted ventilation and chest compression; the organs were preserved with in situ cold perfusion (IGL-1 solution) using an aortic double-balloon triple-lumen catheter ("Gillot sonde") or a normothermic subdiaphragmatic extracorporeal membrane oxygenation. All the harvested kidneys were machine perfused using hypothermic (1-4°C) pulsatile perfusion (RM3, Waters Medical System). Kidneys with RI³0.5 mmHg/mL/min after 6 hours of perfusion were discarded.
Results: There was one PNF, while 37 recipients (84.1%) experienced DGF. Graft survival was 97.6% at 1 and 3 post-transplantation years. Renal function estimated by MDRD and inuline clearance at one year showed excellent results, remarking as the outcomes from uDCD are comparable to those of kidneys from controlled donors. Linear regression models showed that RI values at the end of perfusion were associated with MDRD variations at 3 and 6 months after transplantation (p=0.049 and p=0.010, respectively) and with inuline clearance at 1 year (p=0.030), and in a separate model, with renal fibrosis at 3 post-transplant months (p=0.022). RI at the beginning of perfusion was significantly influenced by the in situ cold perfusion procedure, donor smoking, warm ischemia time; at the end of the perfusion it was influenced by the in situ cold perfusion procedure, donor sex and donor serum creatinine values.
Conclusions: This study confirmed that good clinical outcomes could be obtained transplanting selected uDCD kidneys. The perfusion machine allowed to prevent the deleterious effects of warm ischemia in uDCD grafts and RI values were correlated to graft function.

353.5

Eculizumab for prevention and treatment of the kidney graft reperfusion injury

Michael Kaabak[1], Nadine Babenko[1], Allan Zokoyev[1], Stanislav Schekaturov[1], Valery Sandrikov[1], Alexander Kovaltchuk[2].
[1]Kidney Transplantation, Boris Petrovsky Scientific Center of Surgery, Moscow, Russian Federation; [2]Dialysis, Pediatric City Hospital #13, Moscow, Russian Federation.

Background: Ischemia-reperfusion injury (IRI) is unavoidable during clinical organ transplantation. Activation of terminal complement complex (C5b-9) was shown in IRI in clinical and experimental organ transplantation.
Eculizumab, a monoclonal antibody, blocks the terminal components of complement activation. We report preliminary results of RCT (ClinicalTrials.gov identifier NCT01756508) evaluating eculizumab influence on IRI.
Materials and Methods: Between September 2012 and October 2015, 46 patients (22 male, 7,7±4,1 y.o., 28/18 live/deceased donors) were randomized to receive or not (23/23) eculizumab prior to graft reperfusion. Immunosupression: two dose alemtuzumab followed by tacrolimus and MMF. Patients were followed 654±261 days, 77 surveillance and 14 indication biopsies were performed.
Results: One patient in each group (1.7 y.o. boy and 2.8 y.o. girl) died early post Tx because of cardiomyopathy. One late graft loss was observed in each group (Day 346 and Day 557). Three grafts were lost (boys 2, 3, and 6 y.o) in eculizumab group on day 26-60 as a consequence of flu-like syndrome. Other patients are alive with functioning graft. No difference in GFR at last follow up was found (91±25 ml/min), there were trend in eculizumab group to lower proteinuria (76±54 mg/day vs 108±91 mg/day) and statistically significant less chronic glomerulopathy (0.076±0,1 vs 0.123±0,2, p=0.046). Acute rejections observed in 5 eculizumab patients (including 3 subclinical), and in 7 control patients (including 3 subclinical).
Discussion: we found less signs of chronic/subclinical AMR in eculizumab group – less chronic glomerulopathy, and trend to lover proteinuria. However, our findings have a serious limitations – average time to biopsy since transplantation was lover in eculizumab patients (133±129 vs 235±204 days). Early graft losses in eculizumab patients make evident the need for effective vaccination before transplantation, not only against pneumococcal and meningococcal infections, but against influenza as well.
Conclusion: Combination of eculizumab and alemtuzumab leads to profound immunosuppression early post transplant, which can cause unusual course of flu-like infections when pretransplant vaccination was no complete. Continuation of the study is necessary to clarify potential clinical benefits.

353.6

Impact of pre-existing diabetes mellitus on the immunologic outcome after kidney transplantation

Oliver Staeck, Fabian Halleck, Anna Kleinsteuber, Lukas Lehner, Klemens Budde, Dmytro Khadzhynov.
Nephrology, Charite Universitaetsmedizin Berlin, Berlin, Germany.

Background: There is only limited data available on the immunologic outcomes of kidney transplant recipients (KTR) with pre-existing diabetes mellitus (preDM) at time of transplantation.
Methods: This long-term retrospective single center analysis included 839 deceased donor KTR transplanted 1999-2014. Prevalence and history of DM at time of transplantation were determined. Cox proportional hazard models were applied to examine the association of preDM and immunologic outcomes. Multivariate models were adjusted for recipient age, gender, time on dialysis, prior kidney transplantation, donor age, HLA-mismatches and cold ischemia time.
Results: Mean recipient age was 54±14 yrs, mean post-transplant follow-up was 5.9±3.7 yrs. 16% of all included KTR had preDM, while 9% of KTR had pre-existing diabetic end-organ damage (retinopathy, neuropathy or nephropathy). KTR with preDM had a significantly worse 7-year survival compared to recipients without DM (after 7 years 58.6% vs. 81.6%; p<0.001, respectively). However, preDM had no significant influence on a death censored graft survival in this analysis (p=0.128). Multivariate analysis adjusted for all relevant demographic factors identified DM as significant independent risk factors for death (HR 1.76, p=0.001).
Multivariate cox regression analysis revealed preDM as an independent risk factor for T-cell mediated rejections (HR 1.45, p=0.024). Independent association of preDM with antibody mediated rejections did not reach significance level (HR 2.08, p=0.064). Other significant independent risk factors were HLA mismatches and prior kidney transplantations. Pre-existing diabetes did not contribute independently as risk factor for de novo donor specific antibodies.

TABLE 1. Estimates of predictors for immunologic outcome (Cox proportional hazards models)

		Univariate analysis		Multivariate analysis*	
Outcomes	**Predictors**	**HR (95% CI)**	**P**	**HR (95% CI)**	**P**
TCMR	Recipient age, per year	1.01 (1.00-1.02)	0.076	0.99 (0.98-1.00)	0.018
	Donor age, per year	1.01 (1.01-1.02)	<0.001	1.01 (1.00-1.02)	0.087
	Male sex	1.14 (0.90-1.44)	0.279		
	Prior kidney transplantation	1.22 (0.91-1.63)	0.185	1.55 (1.12-2.14)	0.009
	Time on dialysis, per month	1.00 (1.00-1.01)	0.191		
	Cold ischemia time per hour	1.01 (0.99-1.03)	0.617		
	HLA-mismatches, per mismatch	1.26 (1.17-1.34)	<0.001	1.25 (1.15-1.36)	<0.001
	Diabetes	1.47 (1.09-1.99)	0.012	1.45 (1.05-2.00)	0.024
ABMR	Recipient age, per year	0.96 (0.95-0.98)	<0.001	0.94 (0.92-0.96)	<0.001
	Donor age, per year	0.99 (0.98-1.01)	0.467		
	Male sex	1.24 (0.70-2.17)	0.460		
	Prior kidney transplantation	1.92 (1.04-3.54)	0.037		
	Time on dialysis, per month	1.00 (1.00-1.01)	0.248		
	Cold ischemia time per hour	0.99 (0.94-1.04)	0.660		
	HLA-mismatches, per mismatch	1.26 (1.07-1.48)	0.006	1.52 (1.25-1.86)	<0.001
	Diabetes	1.45 (0.73-2.80)	0.288	2.08 (0.96-4.51)	0.064
de novo DSA	Recipient age, per year	1.00 (0.99-1.00)	0.239	0.98 (0.97-0.99)	0.003
	Donor age, per year	1.00 (1.00-1.014)	0.137		
	Male sex	1.08 (0.83-1.39)	0.570		
	Prior kidney transplantation	2.05 (1.53-2.69)	<0.001	1.78 (1.24-2.54)	0.002
	Time on dialysis, per month	1.00 (1.00-1.01)	0.297		
	Cold ischemia time per hour	0.99 (0.97-1.01)	0.286		
	HLA-mismatches, per mismatch	1.32 (1.22-1.42)	<0.001	1.44 (1.30-1.59)	<0.001
	Diabetes	0.89 (0.58-1.35)	0.571		

*multivariate analysis using stepwise backward elimination with inclusion of recipient age, donor age, prior kidney transplantation, HLA-mismatches, diabetes.

Conclusion: Pre-existing DM is not only associated with increased mortality but also a strong independent risk factor for T-cell-mediated rejections. This emphasizes the need for appropriate post-transplant management.

353.7

Is prolonged cold ischemia a contraindication to using kidneys from acute kidney injury donors?

Robert Stratta[1], Amber Carrier[1], Alan Farney[1], Jeffrey Rogers[1], Giuseppe Orlando[1], Muhammad Khan[1], Hany El-Hennawy[1], Amber Reeves-Daniel[2], Amudha Palanisamy[2].
[1]Surgery, Wake Forest, Winston-Salem, NC, United States; [2]Internal Medicine, Wake Forest, Winston-Salem, NC, United States.

Introduction: Elevated deceased donor (DD) serum creatinine (SCr) levels and prolonged cold ischemia time (CIT) are major independent reasons for kidney discard following organ recovery. Many kidneys are declined by local centers because of acute kidney injury (AKI), which results in delays and longer CIT as these kidneys are offered regionally and then nationally in the US. The study purpose was to analyze the combined effect of these 2 factors on kidney transplant (KT) outcomes.
Methods: We performed a single center retrospective analysis in adult DD KT patients (pts) receiving AKI donor kidneys. AKI was defined as a doubling in the DD admission SCr and a terminal SCr >2.0 mg/dl. Outcomes were stratified according to longer CIT categories. Delayed graft function (DGF) was defined as the need for dialysis for any reason in the 1st week post-KT. All pts received depleting antibody induction with tacrolimus/mycophenolate maintenance therapy ∓ steroids.
Results: Since 1/1/07, we performed 118 KTs from 89 AKI DDs. Mean DD age was 35 years; mean DD admission and terminal SCr levels were 1.2 mg/dl and 3.1 mg/dl, respectively. 27 KTs (23%) had CITs <20 hrs, 52 (44%) 20-<30 hrs, 30 (25%) 30-<40 hrs, and 9 (8%) ≥40 hrs. The proportion of imported kidneys (66% overall) increased with longer CIT category (30%, 67%, 90% and 89%, respectively, p<0.0001). Most kidneys (87%) were managed with both cold storage and machine perfusion (mean pump time 12.2 hours, mean total CIT 26.3 hours). The overall incidence of DGF was 41.5%; the DGF rate with longer CIT category was 30%, 48%, 33%, and 67%, respectively (p=NS). With a mean follow-up of 52 months, overall pt and kidney graft survival rates were 91% and 81%, respectively. Death-censored graft survival (DCGS) rates were 84% and 88% for pts with and without DGF (p=NS). DCGS rates were 92% in pts with CIT <20 hrs compared to 85% in those with CIT ≥20 hrs (p=NS). In the 9 pts with CITs ≥40 hrs, the 4-year DCGS rate was 100%.
Conclusions: These findings suggest that the presence of both AKI and prolonged CIT may not adversely influence intermediate-term outcomes, particularly when imported kidneys are placed on short-term machine preservation prior to KT. The presence of DGF likewise does not affect outcomes in this setting. Discard of AKI donor kidneys because of projected long CIT is not warranted. Consequently, use of these kidneys may be a safe and effective way to expand the donor pool and reduce the KT waiting list.

353.8

Retrospective study of post renal transplant delayed graft function - analysis of multiple definitions and respective prognostic capacity

Samantha Bateman[1], Theresa Nguyen[1], Shreeya Patel[1], Robyn Langham[1,2].
[1]Department of Nephrology, St Vincent's Hospital, Melbourne, Australia; [2]School of Rural Health, Monash University, Melbourne, Australia.

Introduction: Delayed graft function (DGF) is a term often used in describing an acute kidney injury from ischaemia-reperfusion in the post renal transplant (Tx) setting[1]. Though associated with an increase in morbidity, graft loss and increased hospital stay, consensus on the definition of DRG is lacking, with the term often misused and misunderstood. Currently, the reported incidence of DGF varies depending on definition used, with a range in the literature from five to 50 percent[2]. In order to progress meaningful intervention studies to improve the outcomes of DGF, there is a pressing need to develop a consensus on a unifying, clinically relevant and simple definition.
Materials and Methods: A retrospective single-centre analysis of 76 consecutive cadeveric renal transplants was undertaken. 18 published definitions of DGF were applied to the data set. Each definition was correlated to clinical outcome, hospital length of stay and mean serum creatinine (SCr) level at 1 year as a surrogate marker of graft function.
Results: Application of the 18 definitions of DGF to our cohort yielded a wide range in incidence from 10.8 to 68.9 percent. Only three of the 18 definitions predicted a clinically significant worse graft function at one year; 1. Urine output less than one litre and less than 25 percent fall in SCr from baseline in first 24 hours post Tx (incidence 52.7%; SCr 210mmol/L DGF vs 119mmol/L no DGF, p< 0.05); 2. SCr decrease less than 10% per day during the three consecutive days post Tx (incidence 43.3%; SCr 200mmol/L DGF vs 125mmol/L no DGF, p< 0.05) and 3. SCr reduction ratio of less than 25 percent and or need for dialysis (Dx) at 48 hours post Tx (incidence 48.65%; sCr 189mmol/L DGF vs 123mmol/L no DGF p<0.05). The only definition to associate with a significant difference in hospital length of stay was the clinical documentation of DGF (incidence 45.8%; LOS 13.5days DGF vs 10.7 days no DGF, p <0.05).
Discussion: Many definitions of DGF have been applied in the post renal transplant literature, creating confusion both academically and clinically. The inclusion of multiple clinical variables such as SCr, urine output and/or the need for Dx increases the likelihood of useful predictive validity of the definition. Excluding highly sensitive histopathological findings in renal biopsies increases the applicability of a definition. In determining the optimal definition that best predicts 1 year outcome, this study supports the use of a combination of SCr measures and the need for Dx in defining DGF.
Conclusion: Similar to the development of consensus definitions in AKI[3], determination of a consensus framework in defining DGF in renal Tx is needed in both research and clinical terms. Further interventional studies aimed at reducing the incidence and subsequent poorer outcomes of DGF require the development of a robust definition framework that is fit-for-purpose, with clarity and easy applicability.
References:
[1] Perico N, Cattaneo D, Sayegh MH, Remuzzi G. Delayed graft function in kidney transplantation. *Lancet*. 2004;364(9447):1814-1827.
[2] Yarlagadda SG, Coca SG, Garg AX, et al. Marked variation in the definition and diagnosis of delayed graft function: a systematic review. *Nephrology Dialysis Transplantation*. 2008;23(9):2995-3003.
[3] Lopes JA, Jorge S. The RIFLE and AKIN classifications for acute kidney injury: a critical and comprehensive review. *Clinical Kidney Journal*. 2013;6(1):8-14.

353.9

Interim analysis to determine whether preoperative intravenous fluids result in a decrease in donor subclinical acute kidney injury

Ryan Ghita[1], Kieran Lafferty[2], Shona Mackinnon[2], Aitken Emma[1], Marc Clancy[1,2].
[1]Faculty of Medicine, University of Glasgow, Glasgow, United Kingdom; [2]Renal Transplant Department, Queen Elizabeth University Hospital Glasgow, Glasgow, United Kingdom.

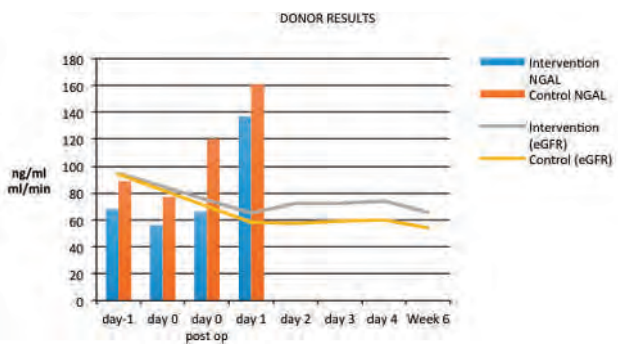
Introduction: Laparoscopic nephrectomy is considered the gold standard for living kidney donation, reducing post-operative pain and faster recovery[1]. Conversely, a pilot study (Aitken et al. 2014; unpublished) found laparoscopic nephrectomy exposes the kidneys to increased hemodynamic stresses due to extreme position and pneumoperitoneum leading to acute kidney injury (AKI). It is hypothesized that active hydration prior to donation counteract these stresses resulting in a reduced subclinical kidney injury and ultimately improving long-term renal function in both donor and recipient.
We are monitoring AKI using neutrophil gelatinase associated lipocalin (NGAL). It has been proven to be a more sensitive marker of AKI compared with creatinine or urine output[2].

Methods:

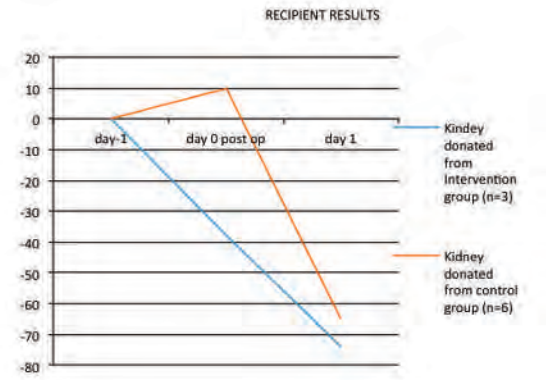
- A single center, single blinded randomized controlled trial
- The intervention cohort receives 3L of Harttmans solution overnight (00:00 to 08:00). The control group will be allowed clear oral fluids up to 06:00. Donor taken to theatre at 08:30
- Blood samples are taken from the donor day-1, day 0 (preop), day 0 (postop), day+1
- Blood samples are also taken from the transplant recipient at the same time points

Results:

- To date there are 8 patients in the control and 7 patients in the intervention
- Donor baseline NGAL dropped by 17% post intervention (p=0.197)
- Immediately post laparoscopic kidney donation NGAL was 66.86ng/ml in the Intervention (a rise of 18.4%(p=0.188)) and 120.38ng/ml in the control (a rise of 55.8% (p=0.003))
- Day 4 post laparoscopic kidney donation eGFR dropped by 21.8%(p=0.003) in the Intervention group compared to only 36.2%(p=<0.01) in the control group. By week 6 this trend had continued
- Recipient of a kidney from the control group has a post operative NGAL increase on average of 10%(p=0.128). Recipient of a kidney from the intervention group has a post operative NGAL decrease on average of 38%(p=0.136)



Recipients of kidneys donated from the control group have an immediate NGAL rise unlike the recipients of kidneys from the intervention group in which the NGAL begins to drop immediately post surgery. This may represent that kidneys donated from the control group are more susceptible to AKI associated with nephrectomy and then transplantation.



Preoperative IV hydration has shown to reduce any subclinical AKI in the donor prior to donation and reduces the degree of AKI associated with laparoscopic donor nephrectomy. There is also a consistent improvement in renal function in the intervention group at 6 weeks post surgery.

Conclusions: Preoperative Intravenous fluids may decrease the severity of AKI associated with laparoscopic donor nephrectomy with a subsequent increase in donor renal function 6 weeks post surgery. However further data is needed from completion of the Randomized Controlled trial to validate results and to see if this will have a longstanding impact on kidney function for both kidney donor and transplant recipient.

References:

[1] Wilson CH, Sanni A, Rix DA, Soomro NA. Laparoscopic vs Open Nephrectomy for live kidney donors. Cochrane Database Syst Revi. 2011; CD006124. doi 10.1002/14651858.CD006124.pub2.
[2] Mishra, J., Dent, C., Tarabshi, R. et al. Neutrophil gelatinase-associated lipocalin (N-GAL) as a biomarker for acute renal injury after cardiac surgery. Lancet. 2005;365(9466):1231-1238.

354.1

Extracorporeal membrane oxygenation practices changes as bridge to lung transplantation: An Institutional Experience and Literature Review

Daniel Absi, Margari Peradejordi Lastras, Juan Osses, Gustavo Parrilla, Mariano Candioti Lehmann, Roberto Favaloro, Alejandro Bertolotti.
Intrathoracic Transplant Department, Favaloro Foundation Universitary Hospital, Buenos Aires, Argentina.

Introduction: Respiratory failure patients requiring support with invasive mechanical ventilation while awaiting lung transplantation present a higher risk of death. Extracorporeal membrane oxygenation (ECMO) has been proposed as an alternative bridging mode to mechanical ventilation and this is an evolving management technique of rapidly progressive pulmonary disease. The aim of this study was to assess the current evidence regarding how the ECMO strategies have changed over the last years.
Methods: Retrospective review of a single-institution's experience placing patients (p) on ECMO in order to bridge them to lung transplant. From 2010 to 2015, we performed 62 ECMO Runnings in 58 p, of these, 25 p were bridge to lung transplant (BTT). We divided this cohort in two groups by era (Group 1: 2010-2012 and Group 2: 2013-2015). Categorical data were shown as frequencies/percentages and continuous data as means with standard deviation (SD). Fisher's exact test and the chi-square analysis were performed to compare continuous and categorical variables, respectively. Actuarial survival was estimated with the Kaplan–Meier method. End points, included successful bridging, duration of extracorporeal membrane oxygenation support and overall survival.
Results: Eight p were BTT in group 1 and 17 p in group 2. Overall median recipient age was 34,7±19,4 years. The etiologies were: cystic fibrosis (n=11), idiopathic pulmonary fibrosis (n=7), secondary pulmonary fibrosis (n=3), pulmonary arterial hypertension group 1 (n=3) and 1 p with pulmonary hypertension group 3. Veno-venous ECMO mode was used in 15 p (60%), veno-arterial in 5 p (20%), arterio-venous in 3 p (12%) and veno-arterio-venous in 2 p (8%). Thirteen p underwent sedated bridging, 7 p awake and 5 p ambulatory ECMO strategy. Eight p were declared futile. Support mean time was 9±6,8 days in group 1 and 19±12,6 days in group 2 ($p<0,05$). Overall successful bridging was 58,8% (20% vs 75% group 1 and 2 respectively, $p<0,05$). All p survive lung transplant except one. Survival rate at 1 year was 70%.
Discussion: Although the early experience and outcomes with ECMO as BTT were unconvincing, latest series demonstrate that better results can be achieved. The main contributors of poor early and late outcomes after transplant are: Pre-transplant deconditioning, prolonged immobilization and heavy sedation. Therefore, changes in ECMO strategies have shown to be an important goal that could improve these outcomes.
Conclusions: Early mobilization of patients on ECMO as BTT is an important goal because it facilitates participation in physical therapy and improves overall patient conditioning for lung transplantation. In carefully selected patients, ECMO is a safe and effective mean of bridging decompensated patients with end-stage lung disease to lung transplantation.
Mrs. Gomez Rosa
References:
[1] Fischer S. Bridge to lung transplantation with the novel pumpless interventionallung assist device NovaLung. *J Thorac Cardiovasc Surg* .2006; 131:719-23
[2] Olsson K. Extracorporeal Membrane Oxygenation in Nonintubated Patients as Bridge to Lung Transplantation. *Am J Transplant* 2010; 10(9):2173-8
[3] Biscotti M. Extracorporeal membrane oxygenation with subclavian artery cannulation in awake patients with pulmonary hypertension. *ASAIO* J. 2014 Nov-Dec;60(6):748-50
[4] Rosenzweig E. Extracorporeal membrane oxygenation as a novel bridging strategy for acute right heart failure in group 1 pulmonary arterial hypertension. *ASAIO J*. 2014 Jan-Feb;60(1):129-33
[5] Del Sorbo L. Extracorporeal membrane oxygenation as "bridge" to lung transplantation: what remains in order to make it standard of care? *Am J Respir Crit* Care Med. 2012 Apr 1;185(7):699-701
[6] Crotti S. Organ Allocation Waiting Time During Extracorporeal Bridge to Lung Transplant Affects Outcomes. *Chest* 2013;144(3):1018-1025
[7] Yusen RD. Al: The registry of the International Society for Heart and Lung Transplantation: thirty-first adult lung and heart-lung transplant report-2014. *J Heart Lung Transplant* . 2014;33(10):1009-24
[8] Fuehner T. Extracorporeal membrane oxygenation in awake patients as bridge to lung transplantation. *Am J Respir Crit Care Med* . 2012 Apr 1;185(7):763-8
[9] Abrams D. Early mobilization of patients receiving extracorporeal membrane oxygenation: a retrospective cohort study. *Crit Care* . 2014 Feb 27;18(1):R38

354.2

Donor CCR2+ cardiac macrophages mediate neutrophil extravasation after heart transplantation through MyD88-dependent production of chemoattractants

Wenjun Li[1], His-Min Hsiao[1], Ruyiji Higashikubo[1], Brian Saunders[2], Ankit Bharat[3], Daniel R.Goldstein[4], Alexander S. Krupnick[1,2], Andrew E.Gelman[1,2], Kory Lavine[5], Daniel Kreisel[1,2].
[1]Surgery, Washington University School of Medicine, St. Louis, United States; [2]Pathology & Immunology, Washington University School of Medicine, St. Louis, United States; [3]Surgery, Northwestern University, Chicago, United States; [4]Surgery, Yale University, New Haven, United States; [5]Medicine, Washington University School of Medicine, St. Louis, United States.

Introduction: Myocardial ischemia reperfusion injury contributes to morbidity and mortality in heart transplant patients. Neutrophilic infiltration into myocardial tissue plays a critical role in promoting damage and can also augment alloimmune responses. The goal of this study was to define cellular and molecular requirements for neutrophilic infiltration into ischemic myocardium.
Materials and Methods: Heart grafts from B6 wildtype (WT), B6 CCR2 DTR (after treatment with DT to deplete CCR2+ cells), B6 MyD88 flox x LysM-cre (MyD88 deletion in macrophages) and CXCL5-deficient mice were transplanted into the necks of congenic B6 CD45.1+ or syngeneic B6 neutrophil reporter LysM-GFP recipients. 2 hours after reperfusion we performed flow cytometric analysis or intravital two photon imaging of heart grafts. RNA expression levels of neutrophil chemoattractants were determined in flow cytometrically sorted cardiac macrophage populations.
Results: Flow cytometric analysis demonstrated donor-derived CD64+CD11b+CCR2- and CD64+CD11b+CCR2+ macrophages in heart grafts 2 hours after reperfusion. RNA expression levels of the neutrophil chemokines CXCL2 and CXCL5 were significantly higher in WT CCR2+ compared to CCR2- macrophages. Intravital two photon imaging revealed that, compared to WT conditions, extravasation of neutrophils into the graft tissue was markedly impaired when we transplanted hearts that lacked CCR2+ cells (extravasated cells: 12.2 ±5% (CCR2-DTR) vs 36.3±3.8% (WT), $p<0.05$). A similar neutrophil trafficking defect was observed when we transplanted hearts that lacked MyD88 selectively in macrophages (Lysozyme M+ cells) (extravasated cells: 1.2 ±1.2% (Myd88 flox x LysM-cre) vs 36.3±3.8% (WT), $p<0.01$). RNA expression levels of CXCL2 and CXCL5 were significantly decreased in MyD88-deficient compared to WT cardiac CCR2+ macrophages. Finally, neutrophils were unable to enter the myocardial tissue when we administered CXCL2-blocking antibody or transplanted CXCL5-deficient hearts (extravasated cells: 1.95 ±1.1% (CXCL2-blocking antibody), 12.7±2.6% (CXCL5-knockout heart), WT 36.3 ± 3.8%, $p < 0.05$ CXCL2-blocking antibody and CXCL5 knockout heart vs. WT).
Conclusion: Our work has identified novel mechanisms that regulate neutrophil trafficking into freshly reperfused cardiac grafts. We have shown that MyD88-mediated production of CXCL2 and CXCL5 by tissue-resident donor CCR2+ macrophages is critical to mediate extravasation of neutrophils into ischemic myocardial tissue. Our results provide the framework for the development of new therapeutic strategies for heart transplant recipients.
References:
[1] Li W, Nava RG, Bribriesco AC, Zinselmeyer BH, Spahn JH, Gelman AE, Krupnick AS, Miller MJ, and Kreisel D. Intravital 2-photon imaging of leukocyte trafficking in beating heart. J Clin Invest. 2012;122(7):2499-508.

354.3

Tertiary lymphoid organs within pulmonary allografts promote tolerance

Wenjun Li[1], Hsi-Min Hsiao[1], Ryuji Higashikubo[1], Alexander S.Krupnick[1,2], Andrew E.Gelman[1,2], Daniel Kreisel[1,2].
[1]Surgery, Washington University School of Medicine, St. Louis, United States; [2]Pathology & Immunology, Washington University School of Medicine, St. Louis, United States.

Introduction: Immune responses after lung transplantation differ from those after transplantation of other organs. We have previously shown that tolerant lung grafts harbor tertiary lymphoid organs (inducible bronchus associated lymphoid tissue (iBALT)), which are rich in CD11c+ and Foxp3+ cells (Li W et al, Mucosal immunology. 2012). Here we explored whether lung grafts can induce systemic tolerance and whether the presence of iBALT is important to maintain lung allograft tolerance.
Materials and Methods: Balb/c lungs were transplanted into B6, B6 CD11c-EYFP/Foxp3-GFP and B6 CD11c-DTR recipients and treated with perioperative co-stimulatory blockade (MR1/CTLA4-Ig). At least 30 days after pulmonary transplantation recipients received Balb/c or CBA hearts. Tolerant lung allografts were removed 2 days before or 4 weeks after heart transplantation. Balb/c lungs were imaged by intravital two-photon microscopy > 30 days after transplantation into B6 CD11c-EYFP/Foxp3-GFP recipients. Tolerant Balb/c lungs were retransplanted into nonimmmunosuppressed B6 or B6 CD11c-DTR secondary recipients at least 30 days after their initial engraftment into immunosuppressed B6 hosts. Secondary hosts were treated with saline or diphtheria toxin (DT) at the time of retransplantation.
Results: Transplantation of donor-specific Balb/c hearts into tolerant B6 recipients of Balb/c lungs resulted in their long term survival with no evidence of allograft vasculopathy (>120 days, n=5). If we removed the tolerant lung graft 2 days before cardiac transplantation, Balb/c hearts were acutely rejected (18.5 days, n=4). If the tolerant pulmonary grafts were explanted 4 weeks after heart transplantation, the cardiac grafts rejected acutely or developed allograft vasculopathy (n=4). Retransplantation of tolerant Balb/c lungs into nonimmunosuppressed B6 recipients resulted in their long-term survival with maintenance of the iBALT within the pulmonary grafts (n=3). Selective depletion of CD11c+ cells from tolerant lungs at the time of retransplantation into nonimmunosuppressed B6 recipients resulted in structural disintegration of the iBALT and graft rejection (n=3). Intravital two photon imaging revealed interactions between CD11c+ and Foxp3+ cells in tolerant lung grafts.
Conclusion: The presence of iBALT in lung grafts is critical to maintain a tolerant state. Induction and maintenance of systemic tolerance after lung transplantation are dependent on the presence of the pulmonary graft. Developing strategies to induce tolerogenic tertiary lymphoid organs within allografts may provide a new therapy for transplant recipients.
References:
[1] Li, W., et al. Lung transplant acceptance is facilitated by early events in the graft and is associated with lymphoid neogenesis. *Mucosal immunology*. 5, 544-554 (2012).

354.4

Effects of brain death (BD) or cardiac death (DCD) on allogeneic lung graft survival in preclinical MHC-inbred CLAWN miniature swine

Yuichi Ariyoshi[1], Hisashi Sahara[1], Mitsuhiro Sekijima[1], Takahiro Murokawa[1], Takehiro Iwanaga[1], Yurika Ichinari[1], Akira Shimizu[2], Kazuhiko Yamada[1].
[1]Division of Organ Replacement and Xenotransplantation Surgery, Center for Advanced Biomedical Science and Swine Research, Kagoshima University, Kagoshima, Japan; [2]Department of Analytic Human Pathology, Nippon Medical School, Tokyo, Japan.

Introduction: We have extensively studied the mechanisms of pulmonary allograft rejection in MHC-inbred MGH miniature swine. To clarify that our newly developed Japanese line of MHC-inbred CLAWN miniature swine represent a highly-valuable preclinical model of transplantation, we established several models of orthotopic lung transplantation.
Methods: Five CLAWN miniature swine received fully MHC-mismatched lungs with 12-days continuous infusion of tacrolimus (constant blood levels 35-45 ng/ml) (Group 1). Additional four CLAWN miniature swine received MHC-matched, minor antigen-mismatched lungs under the same immunosuppressive protocol to investigate the influence of MHC disparity on allograft survival (Group 2). To investigate whether brain death (BD) or cardiac death (DCD) affects the graft survival, three recipients were transplanted with fully mismatched lung grafts from BD donors induced by subdural balloon and subsequently ventilated for 6 h before procurement (Group 3) and six recipients were transplanted with MHC-matched DCD lungs induced by venous injection of potassium chloride and allowed for 90 min before procurement (Group 4). Graft function was monitored by histology and chest X-rays.
Results: All MHC-mismatched grafts was rejected within 63 days with diffuse mononuclear infiltrates associated with intra-alveolar hemorrhage and capillary congestion, consistent with severe cellular and antibody-mediated rejection (Group 1). In contrast to Group 1, all MHC-matched lung in Group 2 was uniformly accepted over one year with occasional presence of minimum acute cellular rejection (the fourth animal is ongoing > 7 months). In Group 3, donor brain death accelerated pulmonary allograft rejection, and all three animals rejected the grafts within 35 days with accelerated development of anti-donor antibodies. In Group 4, three out of six animals eventually rejected the warm ischemic MHC-matched grafts at POD42, 56, 250, respectively.
Conclusions: By taking advantage of the controlled antigenicity of the MHC-inbred miniature swine, we created a reproducible large-animal model of lung transplantation similar to the clinical scenario. We anticipate that this preclinical model using MHC-inbred CLAWN miniature swine will facilitate further systematic study of the pathogenesis and therapy of lung graft rejection.

354.5

Intraoperative vasopressin use as a favorable prognostic factor for early survival after lung transplantation

Kyoung Shik Narm, Hee Suk Jung, Young Woo Do, Jin Gu Lee, Chang Young Lee, Anes Kim, Hyo Chae Paik.
Department of Thoracic and Cardiovascular Surgery, Yonsei University College of Medicine, Seoul, Korea.

Introduction: Lung transplantation has become the standard of care for end-stage pulmonary disease. Despite increasing experience in caring for these patients under extreme stress and improvement of immunosuppressant, the rate of perioperative mortality still remains high. In our institution, to maintain blood pressure during operation, norepinephrine was generally selected as a first drug. Vasopressin had been added to norepinephrine only if norepinephinre alone could not raise blood pressure effectively. Vasopressin was known as selective pulmonary vasodilator[1]. Therefore use of vasopressin might be beneficial to transplanted patient. The purpose of this study was to analyze the impact of intraoperative vasopressin use on early survival following lung transplantation.
Materials and Methods: Between March 2013 and November 2015, we retrospectively reviewed 48 consecutive patients who had undergone double-lung transplantation in Yonsei University College of Medicine. Patients who underwent single lung transplantation, multi-organ transplantation and who received ventilator care or extracorporeal membranous oxygenation(ECMO) support waiting transplantation and who died less than one month after transplantation were excluded. Donor organ procurement and transplantation were performed using standard methods. All operation was performed under ECMO support. All patients received triple drug regimen including tacrolimus (FK 506), mycophenolate mofetil (MMF; CellCept), and corticosteroid postoperatively. Patients were divided into two groups depending on intraoperative vasopressin use. Patients who received vasopressin infusion more than 30 minutes were included vasopressin group. Exact dose for each patient had not been recorded, but usual dose in our institution was 0.1~0.5 unit/min.
Results: 21(43.7%) patients received vasopressin during operation and 27(56.3%) patients did not. Patients characteristics including age, [illegible] preoperative NYHA classification

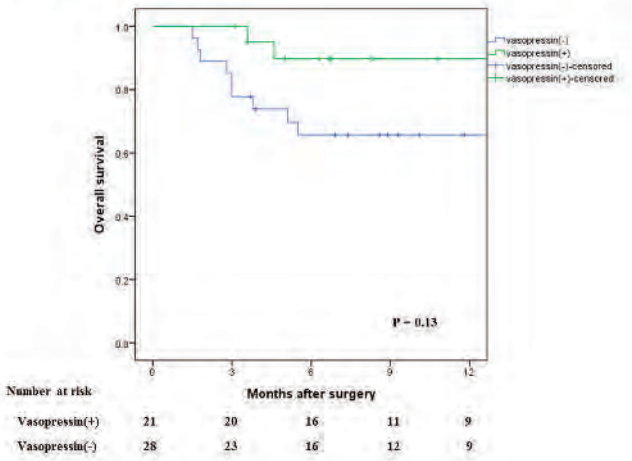
Table. Patients characteristics
ILD : interstitial lung disease, COPD: chronic obstructive pulmonary disease, NYHA: New York Heart Association

variables	Vasopressin use (n=21)	No vasopressin use (n=27)	P-value
Age (years)	48±16	50±11	0.58
Gender			0.26
male	9(42.9%)	16(59.3)	
female	12(57.1%)	11(40.7%)	
Preopetive diagnosis			0.10
Idiopathic pulmonary fibrosis	9(42.9%)	14(51.9%)	
Connective tissue-related ILD	4(19.0%)	7(25.9%)	
COPD	3(14.3%)	0(0.0%)	
Lymphangioleiomyomatosis	0(0.0%)	3(11.1%)	
Bronchiolitis obliterans	3(14.3%)	3(11.1%)	
Others	2(9.5%)	0(0.0%)	
NYHA class			0.87
III	5(23.8%)	7(25.9%)	
IV	16(76.2%)	20(74.1%)	
Operative time (min)	427±74	397±92	0.22
Blood loss during operation (cc)	1800(1150,4050)	1700(1050,3700)	0.92
Mortality	3(14.2%)	9(33.3%)	

Median follow-up period was 14.1 months (range of 1.5 to 32.7 months). Analyzed by Kaplan-Meier method, the 1- year overall survival rates in the vasopressin use group were 89.7% and those in the

no-vasopressin use group were 65.7%, respectively. Vasopressin use group showed tendency to better 1-year survival (p=0.13).

Figure. Overall survival



Conclusion: Our results showed that vasopressin use during operation might be associated with better early survival after lung transplantation. Given that pulmonary vasodilator effect of vasopressin, it could be considerable to use vasopressin earlier in managing blood pressure during operation. Further studies are needed.

References:

[1] Amit Agrawal. Therapeutic applications of vasopressin in pediatric patients. Indian Pediatr. 2012 Apr;49(4):297-305

354.6

The immunosuppressive effect via HGF-CMET pathway of autologous adipose tissue-derived mesenchymal stem cells in a rat lung transplantation model

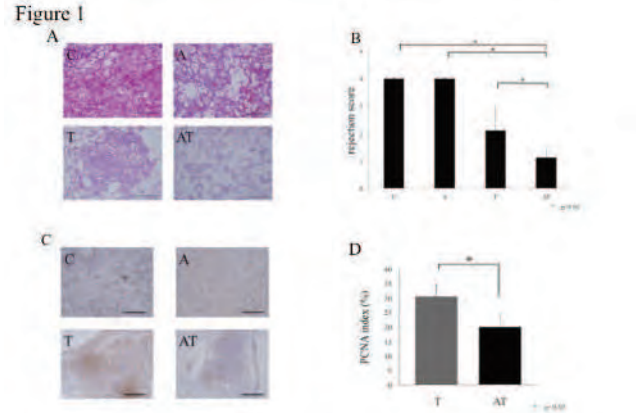
Hironosuke Watanabe[1], Tomoshi Tsuchiya[1], Naoya Yamasaki[1], Takuro Miyazaki[1], Keitaro Matsumoto[2], Hiroshi Yukawa[2], Yoshinobu Baba[1], Takeshi Nagayasu[1].
[1]Division of Surgical Oncology, Department of Translational Medical Sciences , Nagasaki University, Nagasaki, Japan; [2]Nagoya University's FIRST Center Innovative Nanobiodevices, Nagoya, Japan.

Purpose: Immunosuppressants are associated with various complications after lung transplantation, and thus less intense immunosuppression should be attempted. Adipose tissue-derived mesenchymal stem cells (ADMSCs) were recently found to have an immunomodulatory effect in various disease models through the secretion of hepatocyte growth factor (HGF). The purpose of this study was to investigate the effect of ADMSCs on immune response in a rat lung transplantation model.

Materials and Methods: Orthotopic left lung transplantation was performed in the rat using the cuff technique. Before transplantation, ADMSCs were gathered from the rats' abdominal subcutaneous adipose tissue and cultured. The experimental animals were then divided into the following four groups: a control group (C group), in which no treatment was administered after transplantation; an ADMSC group (AD group), in which rats were administered a single intravenous injection of ADMSCs (autologous 1 × 106 cells) after transplantation; a tacrolimus group (T group), in which rats were administered tacrolimus (0.5 mg/kg) every 24 hours after transplantation; and an ADMSC and tacrolimus group (AT group), in which rats were administered ADMSCs (autologous 1 × 106 cells) after transplantation in combination with tacrolimus (0.5 mg/kg) every 24 hours. Allografts were then assessed histologically and serum HGF levels measured.

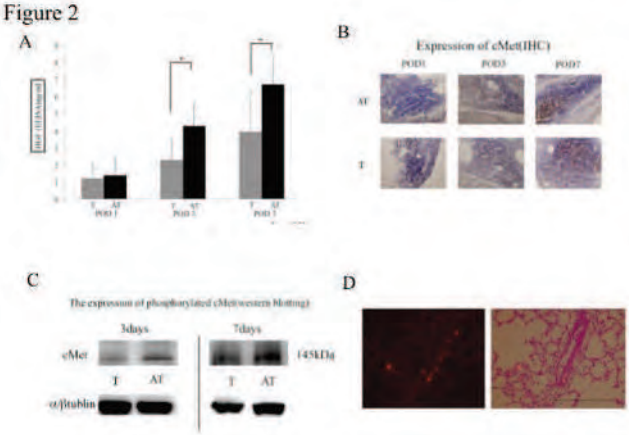
Results: Although no significant differences were found between the C and AD groups, the histologic rejection grade of the AT group was significantly lower than that of the other groups(Figure 1 A, B). The bronchus-associated lymphoid tissue cell proliferating cell nuclear antigen index of the AT group was significantly lower than that of the T group (19.88±4.67 vs. 30.5±3.92 Figure 1 C, D).

Figure 1



The serum HGF level of the AT group was significantly higher than that of the T group at postoperative days (POD) 3 and 7 (4.29±1.34 vs. 2.29±1.25 and 6.71±1.57 vs. 3.94±2.46 ng/mL, respectively (Figure 2 A). The expression of cMet, the tyrosine kinase receptor of HGF, increased with time in the AT group. Programmed death-ligand 1 expression in allografts of the AT group was significantly increased compared with that of the T group(Figure 2 B, C)The administered ADMSCs were detected in allografts at POD 7 using fluorescent quantum dots(Figure 2 D).

Figure 2



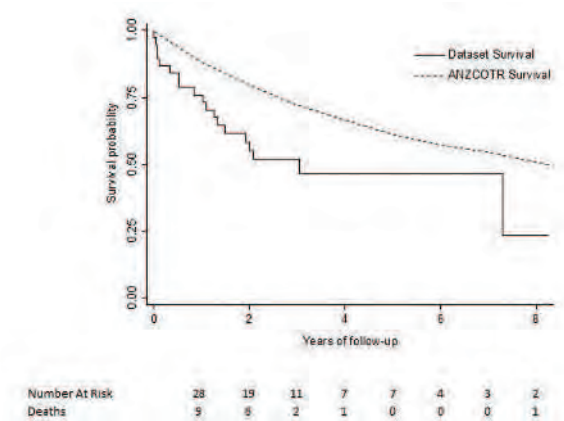
Conclusion: ADMSCs combined with tacrolimus reinforced immunosuppression, possibly due to the secretion of HGF.

354.7

Outcomes of acute kidney injury occurring immediately post lung transplantation: a retrospective single-centre audit

Khai Yang Ooi[1], Eldho Paul[2,3], Bronwyn Levvey[4], Greg Snell[4], Solomon Menahem[1,2].
[1]Department of Renal Medicine, Alfred Hospital, Melbourne, Australia; [2]Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Australia; [3]Clinical Haematology Department, Alfred Hospital, Melbourne, Australia; [4]Department of Allergy, Immunology and Respiratory Medicine, Alfred Hospital, Melbourne, Australia.

Background: Lung transplantation surgery is often complicated by acute kidney injury (AKI) requiring renal replacement therapy, adding to the peri-operative morbidity and mortality. We sought to characterise the risk factors for haemofiltration-dependent AKI occurring in the immediate post lung transplant period and evaluate its impact on survival and long term renal function.
Methods: We performed a retrospective review of all lung transplant recipients at our institution that required haemofiltration in the immediate period post lung transplantation surgery. Demographic, clinical and biochemical parameters were recorded. Kaplan-Meier curve was used to plot survival after transplant as a function of time and this was compared to survival data of all lung transplant recipients from the local Australian and New Zealand Cardiothoracic Organ Transplant Registry (ANZCOTR)[1]. Risk factors associated with mortality were assessed using Cox proportional hazards regression. Change in renal function over time was assessed using Wilcoxon signed rank test.
Results: Of 794 lung transplants performed between May 2000 and April 2015, 38 patients were identified to have developed haemofiltration-dependent AKI immediately post lung transplant. The 1-year survival was 76.1% (95% CI: 59.0-87.8%) and 5-year survival was 46.7% (95% CI: 28.3-63.0%), representing a 12.4% and 15.1% greater mortality risk respectively compared to ANZCOTR data. Cystic fibrosis (HR 4.48 [1.64-12.23], p = 0.003) and duration of inotropic support (HR 1.03 per day [1.003-1.05], p = 0.02) were significantly associated with increased risk of mortality whereas higher BMI (HR 0.88 [0.78-0.99], p = 0.03) was associated with reduced risk. There was no relationship with duration of renal replacement therapy and long term mortality. There was a median decline in renal function of 5.08mL/min/year (IQR -3.43 to 28.19, p = 0.02) in patients who became dialysis free.
Conclusion: This retrospective study demonstrates a greater mortality risk for those who develop haemofiltration-dependent AKI with the impact largely seen in the first year post transplant. It also demonstrates the significant impact AKI has on long term renal function.



References:
[1] Keogh A, Williams T, Pettersson R. ANZCOTR Report 2013

354.8

Renal outcomes in lung transplant recipients treated with the mammalian target of rapamycin (mTOR) inhibitor everolimus

Timothy Coughlan[1], Steve Ivulich[2], Ryan Slack[1], Boyoun Choi[2], Eldho Paul[3], Greg Snell[2], Solomon Menahem[1].
[1]Department of Nephrology, Alfred Health, Melbourne, Australia; [2]Department of Allergy, Immunology and Respiratory Medicine, Alfred Health, Melbourne, Australia; [3]Department of Epidemiology and Preventative Medicine, Monash University, Melbourne, Australia.

Background: Lung transplantation is a treatment option for selected patients with progressive lung disease refractory to other treatment modalities[1]. The calcineurin inhibitors (CNIs) tacrolimus and cyclosporine A (CyA) are the mainstay of most immunosuppressive regimens but are associated with nephrotoxicity[2]. Studies have shown that the use of everolimus as a CNI sparing agent was associated with improvements in renal function, although these studies had relatively short follow-up periods and small numbers[3]-[6]. We sought to describe the renal outcomes of lung transplant patients switched to everolimus in a large single-centre cohort study with prolonged follow-up and determine whether there was an observable deleterious effect of everolimus on lung function.
Methods: We included all lung and heart-lung transplant recipients at the Alfred Hospital with no previous mTOR exposure who were converted to everolimus at any stage after transplantation. Recipients of simultaneous lung-kidney transplants were excluded . Patients who received at least three months exposure to everolimus and who were converted later than one month after transplantation were included for analysis. The study received ethics approval from the Alfred ethics committee.
Demographic and clinical data was collected at four time points: 1. Date of transplantation. 2. Date of conversion to everolimus. 3. 3 months after conversion to everolimus and 4. Date of everolimus cessation, death on everolimus, or last follow-up (end date).
Results: 110 lung allograft recipients at our institution were converted to everolimus of which 67 met the inclusion criteria for analysis. We observed a significant reduction in creatinine clearance between transplantation and conversion in this cohort. Use of everolimus was associated with a reduction or withdrawal of concurrent CNI use.
Following conversion to everolimus, we observed a significant improvement in creatinine clearance at both three months after conversion and at the end date (date of death on everolimus, everolimus cessation or last follow-up). The mean rate of change in creatinine clearance was +13.4 ml/min per year on everolimus, compared to -30.6 ml/min per year between transplantation and conversion (P<0.001).

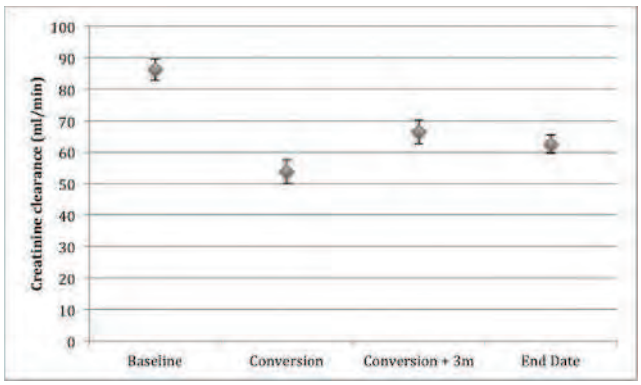


Figure 1: Mean creatinine clearance (+/-CI) by time point
There was no significant difference in FEV1 observed at baseline (first spirometry performed post-transplantation), at conversion to everolimus or at three months after conversion, but there was a significant decline at the end date. There was a mean rate of decline in FEV1 of 0.51L/year between conversion and the end date, compared to a mean rate of increase of 0.15L/year between baseline and conversion (P<0.001).

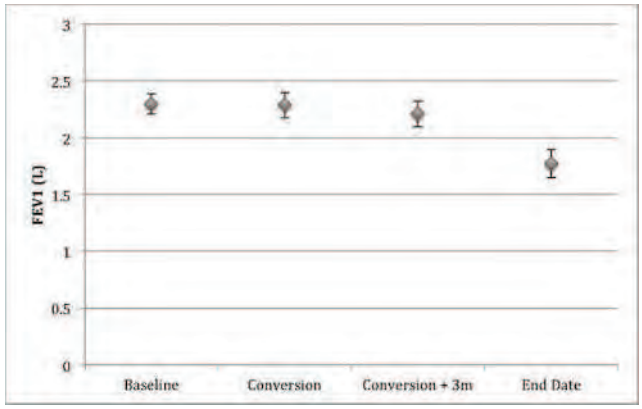


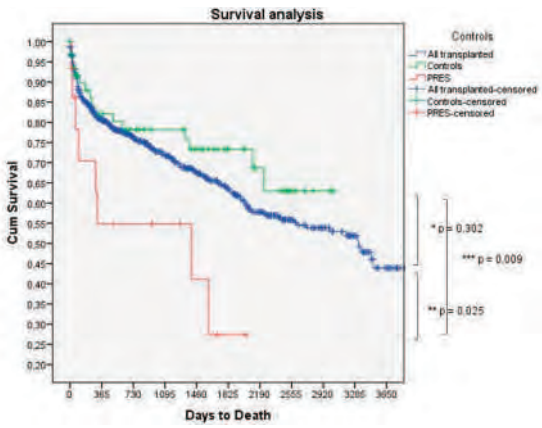
Figure 2: Mean FEV1 (+/-CI) by time point
Our observations are consistent with previous retrospective cohort studies suggesting that the use of everolimus to allow CNI minimization or withdrawal is associated with improved renal function in patients with renal impairment after lung transplantation. Randomised control trials are required to demonstrate whether everolimus is renoprotective when used in lung allograft recipients and whether there is a beneficial or deleterious effect on lung function and patient survival.
References:
[1] Snell GI, Westall GP, Paraskeva MA. Immunosuppression and allograft rejection following lung transplantation: evidence to date. Drugs 2013;73:1793-813.
[2] Naesens M, Kuypers DR, Sarwal M. Calcineurin inhibitor nephrotoxicity. Clinical journal of the American Society of Nephrology : CJASN 2009;4:481-508.
[3] Gullestad L, Iversen M, Mortensen SA, et al. Everolimus with reduced calcineurin inhibitor in thoracic transplant recipients with renal dysfunction: a multicenter, randomized trial. Transplantation 2010;89:864-72.
[4] Parada MT, Alba A, Sepulveda C, Melo J. Long-term use of everolimus in lung transplant patients. Transplantation proceedings 2011;43:2313-5.
[5] Roman A, Ussetti P, Zurbano F, et al. A retrospective 12-month study of conversion to everolimus in lung transplant recipients. Transplantation proceedings 2011;43:2693-8.
[6] Schneer S, Kramer MR, Fox B, et al. Renal function preservation with the mTOR inhibitor, Everolimus, after lung transplant. Clinical transplantation 2014;28:662-8.

354.9

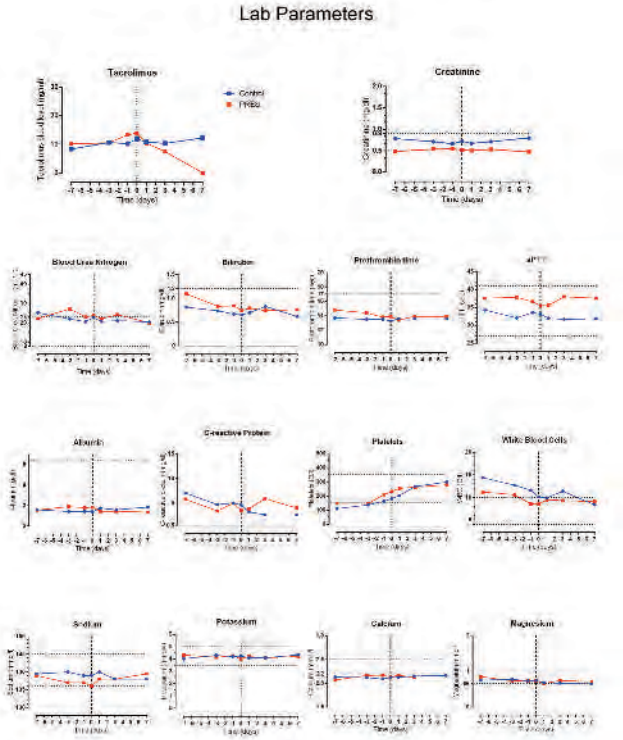
PRES after lung transplantation: A single centre experience

Alberto Benazzo[1], Stefan Schwarz[1], Sophie Bartsch[2], Gabriella Muraközy[1], György Lang[1], Walter Klepetko[1], Peter Jaksch[1].
[1]Department of Thoracic surgery, Medical University of Vienna, Vienna, Austria; [2]Department of Radiology, Medical University of Vienna, Vienna, Austria.

Posterior Reversible Encephalopathy Syndrome (PRES)[1],[2] is a rare but severe neuroradiological complication after solid organ transplantation (SOT). We describe the incidence, risk factors, clinical and radiological presentation and the survival of this syndrome after lung transplantation.
From 1029 patients we retrospectively evaluated 21 cases with a diagnosis of PRES. To chose our control group, 4 patients transplanted in the same period (max. ±50 days) were randomly chosen for each PRES patient (n=84). Two PRES Patients were eliminated for lack of data in matched control patients. All 19 patients received brain MRI and one patient was excluded because of an unclear radiological appearance. We collected demographic, medical, peri-operative and therapeutic data. Lab parameters were also collected at 7 time points: 1 wk, 3 d and 1 d before the PRES event, at the event and 1 d, 3 d and 1 wk after. The time period between surgery and the neurological event was applied to each of the matched 4 control patients to collect control lab values.
Incidence of PRES was 1,9%. Median PRES age was 26,8 yrs (7,4-65,6) and median control age was 47,4 yrs (14,4-66,1) ($p < 0,001$), indicating that young patients are more prone to develop PRES. Significant differences were found in pre-transplant intubation need (p=0,003) and pre- and post-operative ECMO support (p<0,001 and p=0,002), highlighting the more critical conditions in PRES group. Reintubation need (p=0,004) and tracheostomy need (p=0,013) were found significant as well as median ICU time and hospitalization time, respectively 32 d (8-69) vs 7 d (1-105) (p<0,001) and 44 d (20-90) vs 25 d (7-105) (p<0,001). These results, however, may be a consequence of PRES. No significant difference was found between median intubation time of PRES group (5 d, 1-21) vs control (2 d, 0-30) (p=0,81). Univariate conditional logistic regression for PRES within the first 2 months after Tx showed the following results: pre-tx intubation need (OR=7,93, p=0,003, CI=2,02-31,18), pre-operative ECMO need (OR=12, p=0.02, CI=2.42-59.54), post-operative ECMO need (OR=6.09, p=0,003, CI=1.83-20.23) and age<vs>20 (OR=4.069, p=0,022, CI=1,219-13,59). These results indicate that the factors analyzed represent risks for PRES onset. Survival analysis showed 1-year survival of 55% vs 80% and 5-year survival of 30% vs 60% compared to all transplanted patients (p=0,025). Radiologically, correlation was found between the number of affected regions and cytotoxic edema (p=0,03). Subcortical involvement, parietooccipital pattern and asymmetric extension were observed.



PRES is clearly predominant in young patients with severe pre-Tx conditions and complicated postoperative period. The critical conditions of the recipients and the circulatory support may induce a systemic inflammatory status[3] which activates cerebral endothelium and promotes blood extravasation and cerebral edema.



References:

[1] Hinchey J., Chaves C., Appignani B., et al. A reversible posterior leukoencephalopathy syndrome *N Engl J Med*. 1996; 334(8):494-500
[2] Bartynski WS. Posterior reversible encephalopathy syndrome, part I: fundamental imaging and clinical features. *Am J Neuroradiol.*. 2008; 29(6):1036-1042
[3] Bartynski WS. Posterior reversible encephalopathy syndrome, part II: controversies surrounding pathophysiology of vasogenic edema. *Am J Neuroradiol.*. 2008; 29(6):1043-1049

355.1

A Multicentre Randomised Controlled Trial to Compare the Efficacy of Normothermic Machine Perfusion with Static Cold Storage in Human Liver Transplantation: Early Outcomes

David Nasralla, The Consortium for Organ Preservation in Europe (COPE) Liver Research Group, Rutger Ploeg, Peter Friend. Nuffield Department of Surgical Sciences, University of Oxford, Oxford Transplant Centre, Oxford, United Kingdom.

Introduction: The international organ shortage has necessitated the use of sub-optimal organs, which are associated with inferior recipient outcomes. Normothermic machine perfusion (NMP) offers a potential solution to this problem; by perfusing the liver with oxygenated blood, medications and nutrients at 37C, the organ is preserved in a functioning, physiological state. Animal studies[1] suggest several benefits: (i) Repair of damage sustained during the retrieval process; (ii) Extended preservation times; (iii) Assessment of organ viability. If these were achieved in clinical practice, then organ utilisation could be greatly improved without compromise to safety or outcome. We present the first analysis of a randomised controlled trial (RCT) comparing NMP with static cold storage (SCS) in clinical liver transplantation.

Materials and Methods: Under the sponsorship of the University of Oxford, a European Union-funded multi-national RCT was commenced in June 2014 involving 7 transplant centres in England, Belgium, Germany and Spain. Livers from adult DBD and controlled DCD donors were eligible. Recipient eligibility criteria included adult recipients of liver-only transplants. Livers were randomised 1:1 to either NMP or SCS. The trial was powered to demonstrate a 33% drop in peak AST measured in the first week post-transplant, requiring a total of 220 transplanted livers (90% power). Secondary endpoints included: post-reperfusion syndrome; early allograft dysfunction; graft and patient survival; ischaemic cholangiopathy (6 month MRCP); health economic analysis. The flow of participants through the trial is illustrated in figure 1.

Results: No interim analysis is permitted in the design of this trial; preliminary data are therefore not available. However, at the current recruitment rate, enrolment will be complete by the end of February 2016. At TTS 2016, we will be able to present the early outcome data, including: (i) Peak AST (primary outcome); (ii) EAD rates; (iii) Length of ITU stay; (iv) 30-day graft and patient survival.

Conclusion: This is the first clinical study of NMP. If successful, this technology has the potential to greatly increase the pool of transplantable livers. A multi-centre RCT in the USA is scheduled to start in early 2016 as well as other NMP studies in Brazil, Spain and China.

Hynek Mergental; Queen Elizabeth Hospital, Birmingham; Thamara Perera; Queen Elizabeth Hospital, Birmingham; Darius Mirza; Queen Elizabeth Hospital, Birmingham; Wayel Jassem; King's College Hospital, London; Andrew Butler; Addenbrooke's Hospital, Cambridge; Charles Imber; Royal Free Hospital, London; Jacques Pirenne; University Hospitals, Leuven, Belgium; Diethard Monbaliu; University Hospitals, Leuven, Belgium; Andreas Paul; University Hospital, Essen, Germany; Peri Kocabayoglu; University Hospital, Essen, Germany; Juan Carlos Gacia-Valdecasas; Hospital Clinic, Barcelona, Spain; Mihai Pavel; Hospital Clinic, Barcelona, Spain; Annemarie Weissenbacher; NDS, University of Oxford; Carlo Ceresa; NDS, University of Oxford; Rubens Macedo; University Hospital of Sao Paolo, Brazil; Chris Morris; OrganOx Ltd, UK; Les Russell; OrganOx Ltd, UK; Constantin Coussios; IBME, University of Oxford

References:

[1] Brockmann, J., et al., Normothermic perfusion: a new paradigm for organ preservation. Ann Surg, 2009. 250(1): p. 1-6.

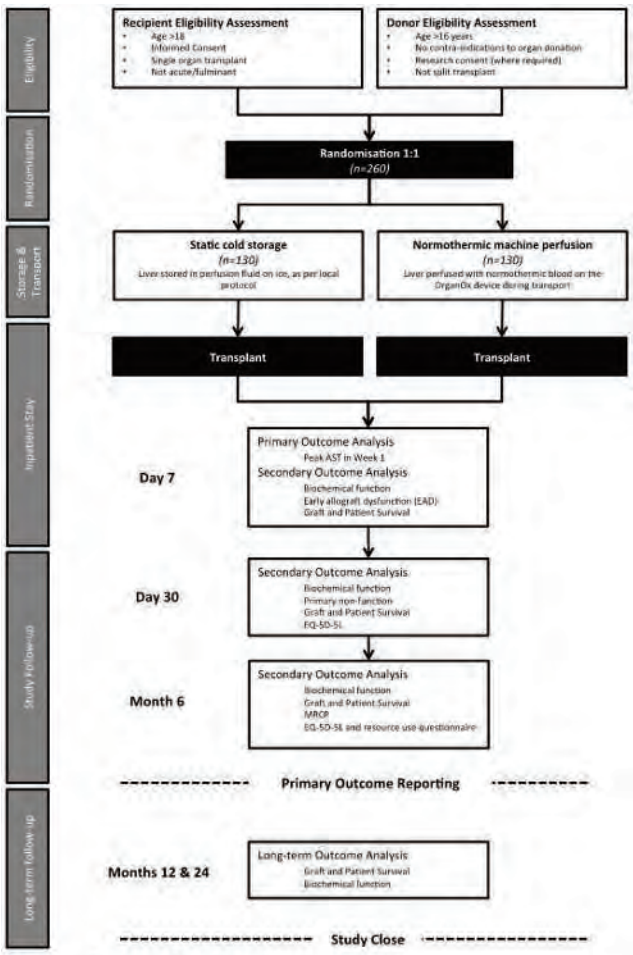


Figure 1. Flow of participants through the trial

355.2

DAMP-associated preservation injury and complications after liver transplantation: proof of concept

Denis Efimov[1], Alijaksei E.Shcherba[1], Olga A.Lebedz[1], Alla I.Kireeva[2], Sergey Korotkov[1], Alexander M.Dzyadzko[3], Oleg O.Rummo[1].
[1]Transplantation Department, RSP Center for Organ and Tissue Transplantation, Minsk, Belarus; [2]Institute of Genetics and Cytology National Academy of science, Minsk, Belarus; [3]Anesthesiology and ICU Division, RSP Center for Organ and Tissue Transplantation, Minsk, Belarus.

Introduction: There is no doubt that the activation of antigen-dependent immune response is the main pathogenetic pathway of immunological complications development following liver transplantation. Recently the attention to antigen-independent injury and subsequent inflammation as triggers of graft rejection was given[1]. High mobility group box 1 (HMGB1), the one of the most important damage associated molecular pattern (DAMP), was shown as an inflammatory trigger in organ transplantation and it was believed that HMGB1 inhibition reduced ischemic reperfusion injury (IRI)[2]-[4].
Aim: The aim of the study was to evaluate the relationship between the expression of HMGB-1 before and after reperfusion, severity of IRI, early allograft dysfunction (EAD) and acute rejection (AR) incidence after liver transplantation.
Materials and Methods: 68 consequent DBD adult liver transplants (LT) from standard criteria donors with static cold storage as conservation type were included in the prospective case-control study; study period: February 2013 – June 2014. Liver graft biopsies taken at procurement operation and 2h after portal reperfusion on LT were assessed: HMGB-1 and CD68+-cells expression, IRI severity, TLR-4 gene polymorphism. End points: early allograft dysfunction (EAD)[5], severe EAD (sEAD)[6], biopsy-proven acute rejection (BPAR) and graft survival were assessed according to standard criteria. Investigation was approved by local ethic committee.
Results and Discussion: Mean cold ischemia time was 441 min (370;510). The overall BPAR incidence was 19.1% (13/68), EAD incidence – 20.6% (14/68), sEAD – 10.3% (7/68). It was revealed that: more severe IRI (sever IRI vs moderate+mild IRI) was associated with larger increment of nuclear HMGB-1 expression (16% (1;24) vs 1% (-4;5), p=0.01) and with greater post reperfusion CD68+ infiltration (65 cell/mm2 (50;93) vs 42 (31;55); p=0.001). Furthermore, heterozygote in donor rs913930 TLR-4 gene (C/T vs CC+TT) were associated with bigger donor nuclear HMGB-1 expression (21% (17;29) vs 16% (10;19); p=0.018) and subsequently with higher sEAD incidence (21.4% (6/28) vs 2.5% (1/40); p=0.017). Alternatively the presence of cytoplasmic HMGB-1 expression (presence vs absence of cytoplasmic HMGB-1) in post reperfusion liver graft biopsy was marginally associated with smaller BPAR frequency (14.6% (7/48) vs 30% (6/20); p=0.1).
Conclusion: HMGB-1 – TLR-4 pathway is important pathogenic mechanism of liver graft injury during cold preservation and subsequent reperfusion. TLR-4 gene polymorphism is associated with sEAD incidence. More severe DAMP-associated preservation liver graft injury was associated with higher incidence of sEAD and lower incidence of BPAR by that proving the concept of antigen independent immune response in EAD and BPAR development after LT.
References:
[1] Daniel N. Mori et al. Inflammatory triggers of acute rejection of organ allografts. *Immunol Rev*. 2014 March; 258(1): 132–144.
[2] Zhu H et al. Hmgb1-TLR4-IL-23-IL-17A axis promote ischemia-reperfusion injury in a cardiac transplantation model. *Transplantation*. 2013; 5:1448– 1454
[3] Li J et al. Neutralization of the extracellular HMGB1 released by ischaemic damaged renal cells protects against renal ischaemia-reperfusion injury. *Nephrol Dial Transplant*. 2011; 26:469–478.
[4] Tsung A et al. HMGB1 release induced by liver ischemia involves Toll-like receptor 4-dependent reactive oxygen species production and calcium-mediated signaling. *J Exp Med*. 2007; 204:2913– 2923.
[5] Olthoff KM et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors.*Liver Transpl*. 2010;16(8): 943-9
[6] Salvalaggio P et al. A proposal to grade the severity of early allograft dysfunction after liver transplantation.*Einstein (Sao Paulo)*. 2013 Jan-Mar;11(1):23-31.

355.3

Biliary epithelial damage, regenerative capacity and local immune response after cold storage of DBD livers determine biliary complications after liver transplantation

Stefan Brunner, Henrik Junger, Hans J. Schlitt, Stefan Fichtner-Feigl.
Department of Surgery, University Medical Center Regensburg, Regensburg, Germany.

Background: The aim of this study was to examine molecular mechanisms of bile duct (BD) damage between organ retrieval and transplantation and its clinical relevance for patients.
Methods: BD samples after donor hepatectomy, cold storage and reperfusion were compared to healthy controls by H&E staining, immunohistochemistry (IH) for tight junctions (TJ), cytokeratin, e-cadherin, laminin, CD4, CD8 and CD14 and by global mRNA expression using Microarray technique. Bacterial infiltration was determined by FISH for bacterial antigens. Further, a BD damage score (BDDS) to quantify biliary epithelial injury was developed and correlated with recipient and donor data and patient outcome.
Results: Patients with major BD damage after cold storage, as quantified by the newly developed BDDS, had a significantly increased risk of biliary complications ($p<0.0001$) and graft loss ($p=0.0004$). After cold storage ($p=0.0119$) and even more after reperfusion ($P=0.0002$), epithelial damage categorized by the BDDS and by TJ architecture was markedly increased. mRNA levels of adherens-junctions ($q=0.003$) and focal-adhesion-molecules ($q=0.04$) in damaged BDs without compared to damaged BDs with biliary complications were increased reflecting enhanced regenerative capacity of the biliary epithelium in the first group. Consecutively, IH showed significantly increased cytokeratin, e-cadherin and laminin expression in this group. FISH analysis demonstrated equal distribution of bacterial infiltration of BDs, however, mRNA analysis detected induced antibacterial immune response ($q=0.00084$) and phagocytosis ($q=0.04$) in BDs with enhanced epithelial regenerative capacity corroborating with significantly increased $CD4^+$ and $CD8^+$ cell-mediated adaptive immune response.
Conclusions: In many cases, the common BD epithelium shows considerable damage after cold ischemia with further damage occurring after reperfusion. The extent of epithelial damage can be quantified by our newly developed BDDS and is a prognostic parameter for biliary complications and graft loss. Following BD damage during cold storage, functional regenerative capacity of the biliary epithelium and enhanced local adaptive antibacterial immune response are able to rescue BDs and prevent biliary complications after liver transplantation.

355.4

Importance of DNA methylation patterns and association with graft injury severity after liver transplantation

Daniel G. Maluf, Ricardo Gehrau, Valeria Mas .
Surgery, UVA, Charlottesville, VA, United States.

Background: Epigenetics modifications in the graft may impact on severity of injury and function post liver transplantation (LT). This study aimed to interrogate the effect of different graft DNA methylation patterns on gene expression profiles that associate with graft injury after LT.
Patients and Methods: The study included 22 deceased donor LT patients with severe (SI, n=11; AST>500 IU/L) and mild (MI, n=11; AST<500 IU/L) early graft injury at 1-day post-LT. Tissue biopsies were collected at pre-implantation (L1) and at post-reperfusion (L2). Genomic DNA was extracted from pre implantation biopsies; bisulfite converted and used in Infinium 450K methylation arrays. Raw data was normalized by SWAN method and analyzed with R bioconductor. Beta scores were converted to M-values. F-test was fit for significant demethylated CpG sites ($q<0.05$). Total RNA was isolated from all biopsies, labeled and used in gene expression microarrays. Probeset summaries were obtained using RMA algorithm. Unpaired ANOVA was fit for deregulated probesets ($p<0.001$, FDR <5%). Molecular pathways were evaluated by IPA tool. CpGs (Methylight) and genes (RT-PCR) were validated.
Results: There was not difference between groups in demographics, graft preservation type, and cold- and warm-ischemia times. In total, 3663 CpG sites (2574 hypomethylated; 1089 hypermethylated) were significantly demethylated and mapping within genic regions. Interestingly, 2251 CpGs (92% hypomethylated, $p<0.0001$) mapped within GC-islands located at promoter regions (1971 genes) in SI grafts. Molecular pathway analysis based on CpGs methylation identified apoptosis activation signaling (TP53, BIM, BAD, BAX, DIABLO, APAF1, CAD, FADD, CASP2, CASP3), ubiquitin protein degradation, and cell cycle regulation (Rb, p27KIP, p18INK4C, CDK, CHK1) ($p<0.0001$). Genes with multiple hypomethylated CpGs anticipated increases in liver cell death (BCL2L11, HSPD1, APP, INHA, TRIM27, BAD) and G1/S cell cycle check-point (SKP2, CDC25A, SIN3A, PA2G4). No genes were differentially expressed at pre-implantation. A unique significantly deregulated molecular signature (94 genes) correlating with CpG demethylation coincided with liver damage (KRT18), apoptosis and cell cycle regulation (PAWR, KRAS, DUSP4, PTPR3) at post-reperfusion in SI grafts. Genes were validated by RT-qPCR.
Conclusion: Hypomethylation of specific genes involved in the regulation of cell death pathways may induce increases in graft injury post-LT.

355.5

Exogenous melatonin promotes graft regeneration in small-for-size liver transplantation

Zhuolun Song, Eleonora Maurizio, Bostjan Humar, Rolf Graf, Pierre AlainClavien, Yinghua Tian.
Department of Visceral and Transplantation Surgery, University Hospital Zurich, Zurich, Switzerland.

Introduction: Live donor liver transplantation increases the liver donor pool, but it is impeded by small-for-size syndrome. Melatonin (MLT) is an endogenous hormone regulating circadian rhythm, which is a strong antioxidant in a pharmacological dose. The aim of the study is to investigate whether MLT enhances liver graft regeneration and prevents small-for-size liver graft failure in model of mouse partial liver transplantation.
Materials and Methods: Male C57BL6 mice were divided into 3 groups: (I) I/R+PH group: 60 min liver ischemia plus 2/3 hepatectomy; (II) I/R+exPH group: 60 min liver ischemia plus extended (86%) hepatectomy; (III) POLT group: arterialized 30% liver transplantation. Each group was subdivided into MLT treated and control groups. Hepatic injury was determined by AST, ALT and histology. The cytokines and histological evidence of liver regeneration were examined by PCR and immunostaining. Serum HMGB1 was measured by ELISA. Survival rate was monitored in I/R+exPH and POLT groups. In mouse primary hepatocytes culture model, hepatocytes were subjected to 4h hypoxia followed by 2h reoxygenation, AST and ALT from the supernatant as well as cytokines from hepatocytes mRNA were measured.
Results and Discussion: The group I disclosed less hepatic injury, improved hepatocyte regeneration and promoted regenerating cytokine release in MLT treated group than controls. HMGB1 was reduced significantly in mice treated by MLT. In group II, 7 day's survival rate was 0% in control mice in comparison with 50% in MLT treated mice. In POLT group, the treatment of MLT increased the survival rate of recipient mice from 0% to 57%. In vitro experiment demonstrated less injury and lower level of pro-inflammatory cytokines in MLT treated hepatocytes. The data indicated that MLT reduces liver ischemia injury and enhances graft regeneration in the setting of mouse partial liver transplantation.
Conclusion: MLT rescues small for size liver graft failure by reducing graft ischemic reperfusion injury and promoting liver regeneration. The enhanced liver graft regeneration is through up-regulated IL-6 and TNF-α by MLT.

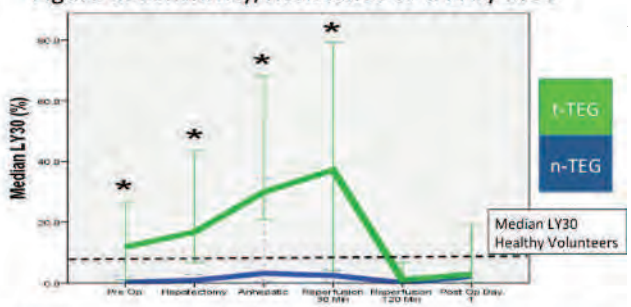
355.6

Reperfusion fibrinolysis shutdown after liver transplantation: Rationale for permissive hypocoagulability in the post-operative setting

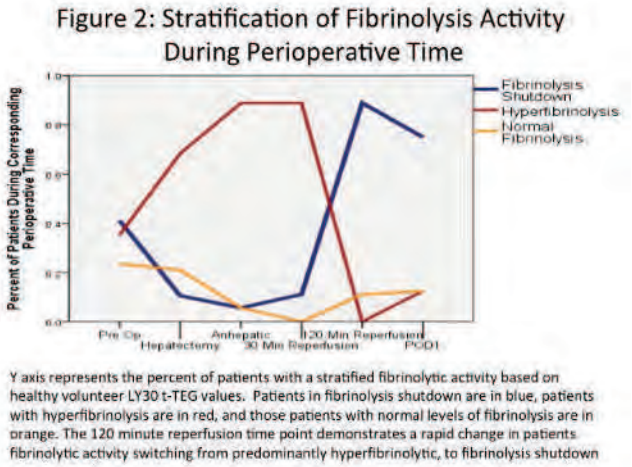
Hunter Moore, Ernest E.Moore, Michael Wachs, Igal Kam, Peter J.Lawson, Benjamin R.Huebner, Thomas Bak, Kendra Conzen, Anirban Banerjee, Christopher C.Silliman, Trevor L.Nydam.
Surgery / Transplant, University of Colorado, Aurora, United States.

Introduction: The mechanisms of post-operative thrombotic events after liver transplant remain poorly understood. Recently it has been appreciated that hypotensive trauma patients develop resistance to clot degradation (fibrinolysis shutdown) within 2 hours of resuscitation. The duration that these patients remain in fibrinolysis shutdown has been associated with thrombosis and other complications. We hypothesize that liver transplant recipients will also transition to a state of fibrinolysis resistance shortly after graft reperfusion.
Methods: Liver transplant recipients had serial blood samples analyzed with citrated native thrombelastography (n-TEG) and a modified TEG assay using tPA (t-TEG). The t-TEG was used to quantify the patient's sensitivity/resistance to fibrinolysis using the lysis at 30 minutes (LY30). Based on the LY30 distribution of 160 volunteers with t-TEG (median LY30 8.2; 25th percentile 4.8; 75th percentile 14.9) fibrinolysis activity was categorized as: fibrinolysis shutdown (<25 %), normal fibrinolysis (25%-75%), and hyperfibrinolysis (>75%). Transplant n-TEG variables were also contrasted to healthy volunteers (R time, angle, MA, LY30). Endogenous tPA levels and its inhibitor [plasminogen activator inhibitor -1 (PAI-1)] levels were measured using ELISA in stored paired patient plasma.
Results: Nineteen patients were enrolled in this study with a median MELD of 30, 37% required >10 units RBC during surgery, and the mortality rate was 5%. When contrasting paired n-TEG vs t-TEG values, the t-TEG LY30 were significantly higher from pre op until 30 minutes of graft reperfusion (all p<0.001). However, at 120 minutes post reperfusion (p=0.212) and post-operative day 1 (POD1 p=0.184), no significant differences were observed (figure 1). Fibrinolysis activity markedly changed during the perioperative period with 70% of patients remaining in fibrinolysis shutdown on POD1 (p<0.001 figure 2). POD1 platelet counts were significantly decreased compared to pre op values (60K vs 75K p=0.04) However, INR and TEG parameters (R, Angle, MA) were not significantly different (although median values pre and POD1 were all abnormal compared to healthy volunteers). Endogenous tPA levels quadrupled from pre op to anheptatic phase of surgery (2.5 to 19 ng/ml p=0.001) and returned to similar pre op levels by 120 minutes post reperfusion (3.6). Free PAI-1 activity levels tripled from pre op to POD1 (5.7 to 16.2 ng/ml P<0.001). Patients who remained in fibrinolysis shutdown on POD1 had longer post-operative

Figure 1: Sensitivity/Resistance to tPA by LY30



Y Axis represents the median LY30 and X axis represents time points during the perioperative period. The dotted line represents the median LY30 of healthy volunteers t-TEG. The green bar represents paired patients samples analyzed with the t-TEG versus n-TEG (blue). LY30 from t-TEGs were significant higher compared to n-TEG until 120 minutes post reperfusion and remained similar on POD1.



Y axis represents the percent of patients with a stratified fibrinolytic activity based on healthy volunteer LY30 t-TEG values. Patients in fibrinolysis shutdown are in blue, patients with hyperfibrinolysis are in red, and those patients with normal levels of fibrinolysis are in orange. The 120 minute reperfusion time point demonstrates a rapid change in patients fibrinolytic activity switching from predominantly hyperfibrinolytic, to fibrinolysis shutdown

Conclusion: Reperfusion fibrinolysis shutdown occurs in the majority of liver transplant patients within 2 hours of graft reperfusion and associated with high levels of PAI-1. Patients who remain in shutdown appear to be at a higher risk of post-operative complications. Resuscitating these patients to a permissive hypocoagulability state post operatively is logical, as over correction to hypercoagulability can result in intravascular thromboses, and the majority of these patients lack the endogenous capacity to clear these clots.

References:

[1] Moore HB, Moore EE, Gonzalez E, Chapman MP, Chin TL, Silliman CC, Banerjee A, Sauaia A. Hyperfibrinolysis, Physiologic Fibrinolysis, Fibrinolysis Shutdown: The Spectrum of Postinjury Fibrinolysis and the Relevance to Antifibrinolytic Therapy J Trauma Acute Care Surg. 2014;77(6):811-7.

[2] Moore HB, Moore EE, Gonzalez E, Hansen KC, Dzieciatkowska M, Chapman MP, Sauaia A, West B, Banerjee A, Silliman CC. Hemolysis Exacerbates Hyperfibrinolysis While Platelolysis Shuts Down Fibrinolysis: Evolving Concepts of the Spectrum of Fibrinolysis in Response to Severe Injury. Shock. 2015;43(1):39-46.

[3] Moore HB, Moore EE, Chapman MP, Gonzalez E, Huebner B, Einersen B, Slaughter A, Sheppard FR, Banerjee A, Silliman CC, Sauaia A. Reperfusion Shutdown: Delayed Onset of Fibrinolysis Resistance After Resuscitation from Hemorrhagic Shock is Due to Increase Circulating Levels of Plasminogen Activator Inhibitor-1. Shock 2016 (Under Review)

355.7

The effect of nafamostat mesilate to minimize ischemic-reperfusion injury of the liver following liver transplantation : a single arm pilot study

Young-Dong Yu, Dong-Sik Kim, Sung-Won Jung, Jae-Hyun Han. Department of Surgery, Division of HBP Surgery and Liver Transplantation, Korea University Medical College, Seoul, Korea.

Background: Ischemia-reperfusion injury in the liver, a major complication of hemorrhagic shock, resection and transplantation, is a dynamic process that involves the two interrelated phases of local ischemic insult and inflammation-mediated reperfusion injury. Nafamostat mesilate inhibits various serine proteases generated during inflammation, coagulation and fibrinolysis cascades and is used for the treatment of pancreatitis and disseminated intravascular coagulation. Although its role in attenuating ischemic reperfusion injury has been studied in animal models, there are few reports regarding its effect on ischemic-reperfusion injury in human liver transplantation. The aim of this study was to examine the effect of nafamostat mesilate to minimize ischemic-reperfusion injury of the liver following liver transplantation.

Methods: Patients that were enrolled in this study underwent additional rinsing with a solution consisting of Nafamostat mesilate following graft perfusion using standard HTK solution. Blood samples were taken from the recipient just before portal vein transection during recipient hepatectomy and 1 hour, 24 hours after reperfusion. Samples were also taken on third and seventh postoperative day. Liver function tests of the study group was compared to a control group match-paired for sex, age, type of transplantation and fatty change of the graft. The levels of TNF, IL-6 and IL-1 were also examined in the study group.

Results: Ten patients were enrolled in this study. The male to female ratio was 7 to 3. The mean age was 55 years. The average MELD score of the recipient was 20.9. The most common indication for transplantation was hepatocellular carcinoma and liver cirrhosis due to hepatitis B. Most patients received emergency deceased donor liver transplantation. The mean cold and warm ischemic time was 373 and 35 minutes, respectively. Although not statistically significant, the mean aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels on the first and third postoperative day were lower in the study group. In addition the mean peak AST and ALT levels were lower in the study group. There were no significant differences in serum bilirubin or prothrombin time.

Conclusion: Although further studies are needed, Nafamostat mesilate showed promise in decreasing ischemic-reperfusion injury and might be useful when using marginal grafts for liver transplantation.

355.8

Impact of a newly developed solution containing artificial oxygen carrier hemoglobin vesicles under subnormothermic conditions with machine perfusion preservation for DCD liver grafts in pigs

Naoto Matsuno[1], Lingtong Meng[1], Tomohiro Suzuki[2], Jo Yamamoto[2], Hiroshi Azuma[3], Yuji Nishikawa[4], Hiromizu Sakai[5], Hiromichi Obara[6], Hiroyuki Furukawa[1].
[1]Surgery and Transplantation, Asahikawa Medical University, Asahikawa, Japan; [2]Pharmacy, Asahikawa Medical University, Asahikawa, Japan; [3]Pediatrics, Asahikawa Medical University, Asahikawa, Japan; [4]Division of Tumor Pathology, Department of Pathology, Asahikawa Medical University, Asahikawa, Japan; [5]Biochemistry, Nara Medical University, Nara, Japan; [6]Mechanical Engineering, Tokyo Metropolitan University , Tokyo, Japan.

Utilization of grafts from donors after cardiac death (DCD) would greatly contribute to the expansion of the donor organ pool. However, the implementation of such a strategy requires the development of novel preservation methods to recover from changes due to warm ischemia. To assess the effects of cell-free oxygen carrier under subnormothermic conditions for porcine livers procured after 60 minutes of warm ischemic time (WIT) were perfused and preserved.
Materials and Methods: Porcine livers were perfused with MP system developed by our group. Liver was perfused with modify UW-gluconate(UW-G) in Group 1 and UW-G containing with artificial oxygen carriers andamino solution(JPS) as in Group 2. All liver grafts were perfused under subnormothermic condition for 4 hours.
Results: A hepatic artery pressure during perfusion was smoothly came down in Group2 rather than in Group1. Lactate level in perfusate was maintained in low level (<3.0mnol/L)I in Group 2. No decrease of PH and no increase of wet tissue weight were seen in Group 2. The AST and LDH levels in perfusate were significantly higher in Group 1. .Histologically, enlarged Disses space was seen in Group 1.
Conclusion: Our perfusion solution containing with artificial oxygen carrying hemoglobin vesicles is expected to facilitate the recovery and resuscitating the function of DCD liver grafts.

355.9

The comparison with donation after cardiac death liver grafts by oxygenated subnormothermic with rewarming machine perfusion in pigs

Lingtong Meng[1], Naoto Matsuno[1], Mariko Furukori[1], Hiroyuki Takahashi[1], Masahiro Hagiwara[1], Tatsuya Shonaka[1], Koji Imai[1], Noriyuki Morito[2], Masamichi Obara[2], Yuji Nishikawa[3], Hiroyuki Furukawa[1].
[1]Surgery and Transplantation, Asahikawa Medical University, Asahikawa, Japan; [2]Mechanical Engineering, Tokyo Metropolitan University , Tokyo, Japan; [3]Division of Tumor Pathology, Department of Pathology, Asahikawa Medical University, Asahikawa, Japan.

Background: The shortage of donor organs would be improved through the use of grafts from donors after cardiac death (DCD). However, the implementation of such a strategy requires the development of novel preservation methods to recover from changes due to warm ischemia. The aim of this study was to evaluate the effectiveness of subnormothermic machine perfusion preservation with rewarming for porcine DCD liver grafts for transplantation.
Methods: Porcine livers were perfused with newly developed machine perfusion (MP) system. The livers were perfused for 4 hours with modified UW-gluconate solution. Group 1: Grafts were preserved with WIT 0 min and simple cold storage (CS). Group 2: Grafts were preserved with WIT 0 min and hypothermic MP (HMP). Group 3: Grafts were preserved with WIT 60 min and CS. Group 4: Grafts were preserved with WIT 60 min and HMP. Group 5: Grafts were preserved with WIT 60 min and rewarming up to 25˚C by MP (RMP). Each graft was preserved for 4 hours. Modified UW-gluconate solution was used.
Results: The aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) in the effluent maintained at lower level in Group 5 compared to Group 4. However, tissue ATP levels maintained in low in Group 3, Group 4 and Group 5. Portal vein pressure during perfusion was lower in Group 4, compared to Group 5. Histologically, the steatotic change and enlarged Disse's space was seen in group 4. Tissue cytokine mRNA level IL-1β, IFN-γ, iNos, and IL-4 were significantly lower in group 5 than group 4.
Conclusion: Potentially, subnormothermic preservation with rewarming is expected to help the recovery of function for DCD liver grafts.

356.1

Donor DCs in chimeric vascularized thymic grafts, but not antigen-presenting host DCs, play an essential role in the induction of tolerance of solid organs in a preclinical large animal model

Kazuhiko Yamada[1,2], Tatsu Tanabe[1,2], Mitsuhiro Sekijima[2,3], Akihiro Kawai[2,3], Vincenzo Villani[2], Masayuki Tasaki[2,3], Akira Shimizu[2,3], David Sachs[1,2].
[1]Columbia Center for Translational Research/Surgery, Columbia University Medical Center, New York, United States;
[2]Transplantation Biology Research Center/Center for Transplantation Science, Massachusetts General Hospital, Boston, United States;
[3]Center for Advanced Biomedical Science and Swine Research , Kagoshima University, Kagoshima, Japan.

Background and Aim of the Study: We have demonstrated that host thymus plays an important role in transplantation tolerance. Euthymic animals accepted a class I mismatched kidney with 12 days of CyA while thymectomized hosts did not in an MHC inbred miniature swine model. Furthermore, we have established technique of isolated vascularized thymic lobe transplantation (VTL) that allows us to study thymic dependent tolerance without changes in thymic structure due to ischemia following thymic transplantation. Utilizing this novel technique, we have reported VTL induced transplant tolerance across allogeneic barriers in miniature swine. In this study, we investigated the mechanisms of thymic dependent tolerance utilizing the VTL technique.
Materials and Methods: MHC-inbred swine were used and allogeneic tolerance was assessed across a class I mismatched barrier using (1) donor-antigen presenting thymi (Donor Ag-VTL) and (2) chimeric thymi containing donor-type dendritic cells (Donor DC-VTL). Donor Ag-VTLs were exposed to donor antigens in long-term tolerant recipients of class I mismatched kidneys. Donor DC-VTLs were prepared by thymic graft parking such that the VTL contained donor DCs at the corticomedular junction but all of the epithelial cells remained recipient-type. Donor Ag-VTLs (Group 1. n=3) or Donor DC-VTLs (Group 2. n=5) were transplanted across class I mismatched barriers with 28 days of FK506. Donor Ag or DCs SLA matched kidneys were transplanted 2 months later without immunosuppression to assess the induction of tolerance.
Results: All eight recipients in both Groups 1 and 2 had donor specific unresponsiveness by in vitro CML assay. However, striking difference was seen following donor-type kidney transplantation without immunosuppression. All recipients of Donor Ag-VTLs rejected donor MHC matched kidneys at day 7, 8 or 9. In contrast, four of five recipients of DC-VTL subsequently accepted the DC matched kidneys without immunosuppression until the termination of the experiment (POD 287, 223, 215 and 187, respectively). One animal lost renal function on POD92, however did not show evidence of donor responsiveness in vitro by CML or development of anti-donor antibodies by FACS. In addition, the results of CD25 depleted CML assays suggested that CD8 T cells were being deleted by donor thymic DCs.
Conclusions: The presence of donor DCs plays an essential role in the induction of tolerance in this model. To our knowledge, this is the first report to indicate that donor DCs induced tolerance of kidneys without immunosuppression in a large animal model.

356.2

The combined effect of stem cell factor and granulocyte macrophage colony-stimulating factor administration after 90% partial hepatectomy in rats

Seung Duk Lee, Yoon Sung Suh, Hyerim Byeon.
Center for Liver Cancer, National Cancer Center, Republic of Korea, Goyang-si, Korea.

Background: After major hepatectomy or liver transplantation, the ability of the liver to regenerate has been used to prevent postoperative hepatic failure. Stem cell factor (SCF) and granulocyte macrophage colony-stimulating factor (GM-CSF) are known to play important roles in liver regeneration with synergistic effects in a previous in vitro study. The purpose of this study was to identify the impact of exogenous SCF and GM-CSF administration after 90% major hepatectomy in rats.
Methods: Sprague Dawley rats underwent 90% major hepatectomy using a bile duct-sparing portal pedicle ligation technique under microscopy. The rats were divided into two groups: group 1 (phosphate-buffered saline) and group 2 (SCF+GM-CSF treatment, each 25 mcg/kg). Treatment was administrated immediately after operation through the inferior vena cava. Liver regeneration capacity and expression of cytokines and their downstream signaling molecules were evaluated at postoperative day 1, 2, 4, and 7.

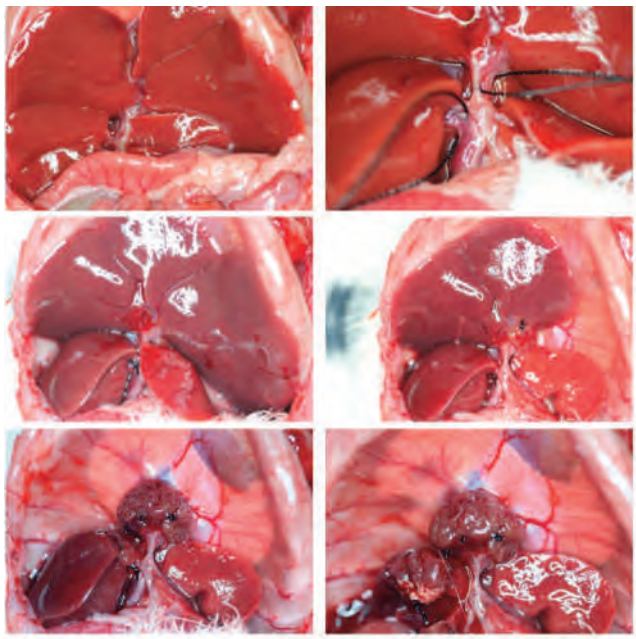


Fig. 1. Surgical technique of 90% partial hepatectomy (PH)
The 90% PH was performed to dissect hilum under microscopy and divide bile duct and portal vein/hepatic artery pedicle entering median/left lateral lobe and right lateral lobe. The pedicles of the lobes were divided into two to three parts and ligated by piercing before the lobes were resected.

Results: The survival rate after 90% hepatectomy in rats using this technique were increased to 95% compared with 55% with conventional parenchymal ligation technique ($p = 0.004$). The combination effect of SCF and GM-CSF was evaluated in in vitro study. Group 2 exhibited a significantly increased liver regeneration index at early period after hepatectomy compared to group 1 (day 2: 287.5 ± 19.6 vs. 513.9 ± 67.1, $p = 0.025$ and day 4: 647.6 ± 108.8 vs. 941.7 ± 53.9, $p = 0.046$). Furthermore, serum liver enzyme levels including total bilirubin, aspartate aminotransferase, and alanine aminotransferase, were significantly lower in group 2 than in group 1 on postoperative days. The expression of Ki-67 and cyclin D1 were significantly higher

in group 2 than in group 1 on postoperative days. Group 2 displayed significant increases of interleukin (IL)-6 and transforming growth factor (TGF)-β expression within 24 h after hepatectomy. Especially, C-X-C motif chemokine 12 (CXCL12)/C-X-C chemokine receptor type 4 (CXCR4) and matrix metalloproteinases 2 and 9 levels in the liver tissue of group 2 were also significantly upregulated according to quantitative polymerase chain reaction on postoperative days.
Conclusions: Our data suggest that the administration of SCF+GM-CSF after major hepatectomy can enhance liver regeneration by liver cell proliferation and mobilization of stem cell modulating IL-6/TGF-β and CXCL12/CXCR4 pathway as well as by matrix remodeling. These findings suggest the possibility of therapeutic treatment using a combination of SCF and GM-CSF in the clinical setting to promote liver regeneration after extreme hepatectomy.

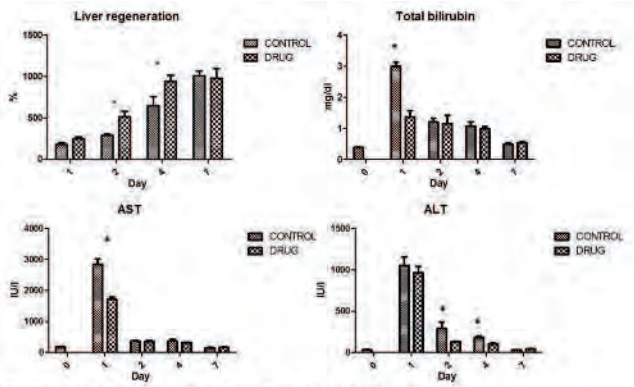


Fig. 3. The results of liver regeneration index and liver function tests between two groups.
The liver regeneration index was statistically significantly increased in SCF+GM-CSF group at postoperative days 2 and 4 (day 2: 287.5 ± 19.6 vs. 513.9 ± 67.1, $p = 0.025$; day 4: 647.6 ± 108.8 vs. 941.7 ± 53.9, $p = 0.046$). Liver enzyme levels including total bilirubin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) at baseline and on postoperative days 1, 2, 4, and 7 between control and SCF+GM-CSF group (*: $p < 0.05$).

356.3

Interleukin-34 is a Treg-specific cytokine and mediates transplant tolerance

Séverine Bézie, Antoine Freuchet, Véronique Daguin, Claire Usal, Ignacio Anegon, Carole Guillonneau.
ITUN, INSERM UMR1064, Nantes , France.

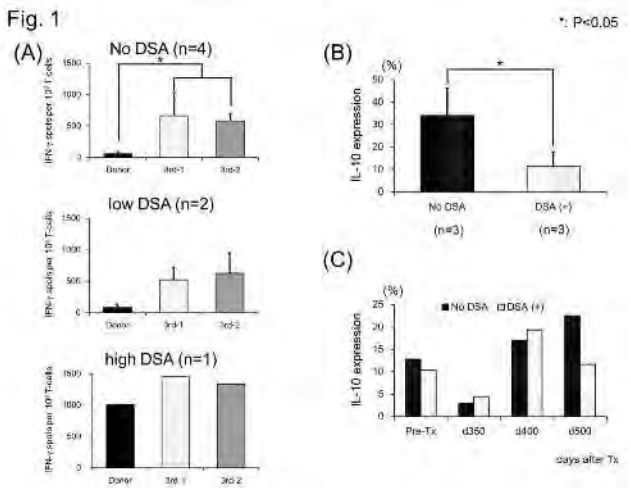
Introduction: Cytokines are powerful tools to regulate immune responses. In transplantation, it is still important to identify new specific and powerful mediators of immune tolerance. Interleukin-34 (IL-34) is a cytokine that binds to CSF1R (the MCSF receptor) and PTPz, and involved in differentiation and survival of myeloid cells. The recent correlation of IL-34 expression level with metastasis development and viral infection permission suggests immunoregulatory properties, but no link with T cell biology nor transplantation has ever been reported.
Materials and Methods: Lew-1A rats were treated with $1x10^{12}$ vg AAVIL-34, 30 days later they were grafted with an allogeneic Lew-1W heart and treated with 0.4mg/day/kg of rapamycin for 10 days. Recipients to cardiac allograft were irradiated at 4.5 Gy for adoptive cell transfers. PBMCs were obtained from blood of healthy volunteers. Monocytes were sorted on CD14/CD16 expression by FACS Aria, cultured for 6 days with 50ng/ml IL-34, and then cultured 14 days with Tregs. NSG mice were infused with human PBMCs and treated with 0.4mg/kg/2days human IL-34 protein or co-infused with expanded Tregs for xenogeneic GVH reaction studies.
Results: We showed that the cytokine IL-34 was expressed by rodent $CD8^+CD45RC^{low}$Tregs and human $FOXP3^+CD45RC^{low}CD8^+$ and $CD4^+$ Tregs. Furthermore, we observed a correlation of IL-34 expression level with the suppressive activity of Tregs and the tolerant status of rat cardiac allograft recipients. Indeed, IL-34 was involved in the suppressive function of both $CD8^+$ and $CD4^+$Tregs and markedly inhibited alloreactive immune responses. In addition, by using a rat IL-34 recombinant AAV vector, we demonstrated that rat IL-34 overexpression associated with a suboptimal dose of rapamycin potently induced tolerance to cardiac allograft in rat with a total inhibition of alloantibody production. In this model, IL-34 promoted allograft tolerance through induction of $CD8^+$ and $CD4^+$ Tregs. Moreover, these Tregs were capable of serial tolerance induction through modulation of macrophages that migrate early into the graft. Translationally, we demonstrated that human IL-34 protein administration into NSG mice infused with human PBMCs efficiently delayed the xenogeneic GVH development. In vitro, we showed that human macrophages cultured in the presence of IL-34 more efficiently expanded $CD8^+$ and $CD4^+$ $FOXP3^+$ Tregs compared to allogeneic APCs or anti-CD3/CD28, with a potentiation of suppressive activity since lower ratios of IL-34-expanded Tregs were sufficient to delay GVH development in humanized mice compared to polyclonally expanded Tregs.
Conclusions: We revealed that IL-34 is a suppressive Treg–specific cytokine that could be considered as a biomarker of immune status of patients. Moreover, we demonstrated the clinical relevance of IL-34 in transplantation as a potent tolerance inducer and as a promising candidate for cell therapy through efficient expansion and potentiation of Tregs.

356.4

IL-10 expression of in vitro activated B cells as a biomarker for B cell tolerance

Tetsu Oura, Kiyohiko Hotta, Benedict A Cosimi, Tatsuo Kawai.
Deaprtment of Surgery, Center for Transplantation Sciences, Massachusetts General Hospital, Boston, MA, United States.

Background: A significant role for regulatory B cell has been suggested in kidney transplant recipients who achieved operational tolerance while receiving conventional immunosuppression. We studied T and B cell responses in recipients in whom tolerance induction was attempted via HLA-mismatched combined kidney and bone marrow transplantation (CKBMT).
Materials and Methods: Five CKBMT recipients who achieved long-term immunosuppression-free survival (>5 years) were retrospectively studied. Two recent CKBMT recipients were also prospectively studied. T cell responses were determined by IFN-g ELIspot assay using irradiated PBMCs (direct) or lysates (indirect). B cell responses were evaluated by IL-10 expression of naïve B cells after stimulation with CD40L/IL-2/CpG/F(ab)'.
Results: Donor specific antibodies (DSA) were never detected in four recipients but three developed low (n=2) or high titer (n=1) DSA. Nevertheless, except for the recipient with high DSA titers, all recipients showed donor-specific hyporesponsiveness in direct IFN-g ELIspot (Fig. 1A). No anti-donor responses were detected by indirect IFN-g ELIspot. In contrast, significantly higher IL-10 expression was observed on in vitro activated B-cells obtained from long-term recipients with no DSA than from recipients with DSA (Fig. 1B). Similar observations were made in the two prospectively followed recipients, one of whom achieved tolerance without DSA while the other developed subclinical rejection and low titer DSA, first detected on day 518, during weaning of his immunosuppression. Although both recipients showed donor-specific hyporesponsiveness on direct IFN-g ELIspot assay, IL-10 expression of in vitro activated B-cells was noted to fall in the rejecting recipient. In contrast, IL-10 expression remained high in recipient who achieved tolerance (Fig. 1C).
Conclusion: Although T cell response assay failed to differentiate "Tolerant" from "non-Tolerant" recipients, IL-10 expression of in vitro activated B-cells appear to be a useful biomarker for B cell tolerance. Studies to identify the B cell specific subsets responsible for the higher IL-10 expression are currently underway.

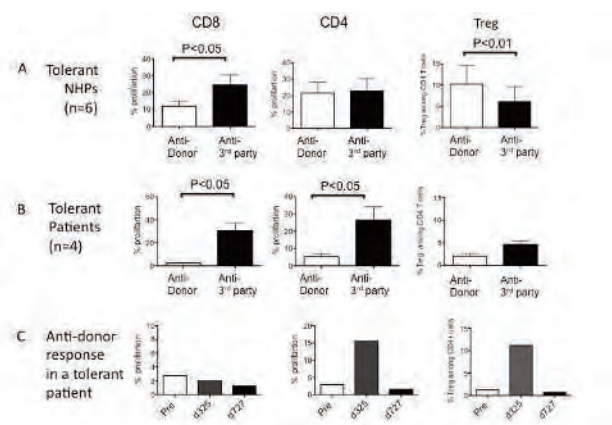


356.5

Immune tolerance monitoring in renal allograft tolerance induced by transient mixed chimerism in nonhuman primates and humans

Kiyohiko Hotta, Tetsu Oura, Kerry A.Crisalli, David H.Sachs, Benedict Cosimi, Tatsuo Kawai.
Center for Transplantation Science, Massachusetts General Hospital, Boston, United States.

Background: Successful induction of allograft tolerance in MHC-mismatched kidney transplantation has been achieved after induction of transient chimerism in both nonhuman primates (NHP) and humans. However, development of a reliable assay to monitor tolerance is critically important to safely taper immunosuppression.
Method: Six NHP and four human recipients of combined kidney and bone marrow transplantation (CKBMT) who achieved long-term renal allograft survival without immunosuppression were studied. Differential T-cell responses were evaluated by CFSE-MLR co-stained with for CD4, CD8 and Foxp3.
Results: NHP tolerant recipients evaluated at 2.1±0.9 years after transplantation showed donor- specific CD8+ T cell hypo-responsiveness but displayed vigorous anti-donor CD4+ T cell proliferation. Interestingly, among these proliferated CD4+ cells, significantly higher Foxp3+ cell proliferation was observed against donor, compareding with the third party stimulators (Fig. A). In contrast, consistently high anti-donor CD8+ and CD4+ T cell responses with limited Treg expansion were observed in rejectors (data not shown). In humans, although assays were performed at significantly markedly later time points (4.7±1.8 years), tolerant patients displayed more significant much greater loss of anti-donor CD8+ and CD4+ T cells responses. However, unlike NHPs, Treg expansion was not significant ands entire CD4+ T cell responses were limited (Fig. B). Finally, we prospectively monitored T cell responses in one recent human CKBMT recipient who achieved renal allograft tolerance. Although anti-donor CD8+ T cell responses gradually disappeared, significant CD4+ responses and Treg expansion were observed at day 325 as observed in most NHP recipients. However, anti-donor Treg responses eventually disappeared by day 727, as observed in other human tolerant recipients (Fig. C).
Conclusion: Regulatory mechanisms were suggested in NHPs at 2 years. Although tested at significantly later time point, mMore robust loss of anti-donor T cell responses werase observed in human tolerant recipients, although these recipients were tested at later time points than the NHP.



356.6

Recently alloactivated CD4+CD8-CD25+Treg express CD8 and are the antigen-specific Treg

Bruce M. Hall[1], Catherine M. Robinson[1], Paul Wilcox[3], Nirupama D.Verma[1], Giang T. Tran[1], Nicole Carter[1], Rochelle A. Boyd[1], Suzanne J. Hodgkinson[1,2].
[1]Immune Tolerance Group, UNSW Australia, Sydney, Australia; [2]Neurology, Liverpool Hospital, Sydney, Australia; [3]Medicine, University of Sydney, Sydney, Australia.

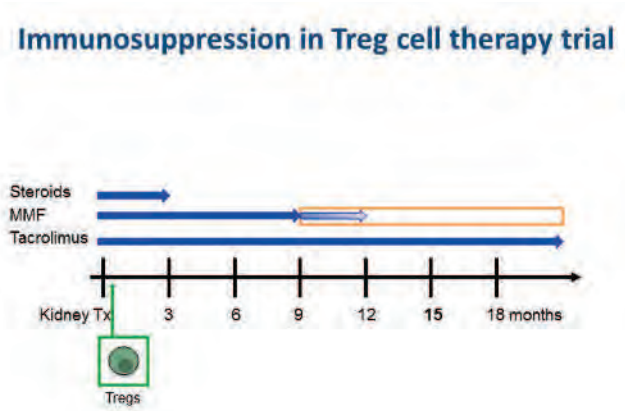
Aim: Transplant tolerance induction requires CD4+CD25+FOXP3+Treg known as thymic Treg (tTreg). Expanding non antigen-specific tTreg for therapy requires impossibly large numbers to induce tolerance. Naïve tTreg with specific TCR for donor can be induced and expanded in vitro, into more potent antigen-specific Treg that could effect tolerance with smaller numbers of cells. After culture of naïve tTreg with alloantigen and IL-2, there is induction of the IFN-gamma receptor (IFNGR) and IL-12 receptor beta2. We called these T suppressor 1 (Ts1) cells. We propose that in the cultures with IL-2 and alloantigen, there are two processes for expansion of tTreg. One, there is polyclonal expansion of tTreg induced by IL-2 that increases the number of tTreg. Second, the small proportion of tTreg with receptor for the specific alloantigens are activated through TCR and expand the antigen-specific activated Treg. In this study we examined if there were phenotypic changes that may identify the antigen-specific Treg.
Methods: Naïve CD4+CD25+Treg from DA rats were cultured with stimulator cells from PVG rats and IL-2 for 3-4 days to induce Ts1 cells. 10-30% of cultured cells expressed CD8, whereas starting population had <1% CD8+ cells. We compared the function of the CD4+CD8+CD25+T cells to the CD4+CD8-CD25-T cells in vitro and in vivo.
Results: Using RT-PCR we examined for differences in the CD8+ and CD8- subpopulation. The increase in IFNGR and IL-12Rb2 that characterizes Ts1 cells was mainly in the CD8+fraction. Further the CD8+ fraction had a marked increase in IRF4, a transcription factor induced by TCR activation. This suggested the antigen specific Treg were induced to express CD8.
The CD8+ fraction had increased suppression in MLC to PVG at 1:1012, and to Lewis at 1:32. The CD4+CD8-FOXP3+T cells had no enhanced suppression to PVG. The unfractionated population suppresses in MLC at 1:32-1:64. In our adoptive transfer assay, IL-2 and alloantigen activated Treg suppress rejection and induce tolerance at 1:10, whereas removal of the CD8+ cells removes their capacity to induce tolerance, suggesting the tolerance is mediated by the CD8+ fraction.
To examine if CD4+CD8+CD25+T cells are induced in vivo, DA rats rejecting PVG grafts or treated to induce tolerance were examined. In these animals 10 days after transplantation, 12-18% of CD4+CD25+T cells expressed CD8, whereas in naïve rats <5% express CD8. These CD25+T cells from both rejecting and tolerance induced rats suppressed MLC to PVG at 1:256-1:512, and to Lewis at 1:16-1:32, showing specific suppression. The enriched CD4+CD8+CD25+T cell suppressed to PVG at 1:1000 in MLC but did not suppress responses to third party Lewis. The CD8- fraction did not suppress.
Conclusion: Expression of CD8 on CD4+CD8-CD25+FOXP3+T cells is a marker of Treg that have been activated by specific antigen. This marker early after tTreg activation may be used to identify and further expand antigen-specific Treg. This de-differentiation to a dual expressing CD4+CD8+T cell may facilitate selection of Treg with TCR that recognize antigen expressed by either class I or class II MHC. Such dual expressing CD4+CD8+ T cells have been observed in man after culture with IL-2.

356.7

The UK ONE Study trial: safety and feasibility of regulatory T (Treg) cell therapy in renal transplantation

Andrew R. Bushell[1], Jeroen van der Net[1,2], David S. Game[3], Rachel Hilton[3], Sarah J.Thirkell[4], Joanna Hester[1], Katie Lowe[4], Laura Fry[4], Peter J. Friend[2], Edward Geissler[5], Kathryn J. Wood[1], Paul N. Harden[2], Giovanna Lombardi[4].
[1]Transplantation Research Immunology Group, Nuffield Department of Surgical Sciences, University of Oxford, Oxford, United Kingdom; [2]Oxford Transplant Centre, Oxford University Hospitals NHS Foundation Trust, Oxford, United Kingdom; [3]Nephrology and Transplantation, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom; [4]Immunoregulation Laboratory, MRC Centre for Transplantation, King's College London, Guy's Hospital, London, United Kingdom; [5]Department of Surgery, University Hospital Regensburg, University of Regensburg, Regensburg, Germany.

Introduction: The UK ONE Study trial investigates the safety and feasibility of regulatory T (Treg) cell therapy in renal transplantation.
Materials and Methods: Eleven (9m;2f) living donor renal transplant recipients (RTR), median age 42 (28-71) years, were enrolled and received Treg cell infusions. 350 ml whole blood or 55 ml of leukapheresis product was obtained 6-10 weeks before transplant. Treg were enriched by CD25 positive selection combined with CD8 negative selection and then expanded by stimulation with CD3/CD28 beads + IL-2 in the presence of Rapamycin at the GMP facility GMP facility at Guy's Hospital, London. Final cell products were cryopreserved at the specified dose and delivered to either the Oxford Transplant Centre in a dry shipper (-190ºC) or to The Clinical Research Facility at Guy's Hospital. 5 days after transplantation; 2 ml cell product was thawed, suspended in 5% HSA, and administered over 30 minutes as an IV infusion. A dose-escalation of three participants at each of 1x10E6, 3x10E6, 6x10E6 and 10x10E6 cells/kg was performed. Immunosuppression comprised Mycophenolate Mofetil, Tacrolimus and Prednisolone. No induction therapy was given (Figure 1).



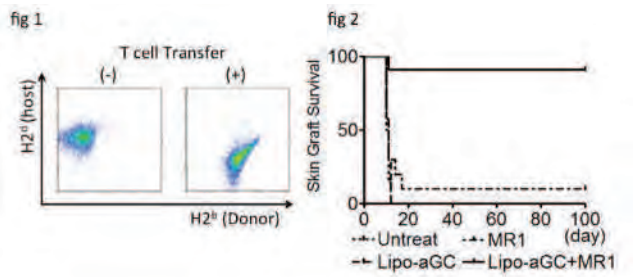
Results and Discussion: No cell-infusion-related adverse events were noted. During a median follow-up of 10 (1-15) months, no biopsy-proven rejection episodes were observed. All patients have stable transplant function (median latest serum creatinine of 128 (90-176) µmol/l).
Conclusion: This study reports the outcome of the first eleven RTR to receive expanded Treg in the UK. The data confirm the feasibility and safety of Treg infusion in RTR to a dose of 10x10E6 cells per kg. These results demonstrate the feasibility of Treg therapy in solid organ transplantation, and establish the need for future trials investigating the efficacy of Treg therapy in renal transplantation.

356.8

Combination of veto cell transfer and iNKT cell therapy establishes complete hematopoietic chimerism in non-myeloablative BMT recipients

Toshihito Hirai[1], Rumi Ishii[1], Yusuyukiu Ishii[2], Masayoshi Okumi[1], Kazunari Tanabe[1].
[1]Department of Urology, Tokyo Women's Medical University, Tokyo, Japan; [2]Laboratory for Vaccine Design, RIKEN IMS-RCAI, Yokohama, Japan.

Hematopoietic chimerism after non-myeloablative bone marrow transplantation (nmBMT) has been found beneficial for establishment of transplant tolerance. Since bone marrow cells (BMCs) are endowed with "veto" activity, reduction in the number of transferred BMCs usually results in engraftment failure in nmBMT. We previously reported that BM engraftment could be promoted by (1) enhancing Treg activity via iNKT cell stimulation with a liposomal α-galactosylceramide (lipo-aGC), and (2) inducing donor-specific peripheral deletion with CD40/CD40L blockade (MR1). Here, we implemented a 3rd strategy: additional transfer of donor-derived T cells as veto cells to facilitate BM engraftment. 3 Gy-irradiated BALB/c ($H2^d$) recipient mice were administered B6 ($H2^b$) BMCs (5×10^6) followed by lipo-aGC plus MR1. On the following day, skin grafts from B6 and C3H ($H2^k$) mice were simultaneously transplanted on the recipient mice. When this clinically practical number of BMCs was used, all mice rejected both B6 and C3H grafts. However, when 5×10^5 splenic T cells obtained from an $H2^{b+}$GFP-Tg donor were additionally administered, complete chimerism was established in the mice (fig 1) and they accepted the skin allograft permanently in an $H2^b$-specific manner. Only $H2^{b+}CD8^+$T cells showed this veto effect, whereas transfer of $H2^{b+}CD4^+$T cells or 3rd party T cells did not facilitate BM engraftment. Concordantly, early expansion of $GFP^+CD44^{hi}CD62L^{lo}$effector $CD8^+$T cells was observed in many lymphoid organs, including the BM and thymus. BM-derived $H2b^+GFP^-$T cells immediately regenerated thereafter and had completely replaced GFP^+T cells until day 60. Therefore, complete chimeric mice did not show GvHD physically and pathologically. Lack of host reactivity was also confirmed in an in vitro proliferation assay. The mice treated with lipo-aGC or MR1 alone rejected BM and skin grafts despite T cell transfer (fig 2), suggesting that Treg cells, peripheral deletion, and veto T cells act in concert for establishing tolerance in nmBMT recipients.



356.9

Natural Killer cells demonstrate functional and receptor diversity in response to alloantigen

Olivia Hatton[2,3], Todd Shawler[2], Catherine Blish[1,3], Olivia M. Martinez[2,3], Sheri M. Krams[2,3].
[1]Department of Medicine, Stanford University School of Medicine, Stanford, CA, United States; [2]Department of Surgery, Division of Abdominal Transplantation, Stanford University School of Medicine, Stanford, CA, United States; [3]Stanford Immunology, Stanford University School of Medicine, Stanford, CA, United States.

Natural killer (NK) cells play an important role in the first-line defense against tumor and virus-infected cells. Although "missing-self" suggests NK cells would target foreign organ allografts, the prevailing dogma has been that NK cells are not important in the rejection of solid organ allografts. However, studies now suggest NK cells participate in both acute and chronic rejection while paradoxically also facilitating tolerance to an allograft. In clinical studies, however, a clear relationship between predicted NK cell alloreactivity and graft survival was not observed suggesting NK cell function in response to alloantigen is complex. NK cell activity is tightly regulated by a repertoire of inhibitory and activating receptors. The goal of this study were: 1) to determine if NK cells recognize and respond to allogeneic targets, and 2) establish the diversity of NK receptors utilized in the alloimmune response.

To assess the ability of NK cells to recognize and respond to allogeneic targets, we generated B lymphoblastoid cell lines (LCL), from a cohort (n=10) of healthy donors. Primary human NK cells ($CD3^-CD14^-CD19^-CD56^+$) from the same cohort, were isolated by negative selection, and co-cultured with autologous (control) or allogeneic LCLs. NK cells were then assayed by flow cytometry for the degranulation protein CD107a, the NK cell maturation marker CD57, the NK cell receptors NKG2A, NKG2C, NKG2D, 2B4, and CD16, and the cytokine IFNγ.

NK cells from nine of 10 donors specifically recognized and responded to the panel of allogeneic LCLs and this was not specifically correlated to HLA and/or KIR ligand mismatches. NK cells from seven donors showed both cytolytic and IFNγ responses whereas the other 2 donors had either a cytolytic or IFNγ response. Visualization of the frequencies of 64 different NK receptor combinations on NK cells co-cultured with allogeneic LCL from each of the 10 donors indicate that 2B4 and NKG2D were highly expressed (>90%) on NK cells that produced IFNγ, CD107a or were polyfunctional ($IFN\text{-}\gamma^+$, $CD107a^+$) whereas expression of the activating receptor, NKG2C was generally absent. Variable expression of CD16, CD57, and the inhibitory receptor, NKG2A was observed on alloresponsive NK cells.

Our study, using a multi-parameter approach to examine NK cells suggests diversity in the NK receptors responding to alloantigen. These findings may provide insight into the varied role of NK cells in alloimmunity and will lead to novel strategies to prevent graft rejection and promote tolerance to an allograft.

357.1

Cell-free mitochondrial DNA activates DCs in an age-dependent fashion and compromises the survival of older grafts

Midas Seyda[1,2], Hong M.Li[1], Guangxiang Liu[1], Markus Quante[1], Hirofumi Uehara[1], Jeroen M.Schuitenmaker[1], Abdala Elkhal[1], Stefan G.Tullius[1].
[1]Division of Transplant Surgery and Transplant Surgery Research Laboratory, Brigham and Women's Hospital/Harvard Medical School, Boston, MA, United States; [2]Institute of Transplant Immunology, IFB-Tx, Hannover Medical School, Hannover, Germany.

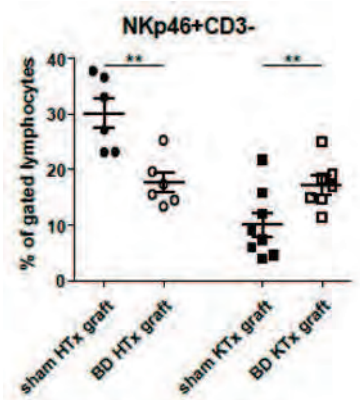
The world population is aging at a dramatic pace and the need for organ transplantation has, at least in part, been compensated by the utilization of older donor organs. We submit that age-dependent immunogenicity in addition to injury and repair will impact alloimmunity and transplant outcomes. Following ischemia and reperfusion injury (IRI), cell-free DNA is shed into the circulation potentially impacting pro-inflammatory responses via toll-like receptors (TLRs) of primarily dendritic cells (DCs) and macrophages. While cell-free DNA has very recently been implicated as an emerging biomarker in transplantation, we tested the impact of cell-free mitochondrial DNA (mtDNA) in DC activation and graft rejection in an age-dependent fashion.
Circulating mtDNA levels were tested by quantitative PCR in old and young mice. In addition, DC activation was characterized by flow cytometry. IRI was performed by bilateral clamping of the renal pedicles for 23 minutes. Sorted old or young CD11b+ CD11c+ DCs were adoptively transferred into young DBA/2J cardiac allograft recipients to determine in-vivo consequences and subsequently received young or old cardiac allografts.
DC activation was associated with highly elevated levels of circulating cell-free mtDNA in naïve old mice. In sharp contrast, young animals had no detectable mtDNA levels. Strikingly, following renal IRI the circulating mtDNA proportion of total cell-free DNA was similarly elevated in old mice. In vitro, DCs demonstrated an upregulation of CD40 and CD80, two costimulatory surface molecules involved in T cell activation when co-cultured with cell-free plasma DNA of old animals. Moreover, DCs cultured in presence of isolated mtDNA were characterized by an upregulation of CD40 that was dose dependent. Notably, the addition of a TLR9 antagonist abrogated the upregulation of co-stimulatory molecules on DCs. While we showed that naïve old DCs expressed augmented levels of costimulatory molecules, they furthermore promoted pro-inflammatory IFN-γ and IL-17 responses of allogeneic T cells in vitro. Finally, we demonstrated the relevance of our findings in a cardiac transplant model by showing that the adoptive transfer of old DCs but not young DCs prior to heart transplantation reduced graft survival significantly. This effect was associated with an increased capacity of old DCs to stimulate proliferative responses of alloreactive T cells in vitro. Collectively these results suggest that aging increases cell-free mtDNA, thereby favoring DCs activation that in turn can orchestrate pro-inflammatory T cell responses and consequently reduces the survival of older grafts.

357.2

Donor brain death influences early NK cell migration in murine heart and kidney transplantation

Paul Ritschl[1], Muhammad Ashraf[1], Rupert Oberhuber[2], Vanessa Mellitzer[2], Cornelia Fabritius[2], Thomas Resch[2], Susanne Ebner[2], Martina Sauter[3], Karin Klingel[3], Johann Pratschke[1], Katja Kotsch[1].
[1]Department of General, Visceral and Transplantation Surgery, Charité – Universitaetsmedizin Berlin, Berlin, Germany; [2]Center for Operative Medicine, Department of Visceral, Transplant and Thoracic Surgery, Medical University of Innsbruck, Austria, Innsbruck, Austria; [3]Department of Molecular Pathology, University Hospital Tübingen, Tübingen, Germany.

The majority of organs used for solid organ transplantation (SOT) is retrieved from brain dead (BD) donors. Donor BD has been defined as irreversible loss of brain and brain stem function resulting in a number of pathophysiological changes suggesting an impaired graft outcome in the long-term. However, detailed information regarding the influence of BD in combination with ischemia reperfusion injury (IRI) on various immune cell subsets within solid organs and their comparative immunological profile is still missing. We therefore characterized the BD-related impact on distinct inflammatory expression patterns and passenger leukocyte composition in solid organs applying a murine model of BD. Following 4 hours of BD investigated organs were characterized by a defined inflammatory profile. For instance, after onset of BD lipocalin 2 (LCN2), a marker described for acute kidney injury was exclusively induced in the liver and not in the kidney. Moreover, BD livers were characterized by a significant decrease of CD3+CD4+ and CD3+CD8+ T cells ($p<0.01$) as well as NK cells compared with sham controls ($p<0.05$). BD derived hearts showed the highest MCP-1 and P-selectin expression compared with BD kidneys and livers, whereas both, BD hearts and kidneys showed significant lower numbers of CD11c+MHC class II+ dendritic cells ($p<0.05$, $p<0.01$). This yet unknown organ specific effect of BD on lymphocytes was further investigated in a murine kidney (KTx) and heart (HTx) transplantation model. Interestingly, 20 hours post reperfusion transplanted BD hearts were characterized by significant lower NK cell frequencies compared with sham derived hearts whereas BD kidneys illustrated significant higher levels of NK cells ($p<0.01$, respectively, Figure 1). Contrarily, T cells or dendritic cells were not affected and no histological differences between BD or sham derived organs in both models were noted. We therefore hypothesize that NK cells present a possible target of future therapy approaches in order to alleviate BD-related organ damage in SOT.
In conclusion, our data illustrate the varying impact of donor BD on solid organs underlining the necessity of organ-specific treatment schemes to improve donor organ quality to close the existing gap between organ demand and available organs.

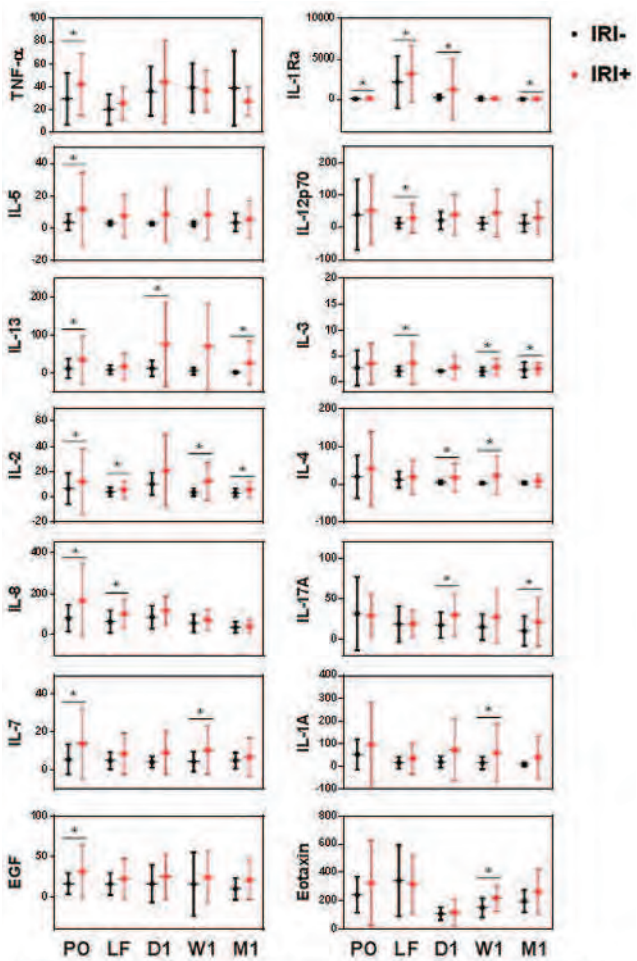


357.3

Evolution of innate and adaptive cytokine responses in ischemia reperfusion injury in orthotopic liver transplantation

Ali Zarrinpar[1], Rebecca Sosa[2], Ping Rao[2], Charles Lassman[2], Ronald Busuttil[1], Yuan Zhai[1], Ying Zhang[2], David Gjertson[2], Jerzy Kupiec-Weglinski[1], Elaine Reed[2].
[1]Surgery, UCLA, Los Angeles, CA, United States; [2]Pathology and Lab Medicine, UCLA, Los Angeles, CA, United States.

Background: Ischemia reperfusion injury (IRI) in orthotopic liver transplantation (OLT) can lead to primary graft non-function, re-transplantation or recipient death. Data from experimental models indicate that OLT-IRI is mediated by complex interactions between the innate and adaptive arms of the immune system. This study aims to determine the evolution of cytokine response over time following OLT-IRI.



Measured Analytes: VEGF, sCD40L, EGF, Eotaxin, FGF-2, Flt-3 ligand, Fractalkine, G-CSF, GM-CSF, GRO, IFN-α2, IFN-γ, IL-1α, IL-1ß, IL-1RA, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17A, IP-10, MCP-1, MCP-3, MDC, MIP-1α, MIP-1ß, TGF-α, TNF-α, TNF-ß;
PO=pre-operative, LF=liver flush, D1=day 1, W1=week 1, M1=month 1;
*indicates p≤0.05 by Wilcoxon Nonparametric Rank-Sum Test

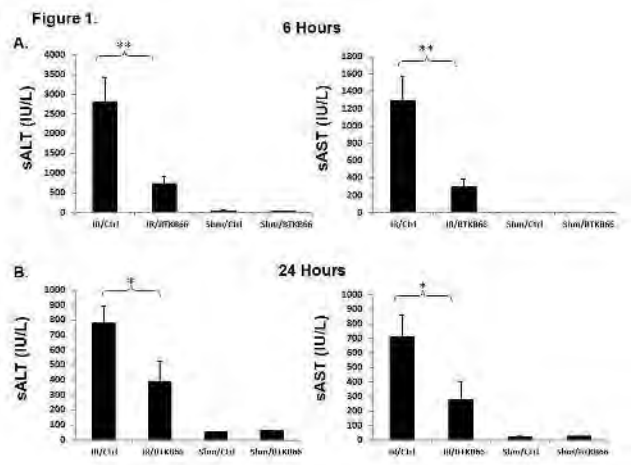
Methods: Blood samples from 53 adult OLT recipients were collected pre-operative, intraoperative (blood liver flush, LF) and post-transplant on day 1, weekly for 1 month, and bi-monthly for 3 months. Graft biopsies were collected pre-transplant and 2 hours after reperfusion. LF and plasma were analyzed using Luminex 38-plex cytokine/chemokine arrays (Millipore). H&E stained slides of liver biopsies were graded for IRI (0=none, 1=minimal, 2=mild, 3=moderate, 4=severe) based on the following parameters: necrosis, inflammatory infiltrates, large droplet macrovesicular steatosis, congestion, and ballooning. IRI scores and cytokine levels were analysis to detect significant associations.
Results: 47% of transplant recipients (25/53) were IRI+ (score ≥2). Pre-operative blood from IRI+ patients showed increased expression of the pro-inflammatory cytokines TNFa, IL-2, IL-8, IL-7, EGF. The intraoperative liver flush from IRI+ patients showed increases in innate immune cytokines IL-2, IL-8, IL-12p70 and IL-3. Early post transplantation (day1) adaptive cytokines/chemokines IL-13 and IL17a were significantly higher in IRI+ patients compared to IRI-. Late after IRI (week 1 and week 4 post-transplantation), adaptive cytokines/chemokines dominate the immune response in IRI+ recipients showing significant increases in IL-17A, IL-2, IL-4, IL-7, IL-3 and eotaxin. Persistent IL-1Ra was found in IRI+ recipients across most time-points tested. Notably, IRI+ patients exhibited increased IL-1a expression at week 1 post transplant that was accompanied by a significant decrease in IL1-Ra implying a resurgence of an acute inflammatory response.
Conclusion: Activation of innate immunity during human OLT-IRI triggers adaptive immunity and correlates with the degree of IRI injury.

357.4

Bruton's tyrosine kinase inhibition attenuates mouse liver damage due to ischemia and reperfusion

Tiziana Palumbo[1], Kojiro Nakamura[1], Charles Lassman[2], Ronald Busuttil[1], Jerzy Kupiec-Weglinski[1], Ali Zarrinpar[1].
[1]Surgery, UCLA, Los Angeles, CA, United States; [2]Pathology and Lab Medicine, UCLA, Los Angeles, CA, United States.

Introduction: Bruton's tyrosine kinase (Btk) is a central player in multiple signaling pathways in lymphoid and myeloid cells, including neutrophils. Neutrophils are crucial effectors in the pathophysiology of organ ischemia/reperfusion injury (IRI). BTKB66 is a highly selective, irreversible inhibitor of Btk. In this study, we tested this small molecule in in vitro and in vivo assays of neutrophil activation and hypothesized that it would reduce warm hepatic ischemia-reperfusion injury (IRI).
Materials and Methods: BTKB66 was tested in an in vitro model of LPS-mediated neutrophil activation. To further assess its efficacy in vivo, BTKB66 was administered for seven days to mice in their drinking water prior to subjecting them to 90 minutes of warm hepatic ischemia followed by 6 or 24 hours of reperfusion. Clinical and pathologic features of these mice were examined following IRI, including AST, ALT, and a panel of cytokines and chemokines.
Results: BTKB66 potently inhibited LPS-mediated activation of bone-marrow derived neutrophils at 1 μM. It also reduced the amount of IRI as determined by AST and ALT levels, as well as in immunohistochemical analyses, at a dose of ~60 mg/kg/day. There were significant decreases in markers of inflammation in the liver, such as CXCL1, CXCL2, and CXCL10. There were also significant decreases in serum markers of the activation of myeloid cells, such as CCL5, CCL11, and CXCL5.



Conclusions: BTKB66 is effective in inhibiting warm hepatic IRI. These finding confirm that neutrophil recruitment and activation plays a large role in injury during IRI and that targeting Btk activity could provide a useful approach for preventing liver IRI and improving overall outcomes in liver transplantation.

357.5

Pre-treatment of heart allografts with the cytotopic anti-thrombin inhibitor Thrombalexin prolongs transplant survival and augments the therapeutic efficacy of adoptively transferred regulatory T cells

Qi Peng, Anna Nowocin, Lesley Smyth, Richard A.Smith, Anthony Dorling, Robert Lechler, Giovanna Lombardi.
MRC Centre for Transplantation, King's College London, London, United Kingdom.

Cardiac transplantation is the treatment of choice for end-stage heart failure. Its efficacy is limited by the occurrence of ischemia-reperfusion (IR) injury as well as the development of cardiac allograft vasculopathy. IR of transplanted organs triggers the activation of innate components of the immune system such as complement and coagulation pathways, which are key contributors to early graft injury, local inflammation and graft dysfunction. Limiting the effects of IR injury and preserving heart function by inhibiting the aforementioned is therefore of primary importance.
In this study, we developed a novel therapeutic strategy of a cytotopic anti-thrombin inhibitor (Thrombalexin) targeting thrombin, a major molecule involved in the coagulation pathway and also interacts with the complement system. Thrombalexin contains a Hirulog-like sequence and a membrane-interacting component with specific inhibition of thrombin activity. We then tested the efficacy of this drug using a murine heart allograft transplant model with the perfusion of the heart allografts with Thrombalexin prior to transplantation. Furthermore, the impact of Thrombalexin on the therapeutic efficacy of adoptive regulatory T (Treg) cell therapy was also assessed. Perfusion of the BALB/c heart grafts with Thrombalexin for 15min before transplantation significantly reduces graft injury and prolongs graft survival in C57BL/6 recipient mice (n=8, MST=15 days) as compared to untreated hearts (n=8, MST=11.5 days). This protection was associated with a significantly reduced macrophage and T cell infiltration. To shed some light on the effects observed in vivo, murine heart endothelial cells (EC) were incubated in vitro with Thrombalexin. The pre-treatment of EC with Thrombalexin resulted in down-regulation of TNFα-induced expression of adhesion molecules such as ICAM-1. Finally, this pre-treatment with Thrombalexin significantly augmented the effect of adoptively transferred Treg cells in allograft survival.
In conclusion, coagulation mediated allograft heart IR injury and graft rejection may be prevented/delayed by pre-treatment of the grafts with the anti-thrombin drug Thrombalexin prior to transplantation. The results from our study suggest that localized anti-thrombin inhibition is effective in reducing early local inflammation and provides a window of opportunity to combine Thrombalexin-treatment with adoptive Treg cell therapy to induce transplantation tolerance.
The British Heart Foundation

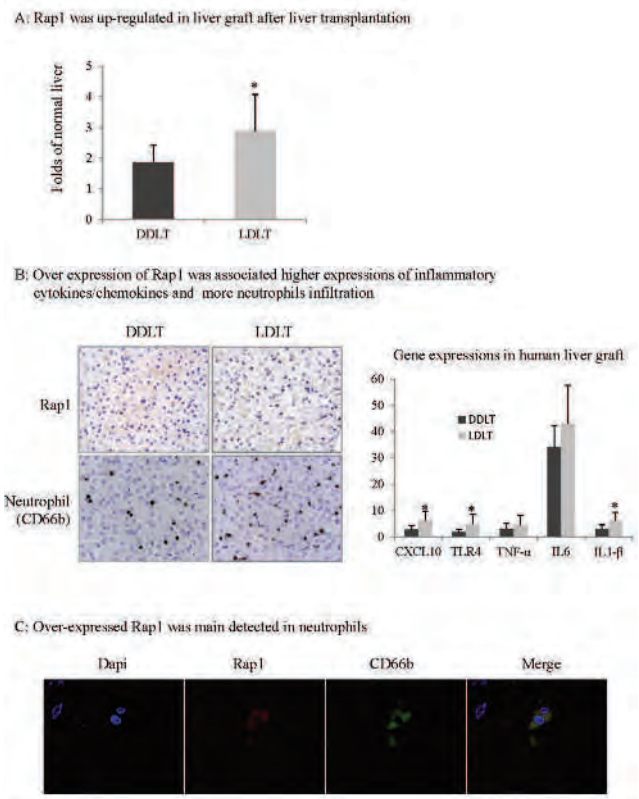
357.6

Repressor and activator protein accelerates hepatic ischemia reperfusion injury by promoting neutrophil inflammatory response

Chang Xian LI[1], Chung Mau Lo[1], Kevin Tak Pan Ng[1], Xiao Bing Liu[1], Xiang Qi[1], Yuen Yuen Ma[1], Qi Zhou Lian[2], Kwan Man[1].
[1]Surgery, The University of Hong Kong, Hong Kong, People's Republic of China; [2]Medicine, The University of Hong Kong, Hong Kong, People's Republic of China.

Background: Increasing evidence showed that hepatic ischemia reperfusion injury (IRI) is a typical inflammatory response involving a complex web of interactions between various cellular and molecular signals. Repressor and activator protein (Rap1) directly regulates nuclear factor-κB (NF-κB) dependent signaling, which is the key player for inflammatory response. Therefore, Rap1 may potentially play an important role in hepatic IRI. We here intended to investigate the effect of Rap1 in hepatic IRI and to explore the underlying mechanisms.

Figure 1

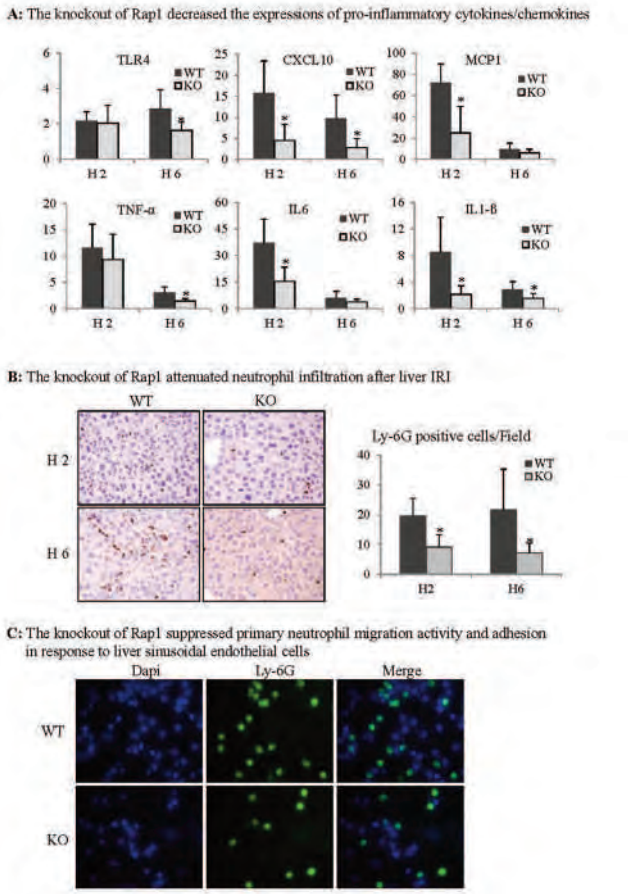


Methods: The association of Rap1 expression with hepatic inflammatory response were investigated both in human and rat liver transplantation. The direct role of Rap1 in regulating hepatic IRI was studied in Rap1 knockout mice IRI model in vivo and primary cells in vitro.

Results: In clinical samples, Rap1 was over-expressed in liver graft and mainly detected in neutrophils after transplantation (Fiugre 1A and C). Over expression of Rap1 was associated with severe histological damage, hepatic apoptosis, higher expressions of inflammatory cytokines/chemokines, more infiltrations of macrophages and neutrophils (Figure 1B). The results were also validated in rat liver transplantation. In mouse hepatic IRI model, the knockout of Rap1 reduced hepatic histological damage, apoptosis and decreased the levels of AST and ALT after liver IRI. Furthermore, the knockout of Rap1 also attenuated hepatic inflammatory response by decreasing the expressions of pro-inflammatory cytokines/chemokines and neutrophil chemoattractants, neutrophil infiltration, and suppressing the activations of inflammation-associated NF-κB and MAPK pathway (Figure 2A and B). In vitro functional study, the knockout of Rap1 suppressed primary neutrophil migration activity and adhesion in response to liver sinusoidal endothelial cells through down-regulating neutrophil F-Actin expression and CXCL2/CXCR2 pathway (Figure 2C). In addition, the knockout of Rap1 also decreased production of pro-inflammatory cytokines/chemokines in primary neutrophils and neutrophil-induced hepatocyte damage.

Conclusions: Rap1 may induce hepatic IRI through promoting neutrophil inflammatory response. Rap1 may be the potential therapeutic target of attenuating hepatic IRI.

Figure 2



357.7

Expression of human CD55 and CD59 reduces complement deposition and preserves renal function in a mouse model of renal ischemia reperfusion injury

David Bo Lu[1], Anjan Bongoni[1], Veena Roberts[1,2], Doreen Fang[1,2], Siddharth V.Rajakumar[1,2], Karen M.Dwyer[1,2], Peter J.Cowan[1,2].
[1]Immunology Research Centre, Vincent's Hospital, Melbourne, Fitzroy, Australia; [2]Department of Medicine, The University of Melbourne, Fitzroy, Australia.

Background: Complement activation plays a key role in antibody-mediated rejection and ischemia reperfusion injury (IRI). We have shown that kidneys from transgenic pigs expressing the human (h) complement regulators CD55 and CD59 are protected from hyperacute rejection in a preclinical baboon model. However, it is not known whether these transgenes have additional benefit by reducing renal IRI. We have addressed this question in a mouse model and shown that transgenic hCD55±hCD59 expression or treatment of wild type (WT) mice with recombinant human CD55 (rhCD55) protects against renal IRI (unpublished). To investigate the mechanism of protection, we examined kidney sections from these mice for deposition of complement.
Aim: To determine whether hCD55-mediated protection from renal IRI correlates with a reduction in complement deposition.
Methods: Transgenic mice expressing hCD55±hCD59 from the broadly active H2Kb promoter were used, with littermate controls. The right kidney was removed from 10-12 week old male mice and the left renal pedicle was clamped for 18 min (mild IRI) or 22 min (moderate IRI). Mice were sacrificed 24 hrs after reperfusion, and samples were collected to assess (i) renal function (serum creatinine), (ii) histological injury score (HIS) on a scale of 0 (no injury) to 4 (severe injury), and (iii) C3b/c and C5 deposition by immunofluorescence staining/confocal microscopy, with quantification as Raw Integrated Density (RawIntDen) using Image J. In a separate study using the moderate IRI model, WT C57BL/6 mice were injected i.v. with 2µg rhCD55 or vehicle immediately after reperfusion.
Results: hCD55 mice were protected from mild IRI (creatinine, $p<0.05$ vs. littermates) but not moderate IRI ($p=0.36$) (see Table). hCD55+hCD59 mice were protected in both models ($p<0.05$). rhCD55 treatment protected WT mice from moderate IRI ($p<0.05$ vs. vehicle). The level of complement deposition correlated well to the degree of injury. In the mild IRI model, C3b/c was lower in hCD55 and hCD55+hCD59 kidneys (both $p<0.05$ vs. littermates), and C5 deposition was lower in the latter ($p<0.05$). In the moderate IRI model, C3b/c and C5 were lower in hCD55+hCD59 kidneys ($p<0.05$, $p<0.01$), and in kidneys from rhCD55-treated mice ($p<0.01$, $p<0.05$ vs. vehicle).
Conclusions: Protection of hCD55 transgenic mice against mild renal IRI is associated with a reduction in C3b/c deposition, consistent with CD55's mid-level regulation of the complement pathway. The additional expression of hCD59, which regulates the terminal stage of the pathway, reduced both C3b/c and C5 deposition and provided further protection. This suggests that activation of the terminal pathway can still occur in IRI even in the context of elevated CD55 expression, possibly by C3-independent mechanisms such as thrombin-mediated cleavage of C5. Our studies further indicate that systemic treatment with soluble rhCD55 is more protective than transgenic expression of the membrane-bound native form.

Mice	Ischemia	n	Mice	HIS	C3b/c (RawIntDen)	C5 (RawIntDen)
hCD55	18 min	11	38.0±4.2	1.60±0.24	2.0 ±0.3 x 10^6	1.8±0.5 x 10^6
hCD55+hCD59		7	34.9±3.8	0.75±0.25	1.4±0.6 x 10^6	1.3±0.4 x 10^6
Littermates		14	59.3±7.7	3.00±0.68	7.8±1.1 x 10^6	7.4±1.2 x 10^6
hCD55	22 min	10	92.5±12.1	Not done [ND]	9.2±2.0 x 10^6	9.3±3.2 x10^6
hCD55+hCD59		11	67.7±6.4	ND	4.4±0.05 x 10^6	5.1±0.9 x 10^6
Littermates		11	96.6±11.5	ND	12.9±0.9 x 10^6	14.5±3.2 x 10^6
Sham		8	20.0±0.4	0		
WT + rhCD55	22 min	6	89.2±21.5	3.67±0.33	5.8±0.9 x 10^6	6.6±1.5 x 10^6
WT + vehicle		6	168.7±10.8	3.00±0.06	12.7±1.1 x 10^6	14.9±3.2 x 10^6

357.8

AMP-activated protein kinase attenuated marginal liver graft injury via promoting mitochondrial biogenesis and respiratory function

Jiang Liu, Kwan Man, Hui Liu, XIANG QI, Xiaobing Liu, Chang Xian LI, Yuen Yuen Ma, See Ching Chan, Chung-Mau Lo.
Surgery, The University of Hong Kong, Hong Kong, Hong Kong.

Introduction and Objective: AMP-activated protein kinase (AMPK) is an energy sensor to AMP/ATP ratio in cells, and its activation can transit cellular anabolism to catabolism to generate more ATP[1]. AMPK was reported to regulate mitochondrial biogenesis of metabolism related enzymes after exercise and training to conquer cell aging. In this study, we aimed to investigate the role of AMPK pathway in marginal graft injury after liver transplantation, and to further explore the underlying mechanism.
Materials and Methods: The expression levels of AMPK, PGC-1α and mitochondrial election transition chain (ETC) components together with liver function were explored in clinical samples from recipients after liver transplantation. Further, a rat orthotopic liver transplantation model with small-for-size fatty liver graft was established to investigate mitochondrial function and correlation with AMPK and PGC-1α expression. The functional role of AMPK in regulating graft injury, mitochondrial biogenesis and respiratory status was studied in AMPKα1 knock-in (AMPKα1+/-) mice underwent partial hepatic I/R injury and semi-hepatectomy.

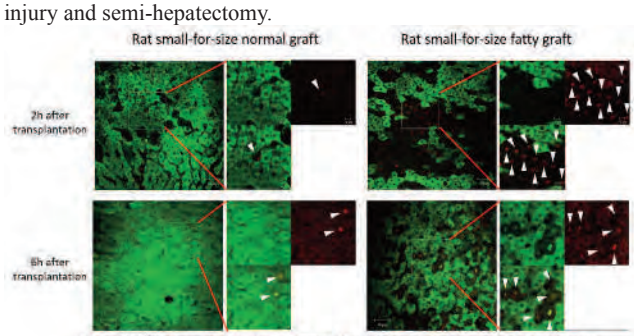


Figure 1. Mitochondrial membranaceous depolarization and hepatocyte necrosis are more severe in fatty graft compared to normal graft after small-for-size liver transplantation in rats.

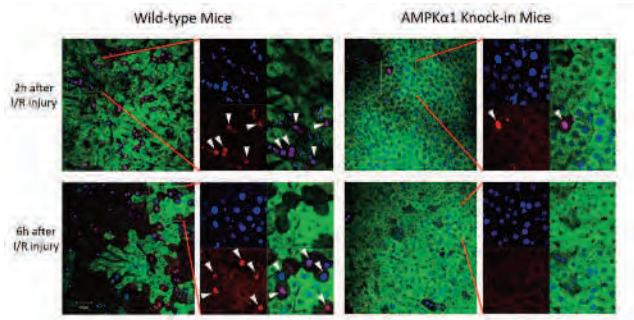


Figure 2. Mitochondrial membranaceous depolarization and hepatocyte necrosis are ameliorated in AMPKα1 knock-in mice compared to wild-type mice during I/R injury.

Results and Discussion: Down-regulation of AMPK and PGC-1α was found in clinical recipients with fatty liver grafts compared to normal grafts, and was correlated with poor acute phase liver function (ALT: 476 vs 346IU/L, AST: 362 vs 275IU/L, TBIL: 193 vs 163mg/dL, LDH: 852 vs 626U/L, $p<0.01$) after liver transplantation. Also, expression of ETC components was suppressed in recipients with fatty liver grafts compared to normal grafts. Consistently, in rats liver transplantation models using small-for-size fatty grafts, lower expression levels of AMPK and PGC-1α were also correlated with more severe mitochondrial membranaceous depolarization, larger proportion of

nonviable cells indicated by intravital imaging analyses (Figure 1), and suppressed function of ETC evidenced by lower ATP level and decreased succinate dehydrogenase (SDH, complex II of ETC) activity than that in normal grafts. In AMPK knock-in mice, there were less nonviable cells, attenuated depolarization of mitochondria (Figure 2), restored expression status of ETC components than in wild type mice. Furthermore, PGC-1α signaling was also maintained in AMPK knock-in mice than in wild type mice.

Conclusion: The AMP-activated protein kinase attenuated fatty graft injury by promoting mitochondrial biogenesis and respiratory function via PGC-1α pathway activation during liver transplantation.

References:

[1] D. Grahame Hardie Genes & Development 2011

357.9

Changes in activin levels and the impact of follistatin treatment in renal ischemia-reperfusion injury in mice

Doreen Fang[1,2], Bo Lu[1], Susan Hayward[4], David de Kretser[3,4], Peter Cowan[1,2], Karen Dwyer[2].

[1]Immunology Research Centre, St. Vincent's Hospital, Melbourne, Australia; [2]Department of Medicine, The University of Melbourne, Melbourne, Australia; [3]Faculty of Medicine, Monash University, Melbourne, Australia; [4]Centre for Reproductive Health, Hudson Institute of Medical Research, Melbourne, Australia.

Introduction: Activins, members of the TGF-β superfamily, are key drivers of inflammation and hence are thought to play a significant role in ischemia-reperfusion injury (IRI), an inherent process in renal transplantation that negatively impacts early and late allograft function. Activin A is a homodimer of the β_A-subunits of the gonadal hormone, inhibin A. Activin B is a homodimer of the β_B-subunits of inhibin B, which has 65% sequence homology with the β_A-subunit. Follistatin (FS) is an activin-binding protein that inhibits its activity. This study examined the changes in activin A and B levels, and the effect of exogenous FS using a mouse model of renal IRI.

Materials and Methods: Mice were treated with intravenous recombinant FS (0.4 μg/g) or vehicle before renal IRI surgery (right nephrectomy and left renal pedicle was clamped for 20 min). Sham mice underwent right nephrectomy only. Serum and kidney activin A, activin B and FS levels, and renal injury parameters, were measured at 3, 6 and 24 h after reperfusion.

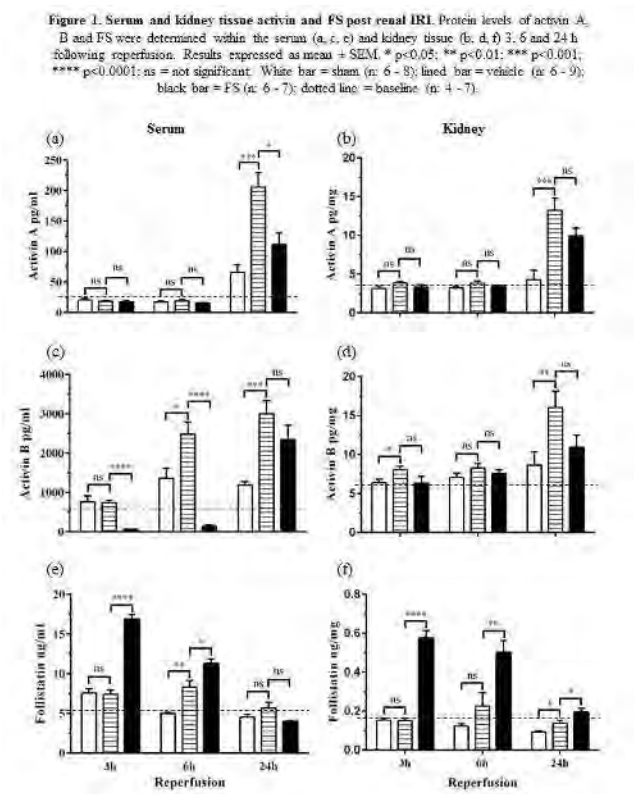


Figure 1. Serum and kidney tissue activin and FS post renal IRI. Protein levels of activin A, B and FS were determined within the serum (a, c, e) and kidney tissue (b, d, f) 3, 6 and 24 h following reperfusion. Results expressed as mean + SEM. * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001; ns = not significant. White bar = sham (n: 6 - 8); lined bar = vehicle (n: 6 - 9); black bar = FS (n: 6 - 7); dotted line = baseline (n: 4 - 7).

Results and Discussion: Serum and kidney activin B levels were increased within 6 h post renal IRI preceding that of activin A (Figure 1a-d). The increase in activin B was accompanied by renal injury illustrated as a 3-fold increase in serum creatinine ($p<0.0001$), and increased mRNA expression of kidney injury molecule-1 (KIM-1) ($p<0.01$) and neutrophil gelatinase-associated lipocalin (NGAL) ($p<0.001$). E-selectin mRNA, a marker of endothelial activation, was

also increased ($p<0.01$). FS treatment blocked the rise in serum activin B (Figure 1c) and reduced the mRNA expression of KIM-1 ($p<0.05$), NGAL ($p<0.05$) and E-selectin ($p<0.01$) but had no effect on serum creatinine. Serum and kidney activin A increased later between 6 and 24 h post renal IRI (Figure 1a-b), with a further increase in creatinine to 5-fold ($p<0.0001$) and in KIM-1 ($p<0.01$) and NGAL ($p<0.05$) mRNA. FS treatment blunted the increase in serum activin A (Figure 1a) and late injury to the kidney, attenuating the increase in creatinine ($p<0.01$), KIM-1 and NGAL mRNA ($p<0.05$), renal tubular injury score ($p<0.05$), and renal cell apoptosis ($p<0.001$). Serum levels of the pro-inflammatory proteins IL-6 and MCP-1 were also decreased by FS treatment. Interestingly, FS treatment had no impact on activin levels in the kidney despite the demonstrable protective renal effect (Figure 1b and 1d).

Conclusion: We propose that activin B initiates and activin A potentiates renal IRI. The renal protection conferred by treatment with FS may be due to dampening of the systemic inflammatory response consequent to reduced serum activin B and A. This suggests a potential clinical application of FS treatment in renal transplantation to improve graft function and survival.

Jacquot Research Entry Scholarship; National Health and Medical Research Council of Australia (NHMRC); Cass Foundation; Oxford Brookes University; Ms Sylwia Glowacka, Department of Medicine, The University of Melbourne; Staff at the Immunology Research Centre, St. Vincent's Hospital Melbourne; Staff at the Bioresources Centre, St. Vincent's Hospital Melbourne

358.1

Time-series variations in calculated panel reactive antibody among kidney transplant candidates

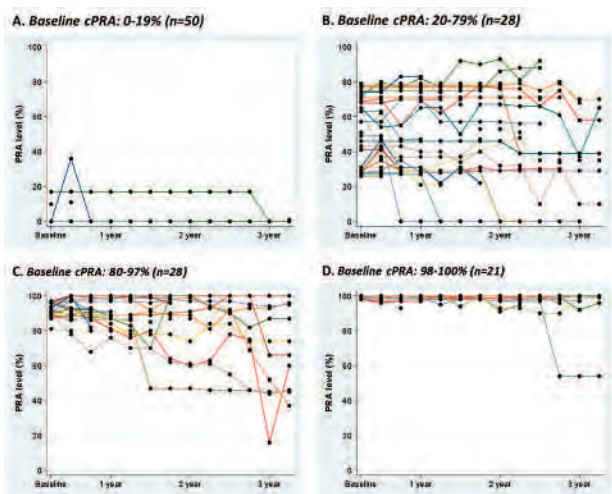
Yoshitsugu Obi[1,2], Akihiro Shimomura[3], Reza FAlizadeh[3], Shiri Li[3], Uttam G Reddy[1,2], Kamyar Kalantar-Zadeh[1,2], Clarence E. Foster[3], Hirohito Ichii[3].
[1]Harold Simmons Center for Kidney Disease Research and Epidemiology, University of California Irvine, Orange, CA, United States; [2]Division of Nephrology & Hypertension, University of California Irvine, Orange, CA, United States; [3]Division of Transplantation, University of California Irvine, Orange, CA, United States.

Background: The Calculated Panel Reactive Antibody (cPRA) calculator has been used to evaluate kidney transplant candidates. However, the time-series variations in cPRA levels and the optimal cPRA evaluation interval remain unclear, potentially resulting in unnecessary tests and increased medical cost.

Method: We retrospectively examined the trajectory of cPRA among 118 end-stage renal disease (ESRD) patients wait-listed for kidney transplantation at our department between January 2011 and December 2014. We measured cPRA levels every 3 months. CPRA levels were then categorized into 0–19%, 20–79%, 80–97%, and 98–100%. Increase or decrease in cPRA levels >10% was used as the primary outcome.

Results: Among 118 wait-listed patients, 50, 28, 19, and 21 patients were categorized as 0–19%, 20–79%, 80–97%, and 98–100%, respectively. One patient with 0% baseline cPRA experienced an increase cPRA levels to 36% at Month 6 but then spontaneously returned to 0% at Month 9. Another patient decreased cPRA from 17% at baseline to 0% at Month 36. Among the other 48 patients in the 0-19% cPRA category, cPRA changes were within 10%. Similarly, cPRA did not change more than 10% among patients in the 98-100% category with an exception of one patient who decreased cPRA from 98% at baseline to 54% at Month 33. Variations in cPRA >10% were observed among 16 (57%) and 9 (47%) patients in the 20–79% and 80-97% category, respectively. Decrease in cPRA was more frequent than increase in cPRA in these middle categories. In particular about half of those with cRPA>80% showed a decline over time without any intervention.

Conclusion: Many wait-listed kidney transplant candidates show a large fluctuation in cPRA over time, while high cRPA tend to decline over time. Further studies are needed to identify predicting factors against cPRA changes and to determine the optimal cPRA evaluation interval that reduces costs and improve efficiency of limited medical resources.



358.2

Early de novo DSA with T cell mediated rejection (TCMR) is associated with allograft dysfunction with chronic injury

Aravind Cherukuri, Rajil Mehta, Puneet Sood, John Lunz, Sundaram Hariharan, David Rothstein.
Thomas E. Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA, United States.

Introduction: De novo anti-donor HLA-specific antibody (DSA) detected post-renal transplantation is associated with adverse outcomes. However, the relationship between early de novo DSA and subsequent T cell-mediated rejection (TCMR) and its impact on allograft survival, remain unknown. In this prospective longitudinal study, DSA was measured longitudinally post-transplantation, and compared to the development of TCMR and subsequent allograft outcomes.
Methods: HLA specific antibodies were analysed longitudinally at 0,1,3,6,9,12 and 18 months post-transplant. Patients had protocol biopsies at 3 and 12months in addition to for-cause biopsies, and the development of rejection was examined in relation to DSA. In a subset of 26 patients where PBMCs were available, B cell subsets and cytokines were examined at 3 months.
Results: 53 (18%) of 299 patients transplanted between dates 1/13 and 7/14, developed de novo DSA (IS: Thymo, MPA+TAC & rapid steroid withdrawal). In 48/53 (90%) of these patients, DSA developed early (<6m). 51.2% of patients who developed DSA had rejection versus 29% of patients without DSA (OR 2.54, p=0.002). Of the patients with DSA and rejection, 74% had DSA at or before the diagnosis of rejection while 15% developed DSA after a rejection episode. Only 3 patients (11%) developed ABMR and were excluded. Patients with de novo DSA had significantly worse renal function at 12 and 18m than those without DSA. Importantly, patients with DSA+TCMR had worse Scr than patients with either DSA or TCMR alone (table 1A). Moreover, patients with DSA had significantly worse chronicity (IFTA & IF+i) scores than those without DSA, predominantly due to patients in the DSA+TCMR sub-group, who had markedly worse chronicity scores than patients with either DSA or TCMR alone (Fig 1).

A

Allograft dysfunction	DSA	No DSA	P-value
Creatinine-12m	1.81 (0.2)	1.46 (0.03)	0.0006
Creatinine-18m	2.04 (0.3)	1.51 (0.06)	0.005
IFTA score (ct+ci)	1.99 (0.16)	1.6 (0.07)	0.05
Interstitial fibrosis and inflammation (IF+i)	55%	39%	0.02

Allograft dysfunction	DSA	DSA+TCMR	P-value
Creatinine-12m	1.42 (0.16)	2.34 (0.2)	0.01
Creatinine-18m	1.48 (0.18)	3.05 (0.5)	0.01
IFTA score (ct+ci)	1.29 (0.2)	2.29 (0.2)	0.001
Interstitial fibrosis and inflammation (IF+i)	7%	89%	<0.0001

B

B cell parameter	DSA	DSA+TCMR	P-value
B cell-IL-10/TNF	**0.73**	**0.26**	**0.02**
T1-IL-10/TNF	**5.9**	**0.9**	**0.0001**
TrB-IL-10/TNF	**2.14**	**0.56**	**0.002**
Naïve B-IL-10/TNF	0.64	0.29	0.2
Memory B-IL-10/TNF	0.33	0.28	Ns

Patients with DSA+TCMR were younger (43 vs. 55yrs, p=0.02) and had a higher incidence of DGF (35% vs. 14.8%, p=0.001) when compared to those with DSA alone and both were independent risk factors (recipient age OR 0.95, p<0.05; DGF OR 6.9, p=0.02). Cumulative MFI levels for the DSA were significantly higher in the DSA+TCMR sub-group (7106 vs. 3212, p=0.03).

Thus, DSA+TCMR identifies patients at high risk for poor graft outcomes. Early diagnosis and treatment could improve outcomes. In this regard, in patients with DSA+TCMR, 50% of the rejections were subclinical (diagnosed on the 3 month protocol biopsy), and 19% presented with late rejection despite a normal 3-month biopsy. We found that transitional B cell (TrB) IL-10/TNFa ratio is a measure of Breg activity and predicts rejection. Patients with DSA+TCMR (16/26) had significantly lower TrB and other B-subset cytokine ratios at 3 months than those with DSA alone (10/26) (Table-1B). Moreover, in patients with DSA, a low TrB cytokine ratio is a strong predictor for presence of concomitant or subsequent TCMR (AUC 0.90, p=0.003; specificity 100%; sensitivity 78%).
Conclusion: de novo DSA with TCMR is associated significantly worse renal function and chronic allograft injury than DSA alone, suggesting that DSA is a marker for a heightened cellular alloresponse. In patients with DSA, TrB cytokine ratios may help identify those at high risk of TCMR allowing early intervention.

358.3

The new OPTN kidney allocation system: A look at the one year transplant trend in the highly sensitized patient cohort

Taba Kheradmand, Chak-Sum (Sam) Ho.
Histocompatibility and Immunogenetics Laboratory, Gift of Life Michigan, Ann Arbor, MI, United States.

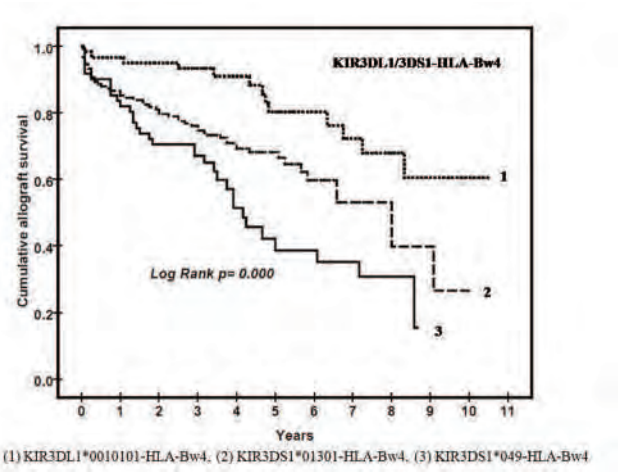
Aim: The new OPTN Kidney Allocation System (KAS) was implemented on Dec 4, 2014, in the U.S. with the primary goals to 1) reduce kidney discards, 2) better match for longevity, and 3) increase transplant opportunities for difficult-to-match recipient candidates, particularly the highly sensitized patient cohort (with 99 and 100% cPRA). Michigan kidney recipient candidates comprise approximately 3% of the United Network for Organ Sharing (UNOS) waiting list, with 9% being highly sensitized. Herein, we evaluated the impact of the new KAS on kidney allocation and transplantation trend in Michigan within the first year after its implementation.
Methods: Data was collected from the 8 Michigan renal transplant centers on recipient candidates with cPRA ≥99%, between Dec 4, 2014 and Dec 3, 2015, and the same time period of the previous three years. In this patient cohort, we investigated: 1) number of kidney offers, 2) transplant rate, 3) number of out-of-state kidney offers, and 4) incompatible positive crossmatch rate (number of positive crossmatch/total number crossmatch performed).
Results: Since the inception of KAS, while the number of kidney offers to recipients with cPRA ≥99% has somewhat increased by 30%, from 6 offers/month pre-KAS, to 9 offers/month post-KAS, the transplant rate in this patient cohort has significantly increased by nearly 8 fold to 45% (47 transplanted out of 105 offers) in comparison to an average of 6% (5 transplanted out of 79 offers) in the past 3 years. Overall our data showed that since the new KAS, 18% of the highly sensitized candidates in Michigan were being transplanted, in comparison to 13% nationally. As a consequence with the new KAS which mandated national/regional sharing for highly sensitized recipient candidates, the majority of the kidney transplant were from out-of-state donors (68%, or 32/47), which demonstrated a 3.5-fold increased compared to the previous 3 years. There did not appear to be a noticeable bias among the transplanted patients with regards to age, sex, race, or their donors' kidney donor profile index (KDPI). Finally, as expected, incompatible positive crossmatch rate was much higher in the highly sensitized patient cohort at 28-48% in the past 4 years, compared to that in all other patients at 21-25%. This could be attributed in part to the high prevalence of HLA antibodies to DP and DQA1 in the highly sensitized patients which were not being accounted for during the kidney allocation process by UNOS.
Conclusions: To date, our data suggested that, despite the higher incidence of incompatible positive crossmatch, the new KAS has greatly enhanced the number of kidney offers for the highly sensitized patient cohort, which led to a significantly higher transplant rate in these difficult-to-match patients.

358.4

Association of KIR3DL1/3DS1 alleles with renal allograft rejection

Raj Kumar Sharma[1], Swayam Prakash[1], Suraksha Agrawal[2].
[1]Nephrology , Sanjay Gandhi Post Graduate Medical Institution, Lucknow, India; [2]Medical Genetics, Sanjay Gandhi Post Graduate Medical Institution, Lucknow, India.

Introduction: Functioning of NK cell is regulated by KIR like inhibitory and activating cell surface receptors. Highly polymorphic KIR3DL1/3DS1 genes affect their corresponding HLA-Bw4 ligand binding affinities. This causes variation in NK cell inhibition and may have an impact on the long term renal allograft survival. Present study evaluated allelic diversity of KIR3DL1/3DS1 and importance of its corresponding HLA-Bw4 ligand affinity with renal transplant cases.
Materials and Methods: KIR3DL1/3DS1 allelic diversity was examined in 100 renal transplant cases and 100 normal controls. All samples were positive for KIR3DL1 or/and KIR3DS1 and possessed HLA-Bw4 ligand. PCR-SSP was used to determine incidence of KIR3DL1/3DS1 genes and sequence based typing method evaluated the pattern of KIR3DL1/3DS1 allele distribution. HLA class-I genotyping was performed using PCR-SSP kits. Relative quantification of KIR3DL1/3DS1 mRNA expression was done by 2-ΔΔCt algorithm with GAPDH as the housekeeping gene.



(1) KIR3DL1*0010101-HLA-Bw4, (2) KIR3DS1*01301-HLA-Bw4, (3) KIR3DS1*049-HLA-Bw4

Results and Discussion: We observed presence of 84 KIR3DL1/3DS1 alleles in the studied north Indian cases and controls. However, only 6 inhibitory KIR3DL1 alleles and 4 activating KIR3DS1 alleles with incidence > 1% were noted. For KIR3DL1*0010101, no-risk (OR=0.37, p=0.0072) association was observed with increased mRNA expression among antibody mediated graft rejection (Fold change=1.48±0.32, p=0.031), and antibody mediated chronic rejection (Fold change=1.12±0.15, p=0.042) cases. Risk was found for KIR3DS1*049N (OR=5.16, $p \leq 0.0001$) and KIR3DS1*01301 (OR=4.27, $p \leq 0.0001$). Both KIR3DS1*049N and KIR3DS1*01301 showed a decreased mRNA level. Looking at the ligand affinity, 3DS1*01301/HLA-Bw4+ (OR=2.12, p=0.0120) and 3DS1*049N/HLA-Bw4+ (OR=3.42, p=0.0051) combinations showed susceptibility. Kaplan-Meier survival analysis performed on a 15 year follow-up data revealed highest overall survival for KIR3DL1*0010101-HLA-Bw4 (cumulative survival=63% at 11 years). Less prolonged survival of was noted for 3DS1*01301/HLA-Bw4+ (cumulative survival=36% at 10 years) and least survival for 3DS1*049N/HLA-Bw4+ (cumulative survival=18% at 8 years).
Inhibitory signal transmitted by KIR3DL1 induces the ITIM motifs giving rise to an inhibitory signaling in KIR positive cells. Inhibition leads to higher T cell response due to protection of T cell from activation induced cell death. During the disease phase, inhibitory

signal loss and resultant activation causes NK cell lysis. This is in contrast to renal allograft donors who lack the KIR3DL1-HLA-Bw4 interaction. Donors carrying this combination posses a functional NK cell subset that induces NK cell inhibition leading to better allograft survival.

Conclusion: These findings highlight the evidence that prolonged survival of the renal allograft depends on presence of inhibitory KIR receptors. Importantly suppressing KIR3DS1 like activating NK cell surface receptors may improve the survival of HLA compatible kidney grafts.

358.5

C1q-fixing antibodies against denatured HLA and MICA antigens are associated with antibody mediated rejection

Junchao Cai[1], Paul I.Terasaki[1], Dong Zhu[2], Nils Lachmann[3], Constanze Schönemann[3], Matthew Everly[1], Xin Qing[4].
[1]Research, Terasaki Foundation Laboratory, Los Angeles, CA, United States; [2]Urology, Zhongshan Hospital, Fudan University, Shanghai, People's Republic of China; [3]Center for Tumor Medicine, Charité-Universitätsmedizin Berlin, Berlin, Germany; [4]Pathology, Harbor-UCLA Medical Center, Torrance, CA, United States.

Background: We have found antibodies against denatured HLA class I antigens in the serum of allograft recipients which were not significantly associated with graft failure. It is unknown whether transplant recipients also have denatured HLA class II and MICA antibodies. The effects of denatured HLA class I, class II, and MICA antibodies on long-term graft outcome were further investigated based on their ability to fix complement c1q.

Materials and Methods: In this 4-year retrospective cohort study, post-transplant sera from 975 kidney transplant recipients were tested for antibodies against denatured HLA/MICA antigens and these antibodies were further classified based on their ability to fix c1q.

Results: Thirty percent of patients had antibodies against denatured HLA class I, II, or MICA antigens. Among them, 8.5% and 21.5% of all patients had c1q-fixing and non c1q-fixing antibodies respectively. There was no significant difference on graft survival between patients with or without antibodies against denatured HLA/MICA. However, when these antibodies were further classified according to their ability to fix c1q, patients with c1q-fixing antibodies had a significantly lower graft survival rate than patients without antibodies or patients with non c1q-fixing antibodies ($p=0.008$). In 169 patients who lost renal grafts, 44% of them had c1q-fixing antibodies against denatured HLA/MICA antigens, which was significantly higher than that in patients with functioning renal transplants (25%, $p<0.0001$). C1q-fixing antibodies were more significantly associated with graft failure caused by AMR (72.73%) or mixed AMR/CMR (61.9%) as compared to failure due to CMR (35.3%) or other causes (39.2%) ($p=0.026$).

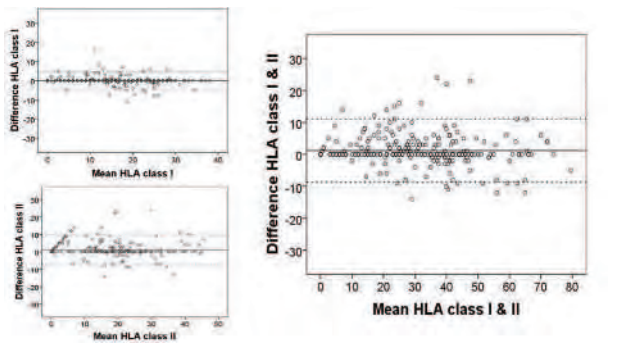
Conclusions: Transplant recipients had antibodies against denatured HLA class I, II, and MICA antigens. However, only c1q-fixing antibodies were associated with graft failure which was related to antibody mediated rejection.

358.6

Agreement between number of donor / recipient eplet mismatches calculated using two-digit serological versus four-digit molecular Human Leucocyte Antigen (HLA)-typing

Samantha Fidler[1], Wai Lim[2], Joshua Lewis[2], Germaine Wong[3]. [1]School of Pathology and Laboratory medicine, University of Western Australia, Perth, Australia; [2]School of Medicine and Pharmacology, University of Western Australia, Perth, Australia; [3]School of Public Health, University of Sydney, Sydney, Australia.

Aim: We aimed to assess the agreement of the number of eplet mismatches at the HLA-A, -B, -C, -DQ and -DR loci determined by 2-digit serological and 4-digit molecular typing. Methods: We included patients who received live or deceased-donor kidney transplants between 2003 and 2007. Donor and recipient serological typing was determined using complement-dependent cytotoxicity, and molecular 4-digit typing determined using sequence based typing (Sanger). The number of eplet mismatches was calculated by converting the 2 and 4-digit HLA-typing using HLAMatchmaker. Correlation and agreement of HLA-A, -B, -C, -DP and -DR mismatches between the two methods was analysed using Spearman rank correlation and Bland Altman plots respectively. HLA-DP can only be determined using 4-digit typing and therefore not included in this study.
Results: Of 264 kidney transplant recipients, 86 (33%) were females. The correlation between class I (HLA-A, -B, -C) and class II (HLA-DQ, -DR) between 2 and 4-digit converted eplet mismatches were 0.966 and 0.931 respectively. Bland-Altman's limits of agreement between class I, class II and combined class I and II eplet mismatches using 2 and 4-digit typing is shown below (figure 1). The number of class I and II eplet mismatches determined by 4-digit conversion exceeded that of 2-digit conversion in 37% of recipients, with 10% exceeding 7 eplet mismatches. One hundred and sixteen (44%) recipients had identical number of class I and II eplet mismatches as determined by 2 and 4-digit typing. Of the 21 "outliers" for both class I and II eplet mismatches, 5 (24%) of patients were females and 8 (38%) of either patients or donors were non-Caucasians. A further 4 (19%) had unusual (non-Caucasian) HLA alleles.



Intraclass correlation coefficients* for consistency and absolute agreement (n=264)

	Consistency	Absolute agreement
2 Digit	4 Digit (95% CI)	4 Digit (95% CI)
HLA-A	0.995 (0.994-0.996)	0.995 (0.994-0.996)
HLA-B	0.983 (0.978-0.987)	0.982 (0.977-0.986)
HLA-C	0.875 (0.843-0.900)	0.875 (0.843-0.900)
Class I	0.969 (0.960-0.975)	0.969 (0.960-0.975)
HLA-DR	0.987 (0.984-0.990)	0.987 (0.983-0.992)
HLA-DQ	0.810 (0.764-0.848)	0.802 (0.748-0.845)
Class II†	0.935 (0.918-0.949)	0.931 (0.908-0.948)

*Two-way fixed intraclass correlation coefficients. †Excluding HLA-DP.

Conclusions: It appears that there is good correlation and agreement between 2 and 4 digit typing for total eplet mismatches at the HLA-A, -B, -C, -DQ and -DR loci. Future research should focus on exploring the clinical significance of total eplet mismatches determined by the two different methods.

Department of Clinical Immunology, PathWest, Fiona Stanley Hospital, Perth

358.7

Careful deselection of low risk unacceptable antigens can facilitate renal transplantation in highly sensitised patients with excessive time on the waiting list

Stuart Falconer[1], Ann-Margaret Little[2], Graeme Pang[3], Colin Geddes[1], Marc Clancy[1].
[1]Renal Transplant Unit, Queen Elizabeth University Hospital, Glasgow, United Kingdom; [2]Histocompatibility and Immunogenetics, Gartnavel General Hospital, Glasgow, United Kingdom; [3]University of Glasgow Medical School, University of Glasgow, Glasgow, United Kingdom.

Highly sensitised patients wait considerably longer for deceased donor transplantation. Modern histocompatibility testing techniques make it possible to monitor the HLA antibody profile of highly sensitised patients. This creates the opportunity to attempt transplantation against previously sensitising mismatched HLA antigens with a low risk of early, severe rejection. We report our early experience of deselecting low risk unacceptable antigens in highly sensitised patients.
Of 294 patients on the local waiting list, 68 had a calculated reaction frequency (cRF) >95% and 18 had been waiting > 6 years. 17 patients were identified in whom it was possible to de-list previously declared unacceptable antigens by the consultant clinical scientist who carefully reviewed each patient and where antigens were felt to be low risk such as historic weakly positive, or a low mean fluorescence intensity (MFI) <3000 by Luminex, these were deselected as 'unacceptable' for transplantation from the Organ Donation and Transplantation database. All patients who then went on to be transplanted had clinical data such as donor/recipient demographics, renal function, rejection and transplant and patient survival recorded.
There were 11 female and 6 male patients in whom it was possible to deselect unacceptable antigens and 10 patients (76.9%) had a reduction in their CRF as a result. 13 of the cohort subsequently received a transplant between 03 Sep 14 and 19 Oct 2015. Of those receiving a transplant, mean age was 52±11.93 years, 8 had received ≥1 transplant previously and mean waiting list time for this transplant was 10.4±5.4 years. There were 10 DBD, 2 DCD and one live non-directed donor with a mean donor age of 49.92±13.94 years. The HLA mismatch levels were 1 at level 1 (000); 4 at level 2 (0DR and 0/1B) and 8 at level 3 (0DR and 2B) or (1DR and 0/1B). All patients had a negative T and B cell complement dependent cytotoxic crossmatch though 2 patients had positive flow cytometry T cell and B cell crossmatch. 7 patients received a transplant as a result of antibody deselection, transplanted against previously unacceptable antigens, and for 6 deselection made no difference in their offer. Mean cold ischaemia time was 17:09h. All but 2 patients were given rabbit anti-thymocyte globulin induction and all patients received prednisolone, tacrolimus and mycophenolate mofetil maintenance immunosuppression. Of the patients who have not received a transplant yet, 2 have been suspended from the list temporarily and 2 patients are still awaiting transplant. 6 patients (46.2%) had delayed graft function. 3 patients (23.1%) had acute rejection following transplantation, one cell mediated and 2 antibody mediated. One patient died of unrelated causes and maintained transplant function. No transplants have failed. Serum creatinines at day 7, 14, 1 month, 2 months, 3 months and 6 months were 301±229.37, 156±298.84, 145.5±219.46, 162±55.81, 143±45.91, 125±53.25 mg/L respectively.
Highly sensitised patients can benefit from deselection of low risk unacceptable antigens to reduce the cRF and facilitate a transplant. This small study has found such patients to have good early transplant function with a low risk of acute rejection. Longer follow up is required to determine if these higher immunological risk transplants will continue to have adequate function and transplant survival.

References:

[1] Picascia, A et al, Lights and shadows of anti-HLA antibodies detected by solid-phase assay *Immunol Lett.* 2014; 162(1 Pt A):181-7

[2] Marfo, K et al, Pretransplant immunologic risk assessment of kidney transplant recipients with donor-specific anti-human leukocyte antigen antibodies. *Transplantation*. 2014 Nov 27;98(10):1082-8

358.8

C1q/C3d single antigen bead assay only detects high titer/avidity class-I anti-HLA antibodies detected by single antigen beads

Manish Gandhi[1], Nicole Henderson[1], Steven DeGoey[1], Justin Kreuter[1], Ripudamanjit Singh[2], Laurie Wakefield[1].
[1]Division of Transfusion Medicine, Mayo Clinic, Rochester, MN, United States; [2]Mayo Clinic, Rochester, MN, United States.

Introduction: Detection of donor-specific anti-HLA antibodies (DSA) by solid phase assay (SpA) has enhanced donor selection by allowing prediction of the actual crossmatch (xM), a process which is referred to as a virtual crossmatch (VxM). At our center we find good correlation (R2=0.85) between flow xM (FxM) and VxM using single antigen bead (SAB) assay to identify DSA. In addition, we and others have demonstrated better post-transplant outcomes in patients that are transplanted with negative xM. The SAB assay is considered a sensitive SpA; however, a concern is that clinically insignificant anti-HLA antibodies may be detected and preclude the recipient from receiving a transplant. The complement fixing (C1q/C3d) SAB assay's has been proposed as a way to minimize the detection of clinically insignificant anti-HLA antibodies. We present a summary of our evaluation of this assay.
Materials and Methods: 20 positive sera, previously used for serological typing, were tested with SAB, at 1:8 dilution with SAB and by C1q and C3d SAB method as per the manufacturer's protocol. Serological anti-HLA specificities were compared with those that were obtained by SAB, C1q SAB and C3d SAB. For the SAB SpA, specificities >5000 MFI were considered as positive, while specificities >500 MFI were considered positive for C1q/C3d assay. In our center SAB >5000 MFI correlates with a positive flow cytometric xM (FxM) and >10000 MFI correlates with cytotoxicity xM.
Results: In total there were 25 anti-HLA antibody specificities as defined by serology, 354 as defined by SAB, 151 as defined by C1q (figure 1)and 60 as defined by C3d. SAB identified all 25 specificities as defined by serology; C1q identified 22/25 and C3d 15/25 (figure 2). SAB specificities not identified by C1q/C3d had MFI between 5000-10,000. C3d SAB MFI were significantly lower as compared to the C1q MFI
Conclusions: For Class I anti-HLA antibodies, C1q/C3d assay only identify high titer/avidity antibodies as defined by SAB. C1q assay identified more specifcities as compared to C3d assay. As DSA with SAB MFI: 5000-10000 can result in a positive FxM, additional investigation is warranted to determine the clinical significance of these anti-HLA antibodies.

Method	Total Specificities
Serological	25
SAB	354
C1q SAB	151
C3d SAB	60

	Serology Positive Specificities	
	N	%
SAB		
Total Positive	25	100
Total Negative	0	0
C1q SAB		
Total Positive	22	88
Total Negative	3	12
C3d SAB	0	
Total Positive	15	60
Total Negative	10	40

358.9

Regulation of microRNA hsa-mir-663a by HLA class I antibodies in endothelial cells

Omar Moussa[1], Kathryn Twombley[2], Satish Nadig[3], Medhat Askar[4], Titi Srinivas[2], Prabhakar Baliga[3].
[1]Pathology and Labratory Medicine, Medical University of South Carolina, Charleston, United States; [2]Medicine, Medical University of South Carolina, Charleston, United States; [3]Surgery, Medical University of South Carolina, Charleston, United States; [4]Pathology, Baylor University Medical Center, Dallas, United States.

Introduction and Aim: The role of HLA antibodies in endothelial cells signal transduction and transplant vasculopathy is well documented by numerous studies. In the current study, we describe a novel mechanism for HLA class I antibodies in endothelial cell signaling by up-regulating microRNA 663a.
Methods: Cultured human umbilical vein endothelial cells (HUVEC) were grown to 80% confluency in EGM media. Following overnight incubation with growth factor free media, cells were treated with humanized monoclonal antibodies (mAbs) directed to a general epitope of HLA class I antigens (invivogen). Following RNA extraction, samples were subjected to global microRNA analysis utilizing Illumina RNASeq platform. MicroRNA 663a expression levels were validated by Taqman real-time PCR (Applied Biosystems). Expression levels were calculated relative to U6 levels. To determine the serum microRNA 663a levels in patients, total microRNA were extracted from patients with antibody mediated rejection (AMR) and evidence of class I donor specific antibodies (DSA) (n=8) and non-AMR controls (n=6) using miRNeasy Serum/Plasma Kit (Qiagen) followed by Taqman real-time PCR assay.
Results: Treatment of cultured HUVECs with class I HLA antibodies resulted in a 3.6 ± 0.7 fold increase in microRNA 663a compared to control cells. Sera from patients with AMR showed increased relative microRNA 663a levels (45.6±13.1) compared to non-ABMR (20.5±3.4) ($P<0.05$). To further understand the functional role of mir-663a in AMR and endothelial cell signaling, we investigated its potential targets using microRNA databases. The heparin sulfate proteoglycan 2 (HSPG2) which also called Perlecan is known to inhibit smooth muscle cell proliferation and play a role in maintaining vascular homeostasis was found to be a direct target for mir-663a. HSGP2 mRNA levels measured by real-time PCR were found to be down-regulated in endothelial cells treated with HLA antibodies.
Conclusion: The current study describes a novel role played by HLA antibodies in modulating endothelial cell signaling by modulating mir-663a and its target HSPG2. Higher serum microRNA 663a in patients with ABMR could be linked to endothelial cell activation following HLA antibody coupling and could be studied as a mechanistic biomarker in future studies.

359.1

Mass cytometry reveals NK cell and T cell subsets in pediatric liver transplant patients with acute rejection

Audrey Lau[1,2], Kelly Haas[1], Todd Shawler[2], Carlos O. Esquivel[2], Olivia M. Martinez[2], Ricardo O. Castillo[1], Sheri M. Krams[2].
[1]Pediatrics, Division of Gastroenterology, Hepatology, and Nutrition, Lucile Packard Children's Hospital/Stanford University, Palo Alto, CA, United States; [2]Surgery, Division of Abdominal Transplantation, Stanford University, Palo Alto, CA, United States.

Introduction: Proper evaluation of the immune status of liver transplant recipients is crucial for appropriate post-transplant management. Current monitoring of graft function relies mainly on non-specific liver enzyme tests and immunosuppressive drug levels without proper assessment of the immune system. Alterations in hepatic enzyme levels can occur for a variety of reasons including acute rejection (AR). Currently the gold standard for diagnosing patients with AR and ruling out other causes of graft dysfunction is liver biopsy. Identifying an immune signature that differentiates patients with AR through a minimally invasive blood test would be preferable.
Materials and Methods: To define an immune signature of AR, we utilized cytometry by time-of-flight (CyTOF, mass cytometry) to comprehensively characterize the peripheral blood mononuclear cells (PBMC) from pediatric recipients of liver allografts (n=11) on conventional immunosuppression (single agent tacrolimus monotherapy). PBMC from patients with biopsy-proven AR prior to treatment (3.8±5.9 years post-transplant, mean age 9.6±8.0 y) were compared to stable pediatric liver recipients (13.0±1.5 years post-transplant, mean age 15.2±4.9 y).
Results and Discussion: Correlation analyses from mass cytometry data was performed using Citrus, a method for unsupervised identification of significant cellular populations, with cell subsets identified using hierarchical clustering of live cells using R. Cells were clustered on the basis of the expression of 22 markers. Significant changes in cell frequency were inferred using the "glmnet" package in R. When the two patient populations were compared, two clusters of significance, a $CD4^+CD5^+CD25^+CD27^+CD44^+$ T cell population and a $CD56^+CD16^+$ NK cell population, were significantly increased in the AR patients as compared to the stable group. Manual gating of the data revealed that $CD3^-CD19^-CD20^-CD8\alpha^+CD16^+CD56^+$ NK cells were significantly increased in the AR group ($p\leq0.05$) and that the NK:$CD3^+$ T cell ratio as well as the NK:$CD4^+CD5^+CD25^+CD27^+$ T cell ratio were significantly increased in the AR group compared to stable patient group ($p\leq0.01$ for both).
Conclusion: Additional studies to further define the phenotype and function of these NK and T populations are necessary. In summary, using mass cytometry, a highly parameterized single cell platform, we have identified that NK cells and a specific T cell subset are significantly increased during AR in pediatric transplant recipients. These results demonstrate the power of mass cytometry to discover significant immune cell populations that may have diagnostic potential.

359.2

Pediatric liver transplantation using segmental grafts in Japan: A single-center experience with 270 patients

Koichi Mizuta[1], Taizen Urahashi[1], Yoshiyuki Ihara[1], Yukihiro Sanada[1], Noriki Okada[1], Naoya Yamada[1], Yuta Hirata[1], Takumi Katano[1], Kentaro Ushijima[2], Shinya Otomo[3].
[1]Department of Transplant Surgery, Jichi Medical University, Tochigi, Japan; [2]Department of Clinical Pharmacology, Jichi Medical University, Tochigi, Japan; [3]Department of Pharmacy, Jichi Medical University Hospital, Tochigi, Japan.

Objectives: This retrospective study evaluated the outcome of pediatric liver transplantation (PLT) using segmental grafts at a single high-volume PLT center in Japan.
Patients and Methods: Two hundred and seventy patients (105 males and 165 females, mean age 3.2 years) who received segmental grafts from living donors or split grafts from deceased donors were retrospectively evaluated between May 2001 and January 2016. The causes of liver disease were biliary atresia (n=195), ornithine transcarbamylase deficiency (n=18), Alagille syndrome (n=11), graft failure (n=11), neonatal hemochromatosis (n=6), and others (n=36). The segmental graft type of living donor liver transplantation (LDLT) was determined according to the recipient's body weight and the donor's preoperative CT volumetry. A summary of the surgical technique for LDLT is described as follows: donor graft hepatectomy was routinely performed with intraoperative ultrasonographic guidance without the Pringle maneuver during the parenchymal transection. The graft biliary duct was resected using intraoperative repeated real-time cholangiography. Recipient hepatic vein anastomosis was performed with 5-0 monofilament sutures to completely clamp the suprahepatic and infrahepatic IVC. The portal vein was reconstructed using 6-0 absorbable sutures by adjusting the alignment of the donor and recipient portal veins. Hepatic artery anastomosis was performed using the microvascular technique with 9-0 Nylon sutures. Biliary reconstruction was performed by hepaticojejunostomy using external biliary drainage with 6-0 absorbable sutures.
Results: The recipients received a total of 276 segmental transplants; 275 were LDLT: left lateral segments (S2,3) in 196 (71%), left lobes (S1,2,3,4 or S2,3,4) in 66 (24%), monosegments (S2 or S3) in 13 (5%), and right posterior segment (S6,7) in 1. The patients' parents comprised 96% of the living donors. One donor encountered re-operation due to postoperative abdominal bleeding, and there was no mortality in any of the living donors. Only one patient received deceased donor split liver transplantation with the left lateral segment. The postoperative surgical complications experienced by the recipients were hepatic arterial complications (HAC; 6%), portal vein stenosis (PVS; 12%), hepatic vein stenosis (HVS; 6%), and biliary stricture (BS; 16%). Regarding the graft type, the incidence of HAC (23%) in the monosegment group, the incidence of HVS (9%) in the left lateral segment group, and the incidence of BS (26%) in the left lobe group were significantly greater than those in the other groups. The graft and patient survivals were 93% and 95%, respectively.
Conclusions: LDLT is an effective and irreplaceable treatment for pediatric end-stage liver disease in Japan. However, the surgical complications may differ according to the graft type.

359.3

Single kidney transplantation from pediatric donors younger than 3 years to adult recipients

Lan Zhu, Gang Chen, Jipin Jiang, Zhishui Chen.
Institute of Organ Transplantation, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, People's Republic of China.

Background: Traditionally, the criteria of splitting pediatric en bloc kidneys for transplantation into two adults includes: donor age > 3 years, donor weight > 12kg, or kidney length > 6cm. To expand the donor pool, we investigated the feasibility and safety of the single kidney transplantation from pediatric donors younger than 3 years to adult recipients.
Methods: From 2014.9 to 2015.9, a total of 21 single kidney transplants from pediatric DCD (donation after citizen death) donors younger than 3 years (8-26 months) were performed in our center. Almost all recipients were adult patients except 2 children. Here we summarized the outcome of these small single kidney allografts in 19 adult recipients.
Results: The average donor age was younger than 2 years (18.0±6.1m), with 4 donors younger than 1 year. The youngest donor was only 8 months. The average donor weight was less than 12kg (11.6±5.2kg), and the average kidney lengths was about 6cm (6.3±0.6cm). The causes of donor death include intracranial tumor (2 cases), severe congenital dysplasia (2 cases), trauma (5 cases), asphyxiation (1 case), and unknown cause (1 case). The average warm ischemia time was 11.5±4.3min. For the 19 adult recipients, the average age was 30.0±9.6 years and the average body weight was 45.8±6.2kg (38-52 kg). The majority of the patients were female (68.4%), and all except one of these patients were transplanted for the first time. The average follow-up time was 12 months. Delayed graft function (DGF) was observed in 3 patients (16%). Most patients (16/19) had a satisfied urine output immediately after the small single kidney transplantation, however, the serum creatinine levels decreased relatively slower in the early period (Fig 1). The average value of eGFR was steadily increased to normal during the 1year follow-up period (Fig 2). Additionally, only one renal graft developed acute rejection (5%), which was successfully reversed by the treatment of high dosed of steroids.

Fig 1 Allograft function of adults receiving small single pediatric kidneys in the early period

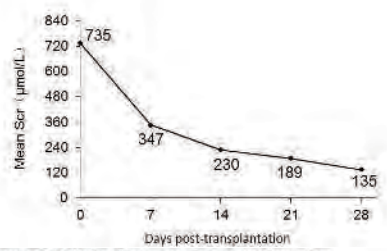
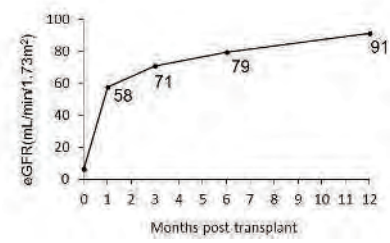


Fig 2 Recipient eGFR during first year after transplantation



Conclusion: By choosing appropriate adult recipients, excellent short-period transplant outcome can be achieved by using small single kidneys from pediatric donors younger than 3 years, which may represent an important way to expend the donor pool for kidney transplantation.

359.4

Overcoming barriers in complex paediatric renal transplantation

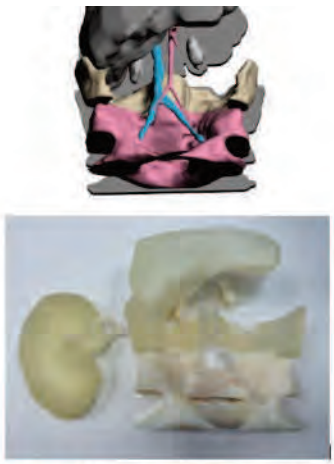
Pankaj Chandak[1], Jelena Stanojovic[2], Nick Byrne[4], Narayan Karunanithy[5], Chris Callaghan[1], Francis Calder[1], Jonathon Olsburgh[1], Martin Drage[1], Geoff Koffman[1], Stephen D.Marks[3], Zubir Ahmed[1], Raphael Uwechue[1], Nizam Mamode[1], Nicos Kessaris[1].
[1]Transplant Surgery, Guy's, Evelina and Great Ormond Street Hospitals, London, United Kingdom; [2]Paediatric Nephrology, Evelina Children's Hospital, London, United Kingdom; [3]Paediatic Transplantation and Nephrology, Great Ormond Street Hospital, London, United Kingdom; [4]Medical Physics and Imaging Sciences, Guy's and St Thomas' and King's College London, London, United Kingdom; [5]Interventional Radiology, Guy's and St Thomas Hospitals, London, United Kingdom.

Introduction: There are increased challenges in transplanting small (<20kg) recipients rendering implantation infeasible or, at best, uncertain. In this context, transplant feasibility is dependent on a multi-disciplinary approach where unconventional interventions may be required. This study evaluates 3 key aspects of transplantation in small children from a large volume combined tertiary UK Paediatric Transplant Centre:
1) A comparative analysis of 350 patients (from 2005-2014) to determine any difference in patient and graft survival in children weighing <20 kg (group1) compared to >20kg (group2) at the time of transplant?
2)Where do we perform graft anastomoses in presence of vascular anomalies?
3)How can we make paediatric transplantation safer? Surgical decisions currently rely on 3D imaging which is limited in evaluating structures by their presentation on 2D screens. As a solution, we assessed the feasibility of using 3D printing technology to fabricate physical models portraying patient-specific anatomy and disease morphology.
Methods: Data was evaluated from a prospectively collected UK Registry (NHSBT) database. We also retrospectively reviewed 5 children with significant vascular anomalies. We prospectivley assessed the value of 3D printing to inform complex transplantation using questionnaires (score 1-5, 1=not useful, 5= very useful).
Results and Discussion: There was no significant difference in graft (p=0.239) or patient (p=1.06) survival between Group1 (<20kg) and 2(>20kg) on Kaplein-Meier analysis. Outcomes for 5 complex vascular cases shown in Fig1. This group had 100% death censored graft survival and median eGFR 46 mls/min/1.73m2 at 10 month median follow up.

Case demographic and vascular issue	Arterial anastomosis site (donor renal artery (RA)→ recipient artery)	Venous anastomosis site(donor renal vein RV→ recipient vein
1. 9yr F, 24 kg : *Mid aortic syndrome- narrowed aorta requiring supra-coeliac to aortic bifucation graft prior to transplant*	Donor RA → Common iliac artery (after inspection of vessels at surgery)	Donor RV→ Common iliac vein
2. 7yr F, 15kg: *Infrarenal IVC occlusion due to recurrent thrombosis*	Donor RA →Aorta	Donor RV→ Suprarenal IVC
3. 6 yr, 18 kg: *IVC occlusion, supra-coeliac- aortic bifurcation PTFE bypass graft for aneurysmal disease aged 2),*	Donor RA→Below aortic PTFE graft just above bifurcation	Donor RV→Left gonadal vein (dilated collateral)
4. 3yr M 14 kg IVC occlusion due to thrombosis	Donor RA→Aorta	Donor RV→Left Ext Iliac vein
5. 17 yr F 34 kg – Mid Aortic Syndrome- narrowed aorta and occluded left iliac artery and common iliac veins	Donor RA→ "bank" deceased donor iliac vessel used as a supra-coeliac aortic conduit to donor artery	Donor RV→IVC

Conclusions: Our large cohort analysis shows transplantation is feasible and safe with comparable outcomes in smaller weight children with respect to graft and patients survival. Furthermore, vascular anomalies do not necessarily preclude transplantation where unconventional anastomotic approaches may be required. Finally, to our knowledge this is the first reported case of using novel 3D printing in paediatric renal transplantation. Our experience proves promising

with clinical translation of these models into the operating theatre, providing the surgical team with full 3D haptic spatial appreciation for making critical decisions – in particular, aiding the accurate placement of adult size donor kidney into the paediatric abdomen.



3D Model Print of the abdomen of a 10.2kg, 2 year old male showing the liver (soft), aorta and IVC and abdominal wall with the bony pelvis (hard) and his father's adult sized kidney. Models used for planning implantation and consenting the family. Prototype design used for segmentation and processing shown above.

359.5

Barriers to preemptive renal transplantation in New Zealand children

Madeleine Didsbury[2], Chanel Prestidge[1], Derisha Naicker[1], Tonya Kara[1].
[1]Paediatric Nephrology, Starship Children's Hospital, Auckland, New Zealand; [2]Centre for Kidney Research , Westmead Hospital, Sydney, Australia.

Introduction: All children with end stage kidney disease (ESKD) in New Zealand are managed at Starship Children's Hospital. We have previously shown that our preeemptive transplantation rates are low, particularly in Maori and Pacific children[1]. The National Renal Transplant Service hopes to increase the rates of preemptive transplantation[2].
We aimed to determine if there are modifiable factors for children having dialysis as first treatment, which may allow for an increase in future rates of Paediatric preemptive transplants.
Methods: We conducted a retrospective review off all entries into ANZDATA from Jan 2006 to Dec 2015 for our centre. We collected demographic and ethnicity data, cause of ESKD, and time from first presentation to first treatment with renal replacement therapy (RRT). Late presentation was defined as requiring RRT within 90 days of presentation, excluding neonates. We reviewed whther those who had dialysis first went onto transplantation, and identified all children who had a medical reason for dialysis as a preferential first RRT.
Results: 65 children were identified via ANZDATA. 9 (13.8%) received a preemptive transplant, none of whom were Maori or Pacific children. Demographic data and medical reasons for dialysis are shown in Table One

		Dialysis n=57	Transplant n=9
Median age at first treatment (years)		8 (range 0 - 16)	15 (range 7 - 16)
Ethnicity	NZ European	29 (50.8%)	8 (89%)
	NZ Maori	13 (22.8%)	0 (0%)
	Pacific Island	10 (17.4%)	0 (0%)
	Asian	4 (7%)	1 (11%)
Median time to ESKD from presentation (months)		7 (range 0 - 182)	59 (range 8 - 204)
Medical reasons for dialysis	Active nephrotic syndrome	16 (28%)	
	Weight <10kg	14 (24%)	
	Oncology stand down	2 (3%)	
	Other Immunesuppression	3 (4.5%)	
	High oxalate level	1 (1.5%)	
	No live donor identified prior to ESKD	2 (3%)	
	Lost to follow up	2 (3%)	
	Late presentation	2 (3%)	

Of the 57 children who recieved dialysis initially, 34 had a medical reason that excluded transplant as first treatment in our centre. However 19 children (29%) presented late, with 10 requiring dialysis within 24 hours of presentation. Of these children 13 (68%) had no medical reason that would have excluded transplant with earlier presentation, and 11of the 13 proceeded to a live donor transplant within the following 12 months, suggesting a potential opportunity for a preemptive transplant if CKD had been identified earlier. Within this group of late presentations 12 (63%) had risk factors for CKD (haematuria, proteinuria, short stature, rickets, genetic diagnoses associated with renal disease and past history of AKI) when previously seen in primary/ secondary care.
Conclusion: The rate of late presentation due to delay in diagnosis of CKD is a potentially modifiable factor to increase our rate of preemptive transplants and offer all children with ESKD optimal care. Improved awareness in primary and secondary care may improve this. Additonally we plan to learn more about families' perceptions of their care via future qualitative research to determine if there are other factors which we can address.
Jane Ronaldson; William Wong

References:
[1] Racial disparities in pediatric kidney transplantation in New Zealand. Grace et al. Pediatr Transplant, 2014 18: 689-697.
[2] http://www.health.govt.nz/about-ministry/leadership-ministry/clinical-groups/national-renal-transplant-service/quality-improvement-metrics

359.6

Association of C1q fixing donor-specific HLA antibodies with late graft failure in pediatric renal transplant recipients

Alexander Fichtner[1], Caner Süsal[2], Britta Höcker[1], Susi Rieger[1], Rüdiger Waldherr[3], Jens H.Westhoff[1], Anja Sander[4], Gerhard Opelz[2], Burkhard Tönshoff[1].
[1]University Children's Hospital, Heidelberg, Germany; [2]Department of Transplantation Immunology, Institute of Immunology, Heidelberg, Germany; [3]Institute of Clinical Pathology Heidelberg, Heidelberg, Germany; [4]Institute of Medical Biometry and Informatics, University of Heidelberg, Heidelberg, Germany.

Purpose: Because the clinical relevance of donor-specific HLA antibodies (DSA) in non- presensitized patients after renal transplantation (Tx) is currently being debated, we investigated the diagnostic and prognostic value of over-all and complement-binding DSA in pediatric patients undergoing clinically indicated graft biopsies and their association with graft outcome and specific histological lesions.
Methods: Sera of 62 pediatric renal transplant recipients at time of indication biopsy ≥ 1 year post-transplant were assessed for DSA and C1q-fixing DSA by Single Antigen-Bead technology.
Results: Twenty-six patients (42%) were DSA-positive at time of indication biopsy, 9 of the 26 (35%) C1q-positive. At 4 years post-biopsy, patients with C1q-positivity had a strikingly low graft survival (11%) compared to DSA-positive, C1q-negative patients (82%, $p = 0.001$) and to DSA-negative patients (88%, $p < 0.001$). The sensitivity of C1q-DSA positivity for subsequent graft failure was 73%, the specificity 93%. The vast majority (89%) of C1q-positive patients were diagnosed with active chronic antibody-mediated rejection. C1q DSA-positivity (adjusted hazard ratio (HR) 6.35), presence of transplant glomerulopathy (HR 9.54) and estimated glomerular filtration rate at the time of indication biopsy (HR 0.91) were risk factors associated with subsequent graft loss.
Conclusions: The presence of C1q-positive DSA in the context of an indication biopsy identifies a subgroup of pediatric renal transplant recipients with a markedly increased risk of subsequent graft loss. Because a fraction of DSA-positive patients escape rejection or graft dysfunction, the C1q assay markedly increases the specificity of a positive DSA result regarding unfavorable transplant outcome.

359.7

Acceptable post-heart transplantation outcomes of children listed in total renal failure

Nassiba Alami Laroussi[1], Manjula Gowrishankar[2], Jennifer A.Conway[1], Paul Kantor[1], Lorie J.West[1], David B.Ross[3], Ivan M.Rebeyka[3], Simon Urschel[1].
[1]Pediatric Cardiology, Stollery Children's Hospital, Edmonton, AB, Canada; [2]Pediatric Nephrology, Stollery Children's Hospital, Edmonton, AB, Canada; [3]Pediatric Cardiovascular surgery, Stollery Children's Hospital, Edmonton, AB, Canada.

Introduction: Renal dysfunction (RD) at heart transplant (HTx) is widely recognized as a poor prognostic factor for survival and post-HTx renal function (RF). There is wide variability of practice in listing patients while depending on renal replacement therapy (RRT). We hypothesized that RD is most frequently pre-renal due to low cardiac output and tends to resolve after HTx. We sought to determine the outcome and long term RF of patients listed for HTx while on RRT.
Methods: This retrospective study included pediatric patients on RRT: (peritoneal dialysis, hemodialysis or hemofiltration) at time of listing for HTx between 2000 and 2014. Data was collected from medical charts and included: RRT pre and post-HTx, mechanical ventricular support pre- and post-HTx and potential risk factors such as ischemic time, donor-recipient size mismatch, HLA-sensitization, ABO-compatibility and concomitant morbidities.
We documented RF by measuring or estimating glomerular filtration rate (GFR) over time until October 2015. GFR was measured by radioisotope, estimated with CKiD formula when cystatin C was available, or Schwartz formula when only creatinine was available.
Results: 12 patients required RRT at time of listing; 11 of which survived to HTx. The median age was 1.1 y ranging [0.01-14.8] at listing, 1.6 y [0.03-15.7] at HTx with a median waiting time of 40 days [1-342]. The primary cardiac diagnosis was congenital cardiopathy in 9/12, myocarditis, dilated cardiomyopathy and ischemic in the 3 others patients. Pre-HTx, they had RRT for a median time of 11 days [3-299]: 7 had continuous hemodialysis/filtration, 3 peritoneal dialysis and 2 both. 9 patients (82%) were on ECMO, 6 (54%) on VAD and 3 patients were highly sensitized prior to HTx (cPRA>50%).
Median post-HTx follow-up of this cohort was 6.4 y [3.3-8.8]. Actuarial post-HTx survival was 90% at 2 weeks, 73% at 1 month, 73% at 6 months, and 55% at 1 and 5 years. One patient was delisted and subsequently died. 4/5 patients died from cardiac failure and one from RD. 2 patients required ECMO for a mean duration of 19 days and one patient required VAD post-HTx. 8/11 patients required RRT for a median time of 17 days [0-240] post-HTx. All survivors were weaned off RRT and most recent mean GFR was 62±9 mL/min/1.73 m2.; which represent mild renal dysfunction (CKD stage II).
Conclusion: RD is associated with a high post-HTx mortality of 45% in our experience, however, death was mainly not due to RF. All survivors recovered RF with only mild impairment in the long-term follow-up.
Dependency on RRT at the time of HTx assessment should not, as a sole factor, preclude patients from being listed.

359.8

Contributing factors to transplant medication errors in pediatric patients

Chow Fen Tang[1], Lee Jin Koh[1], U. Mya Than[1], Kar Hui Ng[1,2], Perry Y.W. Lau[1], Min Yie Gong[1], Jolene R.Y.Ong[1], Qiao Zhi Chee[1], Hui Kim Yap[1,2].
[1]Shaw-NKF-NUH Children's Kidney Centre, KTP-National University Children's Medical Institute, National University Hospital , Singapore, Singapore; [2]Paediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore.

Introduction: Medication errors which impact on patient safety may result in a significant burden to healthcare systems. Medication discrepancy is the most common type of medication error, and is especially worse with polypharmacy. The aim of this study was to define the factors contributing to medication discrepancies during transition of care between different clinical settings in a Paediatric Renal Transplant Programme.
Materials and Methods: Renal transplant recipients on follow-up at the Shaw-NKF-NUH Children's Kidney Centre, Singapore were recruited. At each patient-provider encounter (in the clinic, on admission to the ward, or on discharge from the hospital), medicine reconciliation was performed to determine the occurrence of medication errors. The medications were reconciled using inpatient and outpatient electronic records, as well as by patient or parental verbal confirmation. A 65-item Medication Regimen Complexity Index (MRCI)[1] was obtained for each patient. The primary outcome was discrepant medicine reconciliation events (DMRE) defined as the presence of any discrepancy in drug name, dose or dosing interval for any drug that the patient was taking in that encounter. Multivariate analysis was performed using binary logistic regression to examine the association between the presence of DMRE and demographic/clinical parameters including age, gender, duration of end-stage renal disease (ESRD), patient educational and socioeconomic status and MRCI. Receiver-operating characteristic (ROC) curve analysis was used to determine the MRCI which could predict the likelihood of occurrence of DMRE.
Results and Discussion: 43 patients (19 males and 24 females), with median age 23.0 years (range 8.3-37.4 years), had medication reconciliation performed at a median of 2 (range 1-6) encounters. The median MRCI score was 41 (range 19-80). In total, medication reconciliations were performed at 95 encounters. 30 (31.6%) of these encounters had DMRE. Of these 30 DMRE, 11 (36.7%) involved immunosuppressive drugs, 10 (33.3%) and 19 (63.3%) were patient- and doctor-related errors respectively. 16 (37.2%) patients had at least one DMRE. In these patients, the median number of DMRE per patient was 1.5 (range 1-4). Binary logistic regression showed that male gender (OR 7.8; 95% CI 1.05-58.14) and higher MRCI (OR 1.08, 95% CI 1.01-1.16) were associated with DMRE. There was no significant association with age of patient, duration of ESRD, and patient educational status. The area under the curve (AUC) for MRCI as a predictive factor for the presence of DMRE was 0.78 (95% CI 0.64-0.92). An MRCI score of 33 predicted the presence of DMRE with a sensitivity of 100%, specificity of 44.4%, positive predictive value of 51.6% and negative predictive value of 100%.
Conclusion: Male patients are at risk of DMRE, especially if the medication regimen is complex with multiple medications and dosing. MRCI was a good screening tool to identify patients at risk of DMRE.
National Kidney Foundation, Singapore
References:
[1] *Ann Pharmacothern* 2004;38:1369-76

359.9

Cytokine profiles in children after pediatric kidney transplantation with acute cellular compared to chronic humoral rejection and stabile patients

Nadja Borsum, Thurid Ahlenstiel-Grunow, Lars Pape.
Division of Pediatric Nephrology, Center for Child and Adolescent Medicine, Hannover Medical School, Hannover, Germany.

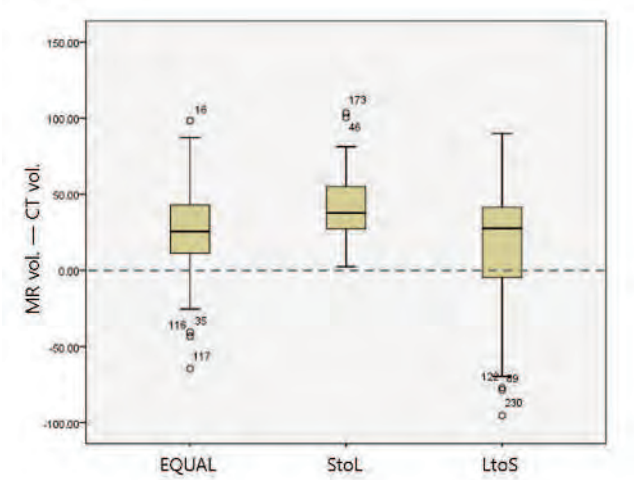
Background: Actually, a diagnosis of acute cellular or chronic humoral rejection after pediatric kidney transplantation (KTX) can only be secured by graft biopsy. Until now, no blood biomarkers have been determined that enable early diagnosis of these two entities. As rejection episodes are associated with an adoption of B- and T-cellular immunity, changes in plasma cytokines can be expected.
Patients and Method: Heparinized blood samples from 24 pediatric kidney transplant patients (11 f, mean age 12.2 ± 4.5 years) were analyzed for interleukin (IL)-2, IL-4, IL-6, IL-10, IL-17A, TNFa und IFNy by flow cytometric analysis (FACS Verse, CBA Human Th1/Th2Th17 Cytokine Kit, BD Biosciences) at time of renal protocol biopsy (6 months after KTX) or indication biopsy. The results of 10 children with acute cellular rejection BANFF ≥ Ia (4 f, mean age 13.8 ± 4.0 years) and 5 children with biopsy proven chronic humoral rejection (3 f; mean age 13.5 ± 3.5 years) have been compared with those of 9 children with baseline creatinine and normal protocol biopsy (4 f, mean age 9.6 ± 4.8 years).
Results: In patients with acute rejection BANFF ≥ 1a, IL-6 concentration was significantly higher (median: 7.85 pg/ml range: 4.87-13.81) than in the control group (median: 3.05 pg/ml range 1.56-5.55, p=0.0007). In contrast, the IL-6 concentration in patients with chronic humoral rejection was significant decreased (median 1.96 pg/ml range 1.03-2.42) compared to the control group (p=0.05) and to the patients with acute rejection (p =0.0007). Measurements of IL-10 concentration showed the same tendency: increased IL-10 concentrations could be detected in patients with acute rejection (median 1.90 pg/ml range: 0.27-20.24 pg/ml, p=0.13) and decreased levels of IL-10 in patients with chronic humoral rejection (median: 0.10 pg/ml, range: 0.00-1.80, p=0.88) compared to the control group (median 0.85 pg/ml, range 0.04-6.86). For IL-2, IL-4, IL-17A, TNFa and IFNy, no differences could be detected between the groups.
Conclusion: The cytokine-analyses in pediatric kidney recipients resulted in elevated levels for IL-6 and IL-10 in children with biopsy proven acute rejection BANFF ≥ Ia compared to controls. In contrast, patients with chronic humoral rejection showed low IL-6 and IL-10 levels. The differences in the IL-6 and IL-10 profile between acute cellular rejection, chronic humoral rejection and controls could be a useful marker to substantiate suspicion of rejection before biopsy.

370.1

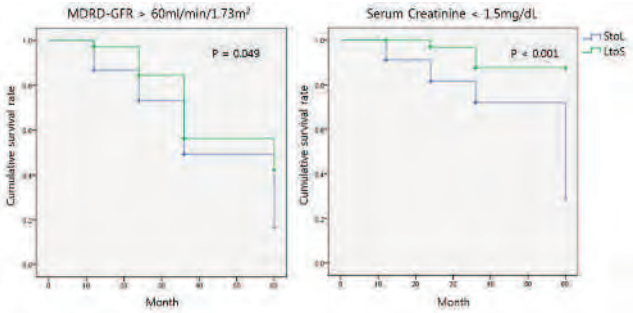
Clinical impact of graft volume change after body weight mismatched kidney transplantation: Based on data of postoperative magnetic resonance volumetry

Mi-Hyeong Kim, Kang-Woong Jun, Hyun-Kyu Kim, Young-Hwa Kim, Kyungjai Ko, Jeong-Kye Hwang, Sang-Dong KIm, Sun-Cheol Park, In-Sung Moon, Ji-Il Kim.
Department of Surgery, College of Medicine, The Catholic University of Korea, Seoul, Korea.

Introduction: Graft volume is an important prognostic factor after kidney transplantation (KT) for representing nephron mass. Optimal graft volume is relative to recipient. Several studies reported preoperative graft volume measurement using CT or MR image, however there is few report about a change of graft volume after KT. We aimed to investigate the change of graft volume after KT between donor and recipient with significant difference in body weight (BW) and to analyze clinical impact of this change.
Methods: We retrospectively analyzed medical records of patients who were undertaken living donor KT, from September 2009 to July 2014. Enrolled patients were divided into three groups according to difference of BW between donor and recipient; EQUAL – BW difference is less than 10kg, StoL (Small-to-Large) – small donor to large recipient with BW difference over 10kg, LtoS (Large-to-Small) - large donor to small recipient with BW difference over 10kg. Donors took CT angiography to evaluate kidney volume preoperatively. We measured real graft weight during the surgery. Postoperative graft volume was assessed using MR angiography at 13th day after KT. To confirm a reliability of MR data, we compared consistency of these three kinds of data and assessed graft volume change after KT among three groups. We evaluated outcome on the basis of MDRD-eGFR > 60ml/min/1.73m2 and serum creatinine < 1.5mg/dl.
Results: Total 236 patients were included this study. CT volume, intraoperative weight and MR volume were well correlated and CT volume was the least and MR volume was the greatest in most cases. The increase of volume between MR and CT, the StoL group was greatest with 41ml, in contrast to EQUAL and LtoS group was 20 and 27ml, and it was statistically significant. (p=0.004 and p<0.001, respectively) The difference of volume between MR and CT had wide variation in EQUAL and LtoS groups with negative and positive values, while every cases of StoL group had positive value (Figure 1).



StoL group, however, had worse graft function than LtoS group in both MDRD-eGFR and serum creatinine (Figure 2).



Discussion: MR angiography can give information about arterial stenosis and perfusion defect which frequently failed to detect by duplex ultrasonography. MR can find complications such as lymphocele and hematoma seated in deep pelvic and retroperitoneal space. This study gives additional information about graft volume, so we planned to investigate usefulness of this data. Gadolinium using MR evaluation is not nephrotoxic but can increase a risk of systemic fibrosis in patient with impaired renal function. We concerned about this, so we checked MR angiography on 13th postoperative day after recipients getting recovered renal function.
Conclusions: Graft volume increment after KT does not mean an increase of renal function, so it is not suitable to estimate graft outcome.

370.2

Long term, single centre experience with simultaneous aortoiliac allogenic reconstruction and kidney transplantation

Tomas Marada[1], Tomas Rohal[2], Libor Janousek[1], Jiri Fronek[1], Ondrej Viklicky[2].
[1]Transplant Surgery, IKEM, Prague, Czech Republic; [2]Department of Nephrology, IKEM, Prague, Czech Republic.

Chronic renal failure is associated with accelerated atherosclerosis. Servere aortoiliac atherosclerosis is often considered to be a relative contraindication for kidney transplantation. In management of those patients should be discussed possibility of simultaneous aortoiliac reconstruction and kidney transplantation. We refer our 18-year experience with aortoiliac reconstruction using fresh arterial allograft and renal transplantation as one-stage procedure.
From 1997 till 2015 we collected data from 15 dialyzed patients treated with aortoiliac arterial reconstruction with simultaneous kidney transplantation. There were 11 men and 4 women with average age 53 +/- 8,6 years. Main reason of renal failure was chronic pyelonephritis (47%). We performed 9 aortofemoral and 6 iliac reconstrunctions with fresh arterial aortoiliac allograft as conduit for consecutive renal transplantation. Mean CIT was 11,8 +/- 4,6 hours. Allograft and kidney graft were obtained from the same brain dead donor. Average age of donors was 33,8 +/- 11 years. Follow up was 2 till 18 years.
Patency of aortoiliac allogenic reconstruction was 100%. 5-year patient survival was 87%. 1 and 5 year graft survival was 87% resp. 73%. Average serum creatinine in 1, 3, 6 months and one year after transplantation was 156, 115, 137 resp. 123 umol/l. DGF was in 2 cases (13%). Lower limb amputation was occured in 2 cases (13%). There was no case of higher limb amputation.
Simultaneous aortoiliac reconstruction and kidney transplantation is safe and useful advance in specific group of dialyzed patients with sever aortoiliac atherosclerosis. Our experiences present succesful surgical treatment of peripheral artery disease and renal failure in one-stage procedure as a chance for patients excluded from an other transplant centres.

370.3

Laparoscopic donor nephrectomy: A comparison of retroperitoneoscopic versus transperitoneoscopic approach

Zi Qin Ng[1], Gabrielle Musk[2], Bulang He[1].
[1]WA Liver & Kidney Transplant Service, Sir Charles Gairdner Hospital, Perth, Australia; [2]Animal Care Service, University of Western Australia, Perth, Australia.

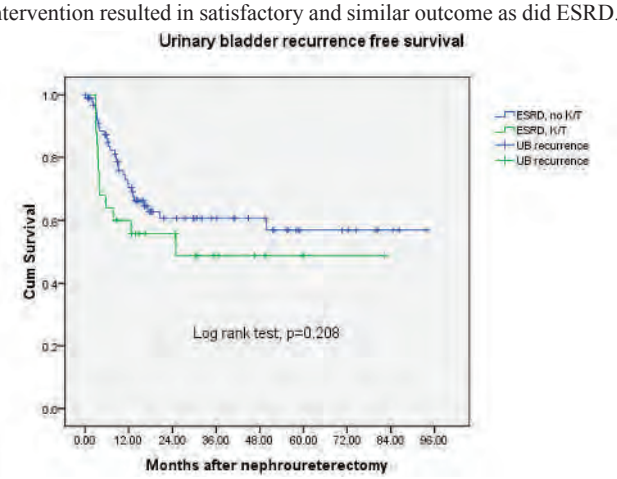
Introduction: Laparoscopic donor nephrectomy has become the standard of care for live kidney donors due to multiple benefits. The techniques include Retroperitoneoscopic Approach (RA) and Transperitoneoscopic Approach (TA). The aim of this study is to determine the superiority of RA versus TA.
Materials and Methods: From January 2010 to December 2015, 93 live donor nephrectomies were performed without hand assistance with both techniques. Of 93, 37 were RA (RA group) whereas 56 were TA (TA group). Donor age ranged from 26 to 75 years (mean 51 years). The variables of peri-operative complications, operative time, the kidney warm ischaemic time (WIT) and length of hospital stay (LOS) were analyzed. All patients received patient-control analgesia for the first 24 hours after surgery. Oral analgesia was then administered. All forms of analgesia were converted to a morphine equivalent for comparison. The average recipient age was 40.3 years (2 to 83 years). 49 recipients underwent pre-emptive kidney transplantation. The graft function and urological complications were also analyzed. $P<0.05$ is considered significant.
Results: All donor nephrectomies were completed successfully with no conversions. There was no blood transfusion and no intra-operative complications were observed in either group. There was no significant difference in operating time, graft WIT (5.3±1.5 vs 4±1.3 minutes), LOS (3.5 ±1.4 vs 3.5±1.1) and requirements for analgesia between RA and TA. Three cases developed mild chyle leakage in the RA group and recovered with conservative management. In the recipients, all kidneys were transplanted successfully and followed-up for between 2 and 61 months. There was no delayed graft function or urine leakage in either group. One recipient developed ureteric stenosis in TA group and required surgical urinary tract reconstruction. Both the patient and graft function recovered well. The mean creatinine level of recipients was not different between the two groups at 1, 3 and 12 months post-operatively.
Conclusion: RA delivers the same outcomes for donor and recipient as TA. Most importantly, RA obviates the entrance into the intra-abdominal cavity and disturbance of the bowel. Therefore, it ultimately prevents the potential risk of adjacent organ injury whilst maintaining a virgin abdomen for the donor.

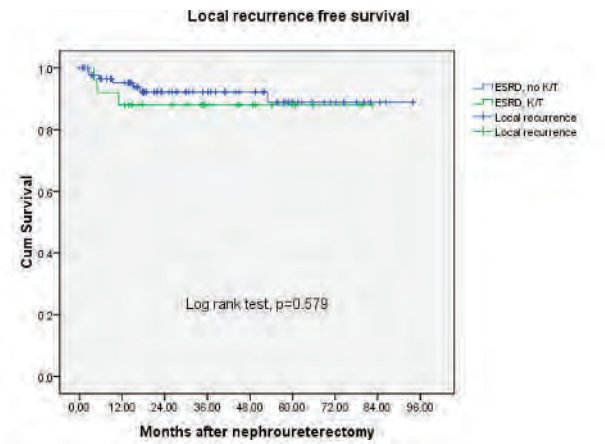
370.4

The upper urinary tract urothelial carcinoma behaviors after kidney transplantation in Taiwan

Chia-Shen Chien, Hao Lun Luo, Yen-Ta Chen, Yuan Tso Cheng, Chou Shu Ling, Po-Hui Chiang.
Department of Urology, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan.

Purpose: To determine upper urinary tract urothelial carcinoma (UT-UC) behavior in patients with end-stage renal disease (ESRD) after kidney transplantation (KT).
Methods: From 2005 to 2012, 117 ESRD patients with localized UT-UC underwent nephroureterectomy at our institution. We divide these patients into two groups according to having had kidney transplantation or not. Kaplan Meier survival plot and multivariate analysis were used to evaluate whether kidney transplantation interfered with the UT-UC outcome.
Results: The mean age was younger in ESRD with KT group ($p=0.001$), but there was no difference in gender, smoking, or previous bladder cancer history between these two groups. The pathological features were more aggressive in group 1 compared with group 2 in regard to incidence of carcinoma in situ ($p=0.025$) and multifocality ($p=0.001$). Kidney transplantation did not interfere with the oncological outcome of UT-UC in ESRD patients. There were no differences in oncological outcome. 5-year metastasis free survival rate was comparable.
Conclusions: In our sample, for patients with ESRD after kidney transplantation, the postoperative onset of UT-UC was younger and the pathological features were more aggressive. Standard surgical intervention resulted in satisfactory and similar outcome as did ESRD.





References:
[1] Wu MJ, Lian JD, Yang CR et al. High cumulative incidence of urinary tract transitional cell carcinoma after kidney transplantation in Taiwan. *Am J Kidney Dis*. 2004; 43(6):1091-1097
[2] Zhang B, Shen C, Han WK et al. Comparison of clinicopathologic characteristics of urothelial carcinoma between patients after renal transplantation and on dialysis. *Transplantation* 2014; 98(5):552-556
[3] Chiang YJ, Yang PS, Wang HH et al. Urothelial cancer after renal transplantation: an update. *Transplant Proc*. 2012; 44(3):744-745
[4] Li WH, Chen YJ, Tseng WC et al. Malignancies after renal transplantation in Taiwan: a nationwide population-based study. *Nephrol Dial Transplant*. 2012; 27(2):833-839
[5] Wu CF, Chang PL, Chen CS et al. The outcome of patients on dialysis with upper urinary tract transitional cell carcinoma. *J Urol*. 2006; 176(2):477-481
[6] Stewart JH, Vajdic CM, van Leeuwen MT et al. The pattern of excess cancer in dialysis and transplantation. *Nephrol Dial Transplant*. 2009; 24(10):3225-3231
[7] Liu GM, Fang Q, Ma HS et al. Distinguishing characteristics of urothelial carcinoma in kidney transplant recipients between China and Western countries. *Transplant Proc*. 2013; 45(6):2197-2202
[8] Kang CH, Chen CH, Chiang PH. Primary urothelial carcinoma of the upper urinary tract in dialysis patients with 5-year follow-up. *Jpn J Clin Oncol*. 2010; 40(3):241-246

370.5

Comparison of baseline co-morbidity and mortality in living donors – 10 year UK cohort study

Nithya Krishnan[1], Lisa Bradbury[2], Neil Raymond[1].
[1]Renal Unit, University Hospitals Coventry & Warwickshire NHS Trust, Coventry, United Kingdom; [2]Statistics, National Health Services Blood & Transplant, Bristol, United Kingdom.

Introduction: Living kidney donation has significantly improved recipient and graft survival world wide. With a move to increase these numbers further, it becomes mandatory to have a better understanding of the long term outcomes and risks of kidney donation.
Aim: To investigate the effect of baseline co-morbidity on all-cause and mortality in kidney transplant living donors – 10 year follow up.
Methods: National Health Service and Blood and Transplant, U.K (NHSBT), obtains informed consent from all patients undergoing a transplant in the UK for continuing data collection and subsequent analyses. The study protocol was reviewed and passed by the Renal Registry (RR) projects advisory group, UK. From January 1, 2001 until December 31, 2013 inclusive, all live kidney donors were included in the study. No formal sample size estimate was produced for the study; all eligible patients records were used. December 31, 2014 was considered the study end, meaning that all patients had at least one year of follow-up. Datasets, based on regular returns from individual transplant centres across the UK, were obtained from NHSBT.
Results: There were 9750 live donor records available. All cause mortality and baseline comorbidity was analysed. 9043 donors had no baseline co morbid condition. The baseline comorbidities analysed were kidney stones (9), microscopic haematuria (9), angina/ischemic heart disease (6), CABG (4), CVA/TIA (5), hypertension (HT) (220), HT on more than 3 medications (1), HT with left ventricular hypertrophy (12). diabetes (2), depression (33), asthma (130), hypercholesterolemia (22) and different BMI bands, BMI <18.5 (82), 18.5 -<25 (3208), 25-<30 (4270), 30-<35 (1449), 35-<40 (158), 40+ (28). There were 48 deaths in total; out of which 2 had baseline HT (2/220), 3 had unspecified comorbidities (3/258) ; and 43 deaths had occurred in the group which had no baseline co-morbidities in the 10 year follow up period. Cox proportional hazards regression modelling, showed no individual baseline comorbidity or "any" combined baseline comorbidity to be a significant predictor of mortality over the follow-up period.
Conclusion: There is no significant association between baseline co-morbidities and mortality in living donors in the 10 year follow up UK cohort study.

370.6

Effectiveness of three-dimensional image analysis for surgical simulation of living donor kidney transplantation with multiple renal artery grafting

Koichi Tomita, Yuki Nakamura, Takayoshi Yokoyama, Naokazu Chiba, Osamu Konno, Yu Kihara, Hitoshi Iwamoto, Shigeyuki Kawachi.
Digestive and Transplantation Surgery, Tokyo Medical University Hachioji Medical Center, Tokyo, Japan.

Background: There are several strategies for the surgical management of kidney transplantation with multiple renal arteries grafting in different institutions. At our center, we have tried to reconstruct as many side arteries as possible, particularly the inferior polar artery that is responsible for ureter perfusion. Small side arteries, which are difficult to reconstruct and perfuse less than 10% of the kidney graft, are sacrificed. However, perfusion areas are based on subjective observations during perfusion in bench surgery. This technique is not very objective and reliable, and there is no available method to evaluate the perfusion area for each renal artery in detail. In the present study, we evaluated the perfusion area of each main and side artery involved in multiple artery kidney grafting using a three-dimensional (3D) image analysis system and determined the validity of the conventional rule regarding the reconstruction of multiple renal arteries at our institution.
Methods: We enrolled 38 consecutive patients who had undergone living kidney transplantation with multiple renal artery grafting. The transplantation procedures were performed at Tokyo Medical University Hachioji Medical Center between 2006 and 2015. All donors underwent dynamic enhanced computed tomography (CT) before transplantation for evaluation of the kidney graft. The CT images were obtained using 64 multidetctor-row CT with a 1.25 mm interval. All CT images were retrospectively analyzed using a 3D image analysis system called Synapse Vincent® (Fuji Medical Systems, Inc., Tokyo, Japan). The volume of the kidney, number of renal arteries, diameter of each artery, and perfusion area for each artery [both volume (mL) and proportion (%)] were assessed in detail. We used the following reconstruction methods: conjoined, side-to-end, individual, simple ligation, or a combination of them.
Results: All grafting procedures were performed using the left kidney of donors. The number of renal arteries was two in 33 patients, three in four, and four in one. The side arteries were classified according to the individual anastomosis method used. There was no significant difference in diameter among the side arteries ($p = 0.062$). The perfusion area for side arteries was statistically equal among the conjoined (42.5 mL, 24.2%), side-to-end (30.8 mL, 18.5%), and individual methods (37.0 mL, 20.5%). On the other hand, the perfusion area for the simply ligated side arteries was 10.3 mL, 6.1%; this was significantly different from the areas obtained with other methods ($p < 0.001$). Considering it was less than 10%, it was concordant with our arterial reconstruction rule. No artery-related complications were observed in our study.
Conclusions: In living donor kidney transplantation, the advantage of using a 3D image analysis system is an accurate visual display of the construction of the renal artery, vein, and ureter and their relationships before surgery. Furthermore, the perfusion area for each artery can be calculated, and the necessity of arterial reconstruction can be predicted preoperatively. These features will promote safe kidney transplantation surgery without complications.

371.1

Which is the predictable values of post-transplant HCC recurrence in patients received down staging therapy or bridging therapy?

Seung Hwan Song, Jee Youn Lee, Su-Kyung Kwon, Jae Geun Lee , Juhan Lee, Dai Hoon Han, Gi Hong Choi, Soon Il Kim, Myoung Soo Kim, Myoung Soo Kim, Dong Jin Joo.
Department of Surgery and The Research Institute for Transplantation, Yonsei University College of Medicine, Seoul, Korea.

Introduction: Liver transplantation (LT) is one of the best treatment for small hepatocellular carcinoma (HCC) in patients with liver cirrhosis. However, many patients do not have available donor. For that reason, many patients received the bridging therapy such as TACE, RFA, and surgical resection during the waiting LT. Furthermore, some patients need the down staging therapy for LT. The aim of this study is to evaluate the predictable values of recurrent HCC in patients received other treatment modality before LT.
Methods: The 261 recipients with HCC who underwent liver transplantation between January 2007 and December 2014 in Severance hospital were retrospectively reviewed. Among 261 patients, 189 patients received the other treatment such as TACE, RFA, chemotherapy, and surgical resection before LT.

Study Characteristics	Univariate OR(95% CI)	p-value	Multivariate OR(95% CI)	p-value
Milan Criteria at diagnosis				
Milan criteria at transplantation				
AFP≥200 at diagnosis				
AFP≥200 at transplantation				
PIVKA≥100 at diagnosis				
PIVKA≥100 at transplantation				
Tumor marker				
AFP≥200 → AFP<200				
PIVKA≥100 → PIVKA<100				
AFP<200 → AFP≥200				
PIVKA<100 → PIVKA≥100				
Within Milan criteria → Above Milan criteria				
Above Milan criteria → Within Milan criteria				

	No HCC recur (n=153)	HCC recur (n=36)	p-value
Age(mean ± SD)			
Sex (M) %			
Etiology (%)			
HBV			
HCV			
HBV + HCV			
Alcohol			
Other			
Above Milan Criteria at diagnosis			
Above Milan Criteria at transplantation			
AFP ≥ 200 at diagnosis			
PIVKA ≥ 100 at Diagnosis			
AFP ≥ 200 at transplantation			
PIVKA ≥ 100 at transplantation			
AFP ≥ 200 → AFP ≥ 200			
AFP ≥ 200 → AFP < 200			
PIVKA ≥ 100 → PIVKA ≥ 100			
PIVKA ≥ 100 → PIVKA < 100			

The Results: The mean age of the HCC recurrence group was younger than non-recurrence group (51.2 ± 6.1 vs 54.6 ± 6.9, $p<0.006$). There was no significant difference of the etiology of HCC between the groups. The patients above Milan criteria showed a higher tumor recurrence rate than those within Milan criteria in both diagnosis and transplantation. In univariate analysis, Milan criteria at first diagnosis,

Milan criteria at liver transplantation, AFP≥200 ng/mL at first diagnosis, AFP≥200 ng/mL at liver transplantation, PIVKA≥100 mAU/mL at liver transplantation affected HCC recurrence. In multivariate analysis, Milan criteria at liver transplantation and PIVKA≥100 ng/mL at liver transplantation affected HCC recurrence. (Odds ratio 3.632 and 5.245, respectively)
Conclusion: PIVKA ≥100 ng/mL and Milan criteria at transplantation are predictable value of post-transplant HCC recurrence in patients received down staging therapy or bridging therapy.

371.2

Ischemic cholangiopathy is the only biliary complication that impacts outcome following donation after cardiac death liver transplantation

Shiva Kumar.
Center for Liver Disease & Transplantation, Aurora St. Luke's Medical Center, Milwaukee, WI, USA &, Digestive Disease Institute, Cleveland Clinic Abu Dhabi, Abu Dhabi, United Arab Emirates.

Donation after cardiac death (DCD) allografts offer the potential of increasing the donor pool for liver transplantation (LT). However, their utilization has been limited by concerns regarding increased morbidity and mortality, primarily due to a higher incidence of biliary complications (BC). A wide spectrum of BC has been reported to occur following DCD LT. However, the relative impact of the various types of BC on graft survival is unclear. Our aim was to determine the frequency and relative impact of the various types of BC following DCD LT on graft survival.
Consecutive patients undergoing LT at a single center over a 7 year period from July 2007- Feb 2014 were included in the analysis. All DCD procurements were done by the same team using standard protocol. Patients undergoing DCD LT were compared to those undergoing donation after brain death (DBD) LT during the same period. The following variables were analyzed: donor age, warm and cold ischemic time, recipient age, MELD score, HCV status, presence of hepatocellular carcinoma (HCC). Ischemic cholangiopathy (IC) was defined as diffuse strictures affecting the allograft biliary system in the absence of hepatic artery thrombosis or stenosis. Modeling for the impact of the various types of BC on graft loss was done to evaluate for an interaction between DCD status and BC to see how they influence graft loss. Categorical variables were analyzed using Chi-square test and continuous variables using Student t-test. Time to event comparison was performed using Kaplan-Meier method.
A total of 196 patients underwent LT during the study period. Among these, 61 (33%) underwent DCD LT. The DCD and DBD groups were comparable with respect to all baseline variables, except median MELD at LT, which was higher in the DBD group (22.4 vs. 28; $p<0.01$). Overall 56 (28.5%) patients developed BC. Among DCD recipients, anastomotic stricture (AS; 42.9%) and bile leak (32.1%) were the commonest type of BC. Others included IC (17.9%), biliary cast disease (3.6%) and isolated intrahepatic small duct disease (3.5%).
Using competing risk analysis to determine incidence, there was trend towards higher risk of BC at 1 year among DCD recipients (21.5% vs. 35.3%; $p = 0.10$). Phreg Models looking at impact of BC on predicting graft survival showed that BC was associated with an increased risk of graft loss at 1 year in DCD compared to DBD recipients (DBD 0.9% vs. DCD 7.8%; $p = 0.02$). Overall, patients undergoing DCD LT that developed BC had a nearly 3-fold increased risk of graft loss compared to DBD recipients. (HR (95% CI): 2.97 (1.42-6.23); $p <0.01$). Modeling, using AS as a reference, to examine which types of BC may increase risk for graft loss compared to others, showed that IC was the only type BC associated with risk of graft loss following DCD LT. IC had an almost 4-fold greater risk of graft loss than AS following DCD LT (HR (95% CI): 3.92; 1.19-12.98, $p=0.02$).
Even though there is an increased frequency of BC after DCD LT, only IC appears to adversely affect graft survival. The pathogenesis of IC in DCD LT is unclear and is presumably due to the effects of warm ischemia on the peribiliary vascular plexus. Strategies to lower the likelihood of development of IC could significantly improve outcomes following DCD LT.

371.3

Impact of donation after cardiac death allografts on outcome following simultaneous liver kidney transplantation

Shiva Kumar.
Center for Liver Disease & Transplantation, Aurora St. Luke's Medical Center, Milwaukee, WI, USA &, Digestive Disease Institute, Cleveland Clinic Abu Dhabi, Abu Dhabi, United Arab Emirates.

Extended criteria donors, including donation after cardiac death (DCD) organs, offer the potential of increasing the available donor pool in liver transplantation (LT). Kidney dysfunction is common in patients with liver failure awaiting transplantation. The number of simultaneous liver kidney transplants (SLK) has increased substantially in the MELD era, mandating the consideration of extended donor grafts for SLK, including DCD. Although the use of DCD donor organs has been shown to be a viable option for kidney transplantation, limited data is available regarding outcomes of SLK using DCD donors. Our aim was to compare outcomes following DCD and donation after brain death (DBD) transplantation in patients undergoing SLK and to determine predictors of outcome.

We performed a retrospective analysis of all liver transplants performed over a 7 year period at a single center (July 2007 - Feb 2014), to compare the outcomes of DCD SLK to DBD and determine the impact of donor and recipient factors on allograft and patient survival. The following donor and recipient variables were analyzed: donor age, warm and cold ischemic time, recipient age, MELD score at LT, hepatitis C (HCV) status, and presence of hepatocellular carcinoma (HCC). Categorical variables were analyzed using Chi-square test and continuous variables using Student's t-test. Survival comparison was performed using Kaplan-Meier method and multivariate analysis of predictors of outcome using Cox regression method.

A total of 196 patients underwent liver transplantation over the study period, of which 33 (20.2%) underwent SLK. All DCD procurements were done by the same team of surgeons using standard protocol. Among the SLK recipients, 23 (Group 1) and 10 patients (Group 2) respectively received DBD and DCD allografts. The outcomes of these two groups were retrospectively compared. The DCD and DBD recipients were comparable with respect to age, gender, HCV status, and presence of HCC. The median MELD score was significantly higher in the DBD group (28 vs. 22.4; p less than 0.01). Liver allograft survival was comparable between DBD and DCD LT recipients of SLK (p= 0.82). Estimates for graft survival among the 2 groups were as follows: 1 year (64% vs. 55.6%), 3 years (64% vs. 55.6%) and 5 years (57.6% vs. 55.6%). Although there was a trend toward decreased graft survival among DCD SLK recipients, this did not reach statistical significance. Multivariable and univariate analysis identified DCD recipients > 50 years old of HCV donors as the only predictor of outcome among SLK recipients following DCD LT (Hazard Ratio (95% CI): 5.08 (1.5-17.19); p = 0.0089).

DCD grafts offer the potential to safely expand the donor pool for recipients of SLK without compromising liver allograft and patient survival. Limiting DCD SLK transplants to non-HCV recipients from younger donors may enable further expansion of the donor pool without compromising graft and patient survival. Although long-term outcomes remain unclear, the utilization of DCD organs should be considered a valid approach to safely expand the donor organ pool in the subset of patients undergoing SLK.

371.4

Ex vivo subnormothermic oxygenated liver perfusion just before liver transplantation from non-heart-beating donors might improve graft viability, in pig model

Yuta Kakizaki, Shigehito Miyagi, Kenji Shimizu, Hiroyuki Kumata, Muneyuki Matsumura, Yuki Miyazaki, Kengo Fukuoka, Satomi Suzuki Uematsu, Yasuyuki Hara, Chikashi Nakanishi, Naoki Kawagishi, Noriaki Ohuchi.
Division of Advanced Surgical Science and Technology, Graduate School of Medicine, Tohoku University, Sendai, Japan.

Introduction: The shortage of donors has become a big problem. Liver transplantation (LTx) from non-heart-beating donor (NHBD) is an effective way to enlarge donor source. However, primary graft non-function and some complications are often caused. We previously reported that short oxygenated warm perfusion (SOWP) before cold storage improved graft function and survival in rat model of marginal donors. In this study, we investigated the effectiveness of short-term subnormothermic oxygenated perfusion just before LTx from NHBD in pigs.

Objectives: The aim of this study is to investigate the effect of subnormothermic oxygenated liver perfusion just before LTx from NHBDs.

Materials and Methods: White landrace pigs, weighing 25-30kg, underwent LTx. Pigs were divided into three groups: (1) the heart-beating (HB) group (n = 5), whose livers were retrieved from HB donors and after 4 hours cold storage in UW solution, grafts were transplanted; (2) the NHB group (n = 5), whose livers were retrieved 20 minutes after cardiac arrest and after 4 hours cold storage, grafts were transplanted; and (3) the ex vivo liver perfusion (EVLP) group (n = 3), whose livers were retrieved the same manner as the NHB group and after 4 hours cold storage, those grafts were perfused by subnormothermic oxygenated Krebs-Henseleit buffer (room temperature about 25℃, 10-15cmH2O for 30 minutes in dripping manner, not machine perfusion. The grafts were transplanted orthotopically.

Results and Discussion: In the HB group, all five recipients survived for more than seven days but in the NHB group, all five cases died within 24 hours. In the EVLP group, two recipients survived for more than seven days and one recipient survived five days. The survival rate was significantly better in the EVLP group than that in the NHB group (p =0.0082, Kaplan-Meier log-rank test). On the other hand, aspartate aminotransferase (AST) in the EVLP group was significantly higher than that in the HB group until 3 postoperative day (POD), but there were no significant differences between those two groups in 7 POD. On the historical examination, the EVLP group in 7 POD showed well-preserved sinusoidal endothelial cells.

Ischemia-reperfusion injury causes AST elevation in the EVLP group until 3 POD. However, Subnormothermic oxygenated perfusion does not cause graft failure and might be considered to reduce oxygen consumption and allow a minimum level of cellular metabolic activity, which leads to protect sinusoidal endothelial cells and support the recovery of microcirculation.

Conclusion: Ex vivo subnormothermic oxygenated perfusion just before liver transplantation might improve graft function and the survival rate.

References:

[1] K Miyazawa. Edaravone, a Free Radical Scavenger, Improves the Graft Viability on Liver Transplantation From Non-heart-beating Donors in Pigs. *Transplantation Proceedings*. 2014; 46: 1090-1094.
[2] Y Hara. A new liver graft preparation method for uncontrolled non-heart-beating donors, combining short oxygenated warm perfusion and prostaglandin E1. *J Surg Res*. 2013; 184: 1134-1142.
[3] S Miyagi. Effects of serine protease inhibitor and prostaglandin I2 on liver transplantation from non-heart-beating rat donors. *Transplantation Proceedings*. 2008; 40: 2152-2155.

[4] E Gringeri. Subnormothermic Machine Perfusion for Non–Heart-Beating Donor Liver Grafts Preservation in a Swine Model: A New Strategy to Increase the Donor Pool? *Transplantation Proceedings*. 2012; 44: 2026-2028.
[5] T Minor. Controlled Oxygenated Rewarming of Cold Stored Liver Grafts by Thermally Graduated Machine Perfusion Prior to Reperfusion. *Am J Transplant*. 2013; 13: 1450-1460.
[6] N Matsuno. Rewarming Preservation by Organ Perfusion System for Donation After Cardiac Death Liver Grafts in Pigs. *Transplantation Proceedings*. 2014; 46: 1095-1098.
[7] P Dutkowski. Novel Short-term Hypothermic Oxygenated Perfusion (HOPE) System Prevents Injury in Rat Liver Graft From Non-Heart Beating Donor. *Ann Surg*. 2006; 244: 968-977.
[8] S Reddy. Non-Heart-Beating Donor Porcine Livers: the Adverse Effect of Cooling. *Liver Transplantation*. 2005; 11: 35-38.
[9] A Schlegel. Warm vs. cold perfusion techniques to rescue rodent liver grafts. *Hepatology*. 2014; 61: 1267-1275.
[10] D Monbaliu. Livers from Non–Heart-Beating Donors Tolerate Short Periods of Warm Ischemia. *Transplantation*. 2005; 79: 1226-1230.

371.5

ABO incompatible dual graft living donor liver transplantation

Jae Hyun Kwon, Sung-Gyu Lee, Shin Hwang, Ki-Hun Kim, Chul-Soo Ahn, Deok-Bog Moon, Tae-Yong Ha, Gi-Won Song, Dong-Hwan Jung, Gil-Chun Park, Jee Yeon Kim, Min Kyu Sung.
Division of Hepatobiliary Surgery and Liver Transplantation, Department of Surgery, Asan Medical Center , Seoul, Korea.

Introduction: ABO blood group compatibility has been regarded as an essential prerequisite for successful adult LDLT. However, the result of ABO-incompatible (ABOi) LDLT has been remarkably improved owing to novel strategies for overcoming ABO blood group barrier. In this report, we adopted the strategies for overcoming ABO-blood group barrier in dual graft LDLT to cope with ABO-incompatibility and small-for-size graft syndrome.
Method: From January 1, 2008, to July 31, 2014, 17 patients underwent dual graft LDLT by using ABOi and ABO-compatible (ABOc) graft combination and 10 patients received both of dual graft from ABOi donors. Clinical data were collected prospectively and analyzed retrospectively. Regeneration rate of each graft was evaluated and calculated by computed tomography (CT) volumetry.
Results: Mean graft-to-recipient weight ratio (GRWR) was 1.0%. All 17 patients who received combination of ABOc and ABOi grafts were survived and mean follow-up period was 50.3 months. One mortality occured in ABOi dual graft LDLT group during mean follow-up period of 30.6 months. Biliary complications showed no significant difference (p=0.365) between ABOc and ABOi grafts. Regeneration rate of each graft for 1 month, 6 months, and 1 year after transplantation were similar between ABOc and ABOi grafts (p=0.215, 0.158, and 0.340, respectively).
Conclusions: There was no episode of antibody-mediated rejection and biliary complication associated with ABO incompatibility. Dual graft LDLT with ABOi and ABOc graft combination can be the feasible solution for overcoming both ABO-blood group barrier and small-for-size graft syndrome simultaneously.

371.6

Inflammation-based prognostic score in living donor liver transplantation for hepatocellular carcinoma

Norifumi Harimoto, Tomoharu Yoshizumi, Shinji Itoh, Toru Ikegami, Hideaki Uchiyama, Tetsuo Ikeda, Yuji Soejima, Yoshihiko Maehara.
Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

Background: Some inflammation-based prognostic score has been attracting attention as a prognostic factor of malignant tumor, such as neutrophils / lymphocyte ratio (NLR), platelet / lymphocyte ratio (PLR), modified Glasgow Prognostic Score (mGPS), Prognostic Nutritional Index (PNI). We previously reported that the NLR is useful to properly assess the patient without decreasing the long-term survival after Living donor liver transplantation (LDLT). We investigated the significance of inflammation-based prognostic score in (LDLT for Hepatocellular carcinoma (HCC).
Methods: Among 213 cases who underwent LDLT for HCC from 1997 until May 2015, 11 cases who died within three months were excluded. Outcomes in 190 patients who has an observation period for more than 6 months were analyzed retrospectively. DFS (disease-free survival) rates after LDLT were calculated, and we divided into the recurrence group and the non-recurrence group and compares NLR, PLR, PNI and mGPS. The risk factors for tumor recurrence were identified in univariate and multivariate analysis.
Results and Discussion: Recurrence we have observed in 28 cases (14.7%). The 1-, 3-, and 5-year DFS rates after LDLT were 92.7%, 85.9%, and 85.1%, respectively. NLR, PLR was significantly higher in the recurrence group. NLR is AUC 0.647, sensitivity 64.29%, and specificity 66.07% of the cut-off 2.66. PLR is AUC 0.704, sensitivity 85.71%, specificity 53.85% of the cut-off 70.44. Univariate analysis showed that DFS was poor significantly prognosis in alpha-fetoprotein concentration ≥300 ng/ml, DCP concentration ≥300 mAU/ml, NLR≥2.66, PLR≥70.44, Platelet count≥4x104/μl, CRP>0.27mg/dl, tumor number ≥5, tumor size ≥5 cm, duration of last treatment of HCC to LDLT <3 months, biolobar distribution and exceeding Milan criteria. Multivariate analysis showed that DCP concentration ≥300 mAU/ml, NLR≥2.66, duration from last treatment to LDLT <3 months were the independent predictor of DFS. Among preoperative factor of inflammation-based prognostic score, NLR was the independent predictor of DFS.
Conclusion: Preoperative NLR in LDLT for HCC is useful as a recurrence predictor.
References:
[1] Mano Y, Shirabe K, Yamashita Y, Harimoto N, Tsujita E, Takeishi K, Aishima S, Ikegami T, Yoshizumi T, Yamanaka T, Maehara Y. Preoperative neutrophil-to-lymphocyte ratio is a predictor of survival after hepatectomy for hepatocellular carcinoma: a retrospective analysis. *Ann Surg* 2013;258(2):301-5.
[2] Harimoto N, Shirabe K, Nakagawara H, Toshima T, Yamashita Y, Ikegami T, Yoshizumi T, Soejima Y, Ikeda T, Maehara Y. Prognostic factors affecting survival at recurrence of hepatocellular carcinoma after living-donor liver transplantation: with special reference to neutrophil/lymphocyte ratio. *Transplantation* 2013;96(11):1008-12
[3] Motomura T, Shirabe K, Mano Y, Muto J, Toshima T, Umemoto Y, Fukuhara T, Uchiyama H, Ikegami T, Yoshizumi T, Soejima Y, Maehara Y. Neutrophil-lymphocyte ratio reflects hepatocellular carcinoma recurrence after liver transplantation via inflammatory microenvironment. *J Hepatol* 2013 ;58(1):58-64.
[4] Yoshizumi T, Ikegami T, Toshima T, Harimoto N, Uchiyama H, Soejima Y, Yamashita Y, Shirabe K, Maehara Y. Two-step selection criteria for living donor liver transplantation in patients with hepatocellular carcinoma. *Transplant Proc* 2013;45(9):3310-3.

372.1

Significant changes in the organ donor pool over the last 10 years: Donors from anoxic cause of death (COD) more than double which includes a 300% increase in donors with a drug overdose

Richard Hasz, Howard Nathan, Sharon West.
Gift of Life Donor Program, Philadelphia, PA, United States.

Purpose: This study characterizes one OPO's expanding anoxic donor pool and donation outcomes over a 10 year period.
Methods: Characteristics examined: cause (COD) and mechanism of death (MOD), age, sex, race, and driver's license designation status. We also examined the outcomes: potential donors, actual donors, and donation rates (actual donors as a percentage of potential donors identified).
Results:

Potential Donors, Actual Donors & Donation Rates by Cause of Death
2006 vs. 2015

	2006	2015	% Change
Potential Donors*	813	857	+5.4%
Anoxia	264	460	+74.2%
Cardiovascular MOD	***192***	***308***	***+60.4%***
Drug Overdose MOD	***32***	***102***	***+218.8%***
Cerebrovascular/Stroke	348	224	-35.6%
Head Trauma	189	163	-13.8%
Actual Donors*	401	483	+20.4%
Anoxia	104	244	+134.6%
Cardiovascular MOD	***69***	***136***	***+97.1%***
Drug Overdose MOD	***19***	***81***	***+326.3%***
Cerebrovascular/Stroke	175	131	-25.1%
Head Trauma	117	102	-12.8%
Donation Rates	49%	56%	+14.3%
Anoxia	39%	53%	+35.9%
Cardiovascular MOD	***36%***	***44%***	***+22.2%***
Drug Overdose MOD	***59%***	***79%***	***+33.9%***
Cerebrovascular/Stroke	50%	58%	+16.3%
Head Trauma	62%	63%	+1.1%

**Total Potential and Actual Donors Exceeds Sum of Anoxic, Cerebrovascular/Stroke, and Head Trauma. Other Causes of Death, Like CNS Tumors are Excluded*

Potential Organ Donor Demographics (Cardiovascular and Drug Overdose Deaths)
2006 vs. 2015

	Cardiovascular Deaths		Drug Overdose Deaths	
Demographic	2006 n=192	2015 n=308	2006 n=32	2015 n=102
Mean Age	46 Y	50 Y	29 Y	32 Y
Age Range	r=1.5 Y - 75 Y	r=2 Days - 79 Y	r=15 Y - 50 Y	r=18 Y - 68 Y
Standard Deviation	sd=18.8 Y	sd=18.1 Y	sd=9.5 Y	sd=9.8 Y
Sex	Male (54%)	Male (52%)	Male (56%)	Male (61%)
Race	White (69%)	White (64%)	White (81%)	White (85%)
Designation Rate*	20%	27%	24%	46%

**Based on Refererred Potential Only*

Discussion: There has been a significant increase in both the proportion (32% vs 53%) and number (264 vs 460, 74% increase) of potential donors with anoxic COD. Actual donors also increased in similar proportion (26% vs 51%) and volume (104 vs 244, 134% increase). These increases were driven primarily by an increase in cardiovascular (+60%) and drug overdose (+219%) MOD in anoxic potential donors. Conversion rates in anoxic deaths increased from 39% to 53 % over the 10 year period. Additionally a higher conversion rate was seen in drug overdose MOD vs cardiovascular MOD in both time periods. Potential donors with MOD of drug overdose were more likely to be donor designated, Caucasian and younger.
Conclusion: Organ donors from anoxic COD more than doubled in a ten year period and make up the majority of the organ donor pool. OPOs

need to continually evaluate changes in the donor pool to optimize donation outcomes and to effectively manage donors with anoxic COD. Many of the potential donors with anoxic COD are cared for in medical/cardiac ICUs and require intense evaluation due to comorbities such as HTN, diabetes, and PHS high risk behavior. OPO should make continued efforts to increase conversion rates in the expanding pool of anoxic COD potential donors.

372.2

Can knowledge and attitudes of critical care professionals influence organ donation? - A Malaysian perspective

James William Foong Zhi Wei[1], Wei Loon Oo[1], Jea Sheng Ong[1], Mohammad Moshaddeque Hossain[2], Nirmala Devi Baskaran[3], Hasdy Haron[4], Yuzana Yusop[4], Lela Mansor[4], Raghu Varadarajan[1].
[1]Department of Surgery, Perdana University - Royal College of Surgeons in Ireland, Serdang Selangor, Malaysia; [2]Department of Epidemiology and Public Health, Perdana University - Royal College of Surgeons in Ireland, Serdang Selangor, Malaysia; [3]Department of Nephrology, Hospital Kuala Lumpur, Kuala Lumpur, Malaysia; [4]National Transplant Resource Centre, Kuala Lumpur, Malaysia.

Background: Malaysian organ donation (OD) rates are amongst world's lowest. Whether critical care professionals' (CCPs) knowledge and attitudes relate to brain death (BD), OD and organ transplantation (OT) underlie these low rates remain unstudied.
Objective: Whether tertiary hospital CCPs' knowledge and attitudes related to BD, OD and OT influence decision to offer OD to families of BD patients and CCPs willingness to donate organs.
Methods: Doctors and nurses in the emergency & neurology departments and general & neurosurgical ICUs were surveyed using a validated questionnaire containing 25 knowledge and 16 attitude questions. Sociodemographic data were collected. Data analysed using Stata 14.1 to assess whether sociodemographic, knowledge and attitude variables were associated with respondents' inclination to offer OD and their willingness to donate.
Results: 413 (73%) of 565 participants responded; 163 (39%) doctors & 250 (61%) nurses. Inclination to offer OD associated with BD knowledge tertiles, p<0.001. BD knowledge scores in 3rd and 2nd tertiles (higher scores) had higher inclination to offer OD than 1st tertile (95% vs 76% vs 65%, p<0.001). Inclination to offer OD associated with OD knowledge scores, p<0.001. Of 359 CCPs convinced of BD, 79% inclined to offer OD compared with 43% of 7 unconvinced counterparts, p=0.001. Of 324 CCPs who believed OT is a good form of treatment, 82% inclined to offer OD compared to 55% of 31 who did not believe in OT, p<0.001. Of 290 CCPs who would accept an organ themselves, 82% inclined to offer OD compared with 54% of 43 who would not accept an organ, p<0.001. CCPs unsure of whether their religion objected to deceased OD were less inclined to offer OD (OR=0.46, 95% CI, 0.24-0.91, p<0.026). Chinese (96%) had highest inclination to offer OD followed by Indians (90%), Malays (70%), p<0.001.
Willingness to donate was associated with BD knowledge scores (OR 1.21, 95% CI, 1.11-1.31, p<0.001) and OD knowledge score (OR 1.13, 95% CI, 0.99-1.27, p=0.05). Of 358 CCPs convinced of BD, 72.4% were willing to donate compared with 28.6% of 7 unconvinced counterparts, p<0.001. Of 324 (79.2%) respondents who believed OT is a good form of treatment, 236 (72.8%) were willing to donate compared with 15 (48.4%) who did not believe in OT, p<0.001. 289 (70.5%) of all HCWs would accept an organ themselves of which 225 (77.9%) were willing to donate compared with 48.8% of 43 counterparts who would not accept an organ (p <0.001). CCPs unsure of whether their religion objected to deceased OD were less willing to donate (OR=0.23, 95% CI, 0.12-0.46, p<0.001). Indians (89.8%) had the highest willingness to donate followed by Chinese (86.0%), Malays (60.8%, p<0.001).
Conclusion: Among tertiary hospital CCPs, both knowledge and attitude were associated with inclination to offer OD and willingness to donate organs. Efforts to increase knowledge with changes in attitude may improve organ donation rates.

372.3

Eye donation in Hong Kong: attitudes of family members of deceased patients

Allie Lee[2], Amanda Luk[1], Jessie Lau[1], Karen Lam[1], Catherine Wong[1], Victoria Wong[2].
[1]Eye Bank, Hospital Authority, Hong Kong, Hong Kong; [2]Hong Kong Eye Hospital, Hospital Authority, Hong Kong, Hong Kong.

Introduction: Cornea transplantation is important in restoring vision among patients with corneal diseases. In Hong Kong, there has been a long-standing shortage of cornea. Under the Hospital Authority (HA), 465 patients are waiting for cornea transplants as of 2014. However, the annual average of donated corneas were approximately 240 over the last 10 years.
Informed consent from family members of a potential deceased donor is mandatory for eye donation in Hong Kong. To enhance eye donation in Hong Kong, we need to understand patient and family factors contributing to organ donation.
Objectives:
• To describe the trends in familial consent for eye donation from 2008 to 2014
• To understand attitudes of the families: reasons for consent and refusal
• To identify factors affecting familial consent in eye donation
Methodology: This is a collaboration between the HA Eye Bank and the Hong Kong Eye Hospital. Family members of potential donors approached by Eye Donation Coordinators from 2008 to 2014 were included. Reasons for consent and refusal, sociodemographics of the deceased and the family member approached were reviewed. The data acquired was summarized using descriptive statistics. Multiple logistic regression was employed to examine determinants for donation among individuals approached from 2013 to 2014.
Results and Outcomes: From 2008 to 2014, a total of 1740 cases were identified. The overall consent rate was 36.8% and the consent rate did not significantly change throughout the years (p=0.237).
Among families who consented to donation, the most common reason was "the wish to help others" (86.0%), followed by "respect deceased's choice" (12.6%). Regarding reasons for refusal, the most common was "traditional Chinese culture to keep the body intact" (42.7%), and other reasons included communication problems (e.g. lack of expressed wish of the deceased or consensus in the family) and beliefs of the family (e.g. fear of donation bringing more sufferings or disfigurement to the deceased, mistrust of hospital).
Among 628 patients from 2013 to 2014, 22 patients (3.5%) were registered donors and all family members of registered donors consented to organ donation. Moreover, family members of the decreased were more likely to consent to eye donation when the deceased was female (OR 1.47, p=0.03), with a do-not-resuscitate (DNR) order (OR 2.20, p<0.001) and was admitted to oncology or palliative wards (OR 2.12, p=0.05).
References:
[1] Usanee Reinprayoon, Nipaporn Maneerat, Sunee Chansangpetch, BPH, Somruk Suntibenchakul, Jittapan Chureeganon, Ngamjit Kasetsuwan. Eye Donation Project: Difference Between Donors Versus Refusers, International Journal of Eye Banking, Vol 3, No 1 (2015)
[2] "Better public understanding helps promote organ donation", a joint press release by Hospital Authority and Department of Health, 20th November 2015 (HKT 16:12)
[3] http://www.organdonation.gov.hk/eng/statistics.html

372.4

Knowledge and attitudes regarding brain death and organ donation among young males in the northeastern Thailand

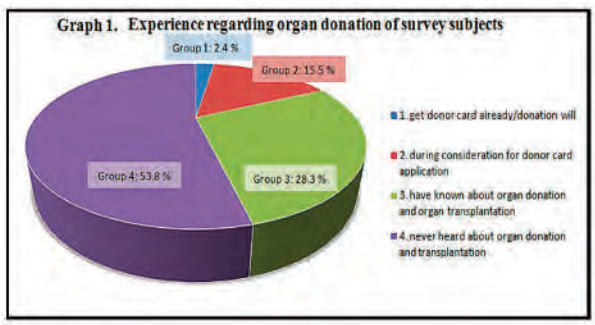
Cholatip Pongskul[1], Jongruk Pongskul[2], Chitranon Chan-on[1].
[1]The Nephrology Division, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand; [2]The Organ transplantation unit, Faculty of Medicine, Khon Kaen University, Khon Kaen , Thailand.

Introduction: The organ donation rate in Thailand was remarkably low with only 3.2 deceased donors per 1,000,000 populations in 2015. Public understanding regarding brain death, organ donation and transplantation affects organ donor rates and the peoples' willingness to donate. The northeastern part of Thailand has the highest deceased organ donation rate in the country. However, there has been little research done to determine public perception of brain death and organ donation in this region. So, the answered questionnaires were reviewed to identify any misunderstanding or negative attitudes in local people.
Materials and Methods: The organ donation campaign exhibition was shown in 15 locations throughout 3 provinces in the northeastern Thailand from July 1st to December 31st 2012. This exhibition was arranged by the Organ Transplantation Unit, Faculty of Medicine, Khon Kaen University. The participants were mostly young male government officers were asked to fill out a self-response questionnaire before receiving correct information about organ donation and brain death policy from well-trained officers.

Figure 1. Demographic data and experience regarding organ donation of survey subjects

Table 1. Demographic data

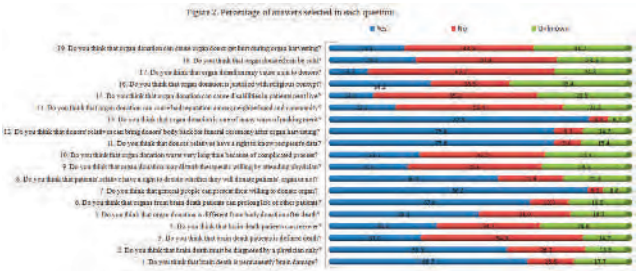
	Number	%
Total subjects	775	100%
Gender		
Male	770	99.4%
Age range		
19-30 years old	769	99.2%
31-45 years old	4	0.5%
46-59 years old	2	0.3%
Education		
1.Lower than master degree	733	94.6%
2 Master degree	41	5.3%
3.Higher than master degree	1	0.1%
Occupation		
1. student	7	0.9%
2. business owner/private	27	3.5%
3. private business employees	47	6.1%
4. bureaucrats/government employee	615	79.3%
5. others	79	10.2%
Marital status		
1. single	690	89.0%
2. married	75	9.7%
3. divorced/seperated	3	0.4%
4. others	7	0.9%



Results and Discussion: There were total 775 subjects who took the survey. 99.4 % were male and 99.2% were between 19-30 years old. Importantly, 54.3% did not feel that brain death truly defined death and 36.3% thought that brain dead patients could recover. 14.8% believed that organ donation could cause disability in the next life and 29.2 % expressed fears that the donated organs may be sold. 26.5 % felt organ

donation is not justified in their religious principles. Critically, our results showed that a high percentage of subject did not trust the donation process, believing it to be a waste of time as well as it might lessen therapeutic willing from physician when the patients turn to the potential donors. Unfortunately, the percentage of subjects in our survey who believed that organ donation could result in organ trafficking (52.7%) was even larger than that shown in the previous survey published in 2002 (34.3%).

Conclusion: While many Thais patients have benefitted from organ transplantation, there is a substantial number of people who has a chance to be organ donors still lack of knowledge about the process, which forms a significant barrier to organ donation.



Somboon Thienthong, MD

References:

[1] Dell Agnolo CM, de Freitas RA, Toffolo VJ, et al. Causes of organ donation failure in Brazil. *Transplant Proc*. 2012 Oct;44(8):2280-2.

[2] Mohamed E, Guella A. Public awareness survey about organ donation and transplantation. *Transplant Proc*. 2013;45(10):3469-71.

[3] Vathsala A. Improving cadaveric organ donation rates in kidney and liver transplantation in Asia. *Transplant Proc*. 2004 Sep;36(7):1873-5.

[4] Newton JD. How does the general public view posthumous organ donation? A meta-synthesis of the qualitative literature. *BMC Public Health*. 2011;11:791.

[5] Anothayanon K, Luanratanakorn P, Teerawattananon Y. Attitude and willingness towards organ donation: the survey for policy recommendations. *Journal of Health Science*. 2002;11:826-35.

372.5

The development and management of China Organ Procurement Organization Alliance

Qifa Ye[1], Xiaoli Fan[1], Yanfeng Wang[1], Francis Delmonico[2], Marti Manyalich[3], Shusen Zheng[4], Jiefu Huang[5].

[1]Zhongnan Hospital of Wuhan University, Wuhan, People's Republic of China; [2]New England Organ Bank, Waltham, United States; [3]Barcelona University, Barcelona , Spain; [4]Zhejiang University, Hangzhou, People's Republic of China; [5]Peking Union Medical College Hospital, Beijing, People's Republic of China.

For the purpose of implementing the Regulations on Human Organ Transplantation and the Regulations on Procurement and Distribution of Organ Donation promulgated by National Health and Family Planning Commission of China (NHFPCC) in August 2013, promoting China Donation after Citizen's Death (CDCD) and realizing healthy, ethical and scientific development of organ transplantation, the National Organ Donation and Transplantation Committee (NODTC) establishes China Organ Procurement Organization Alliance (COPO). COPO strictly abide by the Regulations on Human Organ Transplantation and regulations on organ donation and transplantation; devote itself to industrial management of Organ Procurement Organization (OPO) and scientific norms of CDCD and transplantation, and guarantee that citizen's organ donation is the only source of organ transplantation. COPO is divided into seven regions based on the current situation and geographical factors of organ donation and transplantation in China. COPO responsibilities including: work out the trial measures for industrial norms and scientific management of organ procurement and living related organ procurement; work out a scientific rescue procedure of CDCD donor, a scientific quality assessment system of donated organs and formulate scientific norms of organ procurement; organize industrial experts to work out admittance criteria of OPO staff, OPO responsibilities, application qualifications of OPO medical institute, necessary facility standards of OPO operation and formulate OPO development and quality control standard; organize experts to account necessary costs of organ donation and procurement, and work out charging standard of principle and guidance fee; work out scientific norms and management measures of coordination and operation between OPO and transplant hospital, as well as service scope of OPO and operation code and norms of transplant hospital. A scientific OPO function and management mechanism has been found in China. Medical institute with organ transplantation qualification shall sign a contract with an OPO-qualified medical institute. OPO shall be responsible for organ procurement and ensure that the transplant hospital has priority in using the organ donated, and the transplant hospital shall pay related costs on organ donation and procurement to OPO. Besides, OPO shall sign an agreement with potential organ donation medical institutes and pay for the costs on donor's rescue and maintenance to medical institutes of successful organ donation. Until 2016.01.29 in China, 65837 citizens have registered to be donation volunteers; 16707 organ failure patients have been cured by the organ retrieved from the 6171 successful donation cases. COPO is playing an important role in management of organ procurement in China and prompt the work of China organ donation after citizens' death.

372.6

Outcomes of kidney transplantation using kidneys from expanded criteria donors and donors with acute kidney injury in Thailand

North Techawathanawanna.
Kidney Transplant Institute, Praram 9 Hospital, Bangkok, Thailand.

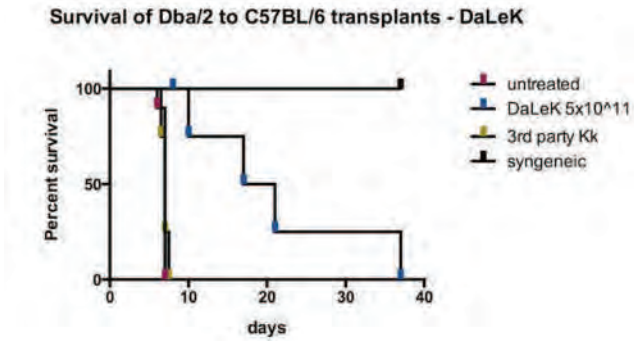
Background: Deceased-donor kidney transplantation using kidneys from expanded criteria donors and donors with acute kidney injury are increasingly utilized in many countries, including Thailand, due to organ shortage. The short-term and long-term outcomes in our country are still not well described.
Methods: We reviewed our database of cadaveric kidney transplants from January 1997 through December 2015, comparing the outcomes of transplantation of kidneys from expanded criteria donors (ECD group, 41 cases), donors with acute kidney injury (AKI group, 63 cases) and standard criteria donors (SCD group, 42 cases). Baseline characteristics of donors and recipients were correlated with short-term and long-term graft function.
Results: One-year graft survivals were 92.68%, 100%, and 100% in ECD, AKI, and SCD group, respectively. Mean five-year estimated glomerular filtration rates were 40.83, 55.29, and 58.99 ml/minute in ECD, AKI, and SCD group, respectively. Risk factors for decreased graft survival included the ECD group and the number of hemodialysis sessions required within the first few weeks after transplantation. Risk factors for decreased glomerular filtration rate included the ECD group, and age of the donor.
Conclusions: Kidneys from donors with acute kidney injury can potentially be used for transplantation with equivalent outcomes to standard criteria donors. ECD kidneys had satisfactory but inferior short-term and long-term graft outcomes.

373.1

Expression of three allogeneic MHC Class I in recipient liver significantly prolongs survival of fully-allogeneic vascularised cardiac allografts

Mamdoh Moawadh[1], Moumita Paul[1], Eithne Cunningham[1], Chuanmin Wang[1], Szun Tay[3], Sharon Cunningham[3], Grant Logan[3], Ian Alexander[3], Patrick Bertolino[2], David Bowen[2,3], Alex Bishop[1], Alexandra Sharland[1].
[1]Transplatation Immunobiology, The University of Sydney, Sydney, Australia; [2]Liver Immunology Group, Centenary Institute, Sydney, Australia; [3]Gene Therapy Research Unit, The Children's Hospital Westmead, Sydney, Australia.

Background and Aim: In previous studies, AAV-mediated gene transfer of a single mismatched donor MHC class I molecule (K^d or K^b) to C57BL/6 or B10.BR recipient liver respectively induced tolerance to skin grafts expressing the same mismatched MHCI molecule. However, such survival is not extended to fully mismatched skin or heart grafts. Tolerance induction may require expression of all mismatched MHC molecules (3 class I and 2 class II). To facilitate expression of multiple MHCI in recipient liver, we created a construct in which the three d-haplotype heavy chains D^d, L^d and K^d were separated by an F2A linker (DaLeK), and then determined the effect of administration of this vector upon heart graft survival.
Methods: DaLeK was packaged into a liver-specific rAAV2/8 vector. Fully-allogeneic hearts from DBA/2 (H-2d) were transplanted into C57BL/6 (H-2b) at either d7 or d14 post-inoculation.
Results: Administration of $5x10^{11}$ vector genome copies AAV-DaLeK to C57BL/6 mice yielded strong expression of Dd, Ld and Kd on hepatocytes. Expression was enhanced by co-transduction with a vector encoding β2 microglobulin, ALT levels remained normal and no inflammatory infiltrates were detected. Survival of DBA/2 hearts transplanted into AAV-DaLeK treated mice was prolonged from a MST of 7 days to 23 days. Administration of a control vector did not alter survival (figure). Rejected grafts showed a mixed inflammatory infiltrate with damage to blood vessels but without cardiac myocyte necrosis. Cardiac myocytes of DBA/2 hearts express only class I whereas vascular endothelium expresses MHC class I and II.
Conclusion: AAV-DaLeK permits expression of multiple MHCI from a single vector, and its administration significantly prolongs survival of fully-allogeneic heart transplants. A combination of AAV-DaLek with vectors expressing CIITA and/or allogeneic MHC class II may produce tolerance to fully-allogeneic grafts.



References:
[1] Cunningham EC Transplantation. 2013 Jan 15;95(1):70-7.

373.2

Heightened expression of HLA-DQB2 and HLA-DPB1 genes in pre-implantation kidney biopsies is associated with poor graft function

Karina L.Mine[1], Tuíla B.Mourão[1,3], Larissa A.Salzedas[1], Érika F.Campos[1], José O.Medina-Pestana[2,3], Hélio Tedesco-Silva[2], Maria Gerbase-DeLima[1,3].
[1]Immunogenetics Institute, AFIP - Associação Fundo de Incentivo à Pesquisa, São Paulo, Brazil; [2]Hospital de Rim e Hipertensão, Fundação Oswaldo Ramos, São Paulo, Brazil; [3]Universidade Federal de São Paulo, São Paulo, Brazil.

Introduction: In a previous gene expression microarray investigation in pre-implantation biopsies (PIB) from kidneys of deceased donors (DD) we showed, for the first time, association between increased expression of HLA class II genes in PIB and poor graft function at 1 year post-transplant (1y post-Tx). This study was conducted in 51 PIB, with the GeneChip® Human Gene 1.0 ST Array, Affymetrix, and the expression of all HLA class II genes, but particularly of HLA-DQB1 (p=0.0001), DQB2 (p=0.0003) and DPB1 (p=0.0007) was higher in PIB of grafts that presented eGFR (estimated glomerular filtration rate, calculated with the abbreviated MDRD formula) < 58.9 L/min (median value for the group) at 1y post-Tx. In the present study, we sought to validate, in a different cohort and by quantitative PCR (qPCR), the relationship between expression of HLA-DQB1, DQB2 and DPB1 and poor graft function.
Materials and Methods: This prospective single-center study included 38 PIB from transplants with DD, performed in 2014 and 2015, in low immunological risk adult recipients who received triple immunosuppressive therapy (a calcineurin inhibitor combined with prednisone and either azathioprine or mycophenolic acid) plus induction with thymoglobulin. The 19 transplants with eGFR < 51.0 mL/min (median of the group) at 6 months post-Tx comprised the poor graft function group. The expression of HLA-DQB2 and HLA-DPB1 was determined by qPCR (TaqMan® assays, Applied Biosystems). HLA-DQB1 expression could not be tested due to the lack of TaqMan® assays able to detect transcripts of most of the HLA-DQB1 alleles. The TATA-binding protein (TBP) gene was used as internal control. Relative expression units (RU) were determined with the 2-Δ ΔCt method and were compared by the Mann-Whitney test.
Results: Both genes presented higher expression in cases with 6-month eGFR < 51.0 mL/min: 2.82 vs 1.01RU, for HLA-DQB2, p=0.04; 1.57 vs 1.00 RU, for HLA-DPB1, p=0.008.
Conclusions: Since there was a highly significant correlation (Spearman correlation test, p=1.55e-007) between the eGFR at 6 months and at 1 y post-Tx in the patients that were included in the previous microarray study, we considered that we have validated the results even if we had to use, in the present cohort, the graft function at 6 months as the end-point, since not all the patients had reached the 1-y post-Tx follow-up at the time of this analysis. A particularly interesting finding of our work was the confirmation of the differential expression of HLA-DQB2, a poorly polymorphic gene, with unknown function, and whose expression has only been so far described in epidermal Langerhans cells (Lenormand et al, 2012). The increased expression of HLA-DPB1 in PIB as an early marker of poor later graft function is consistent with the assumption that heightened expression of HLA genes contributes to increase allograft immunogenicity and consequently affects the long-term graft function and survival.

373.3

Improving perfusion fluid for the next generation of transplants: Identifying agents that lower cellular oxygen consumption and reduce tissue hypoxia

Abul Siddiky, Pavitra Kannan, David Grimes, Thomas Ashton, Ruth Muschel.
CRUK/MRC Centre for Radiation Oncology & Biology, University of Oxford, Oxford, United Kingdom.

Introduction: As the need for transplantation increases, the boundaries of what is a marginal organ is being pushed further back. The development of appropriate perfusion fluid has greatly helped in maximising available organs through reducing the effects of unavoidable tissue hypoxia. We believe we can improve this further.
Materials and Methods: Four colorectal cancer cell lines (COLO320DM, DLD1, HCT116 and HT29) and a non-transformed cell line (MRC5) were investigated. Clonogenic and cytotoxicity assays of a range of agents were used to determine sub-lethal concentrations. The oxygen consumption, mitochondrial and glycolytic function of treated cells were assessed with the XF96 Analyser. Flow cytometry, gene array, western blot and high-performance liquid chromatography (HPLC) were performed to delineate mechanisms of action. The most responsive cell lines and promising agents were progressed to spheroids for 3D hypoxia modelling and then advanced to in vivo and radiosensitivity testing. In vivo tissue hypoxia analysis was achieved with the in vivo imaging system (IVIS).
Results: The oxygen consumption of all cell lines were markedly reduced with a number of the agents. DLD1 and MRC5 cell lines showed the greatest balance of toxicity resistance and reduction in oxygen consumption. Hypoxia imaging of the subsequent spheroids further demonstrated a reduction in hypoxia consistent with drug induced decrease in oxygen consumption. This was further replicated in the xenografts resulting in improved radiosensitivity (see Figure 1).

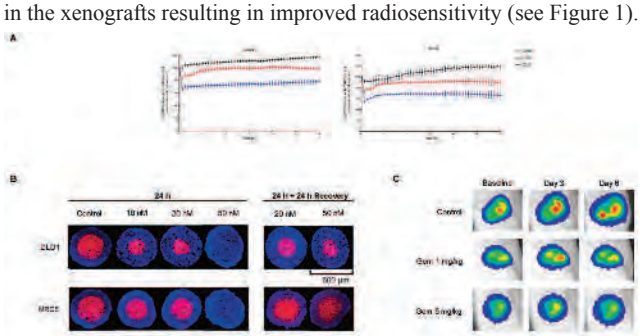


Figure 1. Sub-therapeutic doses of gemcitabine can lower OCR and improve tissue hypoxia in spheroids and in vivo as seen on IVIS. A. Oxygen consumption rate for gemcitabine in DLD1 and MRC5 human cell lines. B. Hypoxia staining of normal spheroid sections for HP5 (red) with Hoechst as a counterstain (blue). C. Representative IVIS images of subcutaneous DLD1 human tumour bearing luminescence (red) in nu/nu mice (mice). Treatments were administered by i.v. via I.P. injection. Each group had at least 6 mice and entered into study once tumour volume reached 80 mm³. Error bars represent standard error.

Discussion: A variety of agents originally identified through our studies on improving tumour hypoxia have shown a reduction in oxygen consumption in malignant and non-malignant cells alike, which has been demonstrated to lead to an increase in oxygen availability and hypoxia improvement. This has been shown in monolayer and 3D in vitro models. We hypothesise that by reducing cellular oxygen consumption, cells will be able to withstand hypoxia for a greater period of time and thus lengthen the preservation period. If the data shown in our work in cancer can be replicated in the transplant setting, we can potentially increase the donor pool by enhancing the preservation period of an organ. Ultimately, this will help reduce the waiting time for patients and improve morbidity.
Conclusion: The preservation period of an organ can potentially be increased through using agents that reduce oxygen consumption. This will improve graft function and increase the donor pool.

373.4

Long-term survival of human stem cell-derived insulin producing cells (SCIPCs) transplanted to the subcutaneous space in a diabetic murine model

Steven Wisel[1], Gaetano Faleo[1], Holger Russ[2], Ryan Chang[3], Tejal Desai[3], Matthias Hebrok[2], Qizhi Tang[1].
[1]Division of Transplantation, Department of Surgery, University of California, San Francisco, San Francisco, United States; [2]Diabetes Center, Department of Medicine, University of California, San Francisco, San Francisco, United States; [3]Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, San Francisco, United States.

Introduction: Recent improvements in stem cell differentiation protocols now allow for reliable production of human Stem-Cell-derived Insulin-Producing Cells (SCIPCs), which mimic the functional properties of human beta cells and represent an unlimited supply of cells for the treatment of Type 1 diabetes. Selection of an appropriate transplant site will be important for human translation, favoring a site which allows for graft monitoring and retrievability in the case of cell de-differentiation and tumor formation. The subcutaneous space provides ease of graft monitoring and an abundance of space, but prior pre-clinical efforts have demonstrated poor graft survival attributable to the relative hypoxia of the subcutaneous tissue. Our group has demonstrated long-term survival of subcutaneous SCIPC transplants. We have also attempted to induce prevascularization of the islet bed prior to SCIPC transplantation by pre-implantation of a thin-film polymer disc, to further optimize graft survival.

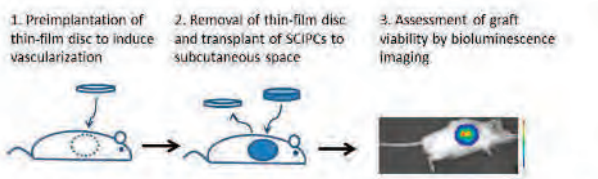


Figure 1. Schematic of transplant site prevascularization (1), SCIPC transplantation (2), and evaluation of SCIPC viability (3).
SCIPC: Stem Cell-derived Insulin Producing Cells

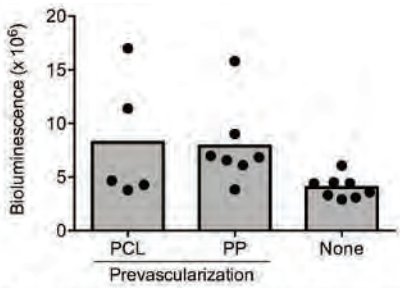


Figure 2. Graft viability at 28 days post-transplant to pre-vascularized sites, as measured by SCIPC luciferase bioluminescence.
SCIPC: Stem Cell-derived Insulin Producing Cells

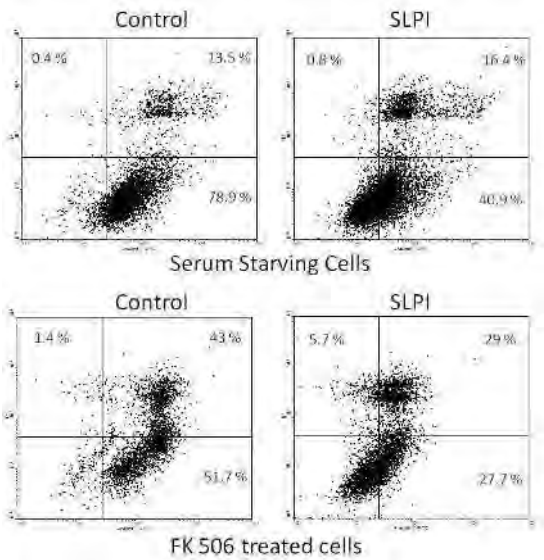
Materials and Methods: Prevascularization of a subcutaneous transplant site was achieved by implantation of a 1.5cm-diameter polycaprolactone (PCL) or polypropylene (PP) thin-film disc to the dorsum of NSG mice for a duration of 10-21 days. At time of transplant, thin-film discs were explanted and 2-3 million SCIPCs were delivered to prevascularized sites (PCL = 6, PP = 6) or to naïve NSG mice as controls (n=3; Figure 1). SCIPCs were differentiated as described previously[1], with constitutive expression of firefly luciferase by this reporter line allowing for in vivo real-time bioluminescence imaging of viability. SCIPC grafts were serially imaged for viability up to 6 weeks post-transplant for evidence of graft viability.
Results: SCIPCs transplanted to the subcutaneous space showed long-term viability, as assessed by luciferase bioluminescence imaging. Prevascularization of the subcutaneous space with PCL or PP showed a trend toward improved graft survival (p=0.058 and 0.087, respectively), while no significant difference was seen between use of PCL versus PP thin-film discs for prevascularization (p= 0.690).
Conclusion: Prevascularization of the subcutaneous space by pre-implantation of a thin-film polymer disc may improve SCIPC viability as one mechanism to overcome the hypoxic milieu of the subcutaneous space. Studies are ongoing to further optimize graft survival and to assess impact of prevascularization on graft function.
References:
[1] Russ HA, Parent AV, Ringler JJ, et al. Controlled induction of human pancreatic progenitors produces functional beta-like cells in vitro. *EMBO J*. 2015;34(13):1759-1772

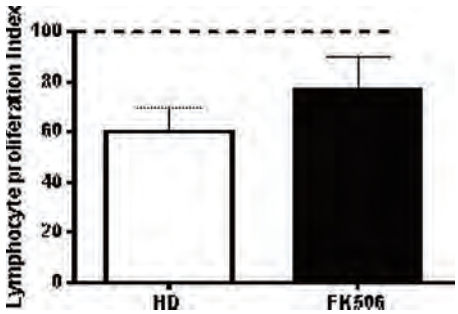
373.5

Secretory leukocyte proteinase inhibitor: A biomarker for acute kidney injury has a protective role in kidney transplantation

Nella Ambrosi[1], Julia R.Rocamora[1], Ana Dotta[3], Diego Guerrieri[1], Fiorella Caro[1], Francisco Osella[3], Juan Salaberry[1], Francisco Sánchez[1], María del Carmen Rial[3], Claudio Incardona[2], Domingo Casadei[3], Eduardo Chuluyan[1].
[1]CEFYBO, Facultad de Medicina, Universidad de Buenos Aires, CABA, Argentina; [2]Fundación Gador, CABA, Argentina; [3]Fundación Argentina de Trasplante de Organos y Tejidos 3er Milenio, Instituto de Nefrología de Buenos Aires, CABA, Argentina.

Secretory leukocyte protease inhibitor (SLPI) is a serine protease inhibitor produced mainly by epithelial cells. It has anti-inflammatory and antimicrobial activity, and enhances wound healing. Recently, it has been proposed that SLPI could be a novel biomarker for acute kidney injury[1]. In fact, we have found that kidney transplanted patients had higher serum values compare with healthy donor ($p < 0.0001$).
The aim of the present study was to assess a putative activity of SLPI on human kidney epithelial cells and lymphocyte proliferation. First, the activity of SLPI on kidney epithelial cell apoptosis was determined by *in vitro* culture human proximal tubular epithelial cell line (HK-2) under serum starving conditions or by treating the cells with FK506, in the presence of SLPI (40 ng/ml). Apoptosis was examined by flow cytometry with annexin V/propidium iodide and MTT assays. Either FK506 or serum starving conditions induced a high degree of apoptosis. However, when cells were incubated with SLPI, the apoptosis induced by both treatments were statistically reduced in both apoptosis assays. Next, lymphocyte proliferation were assessed by culture human peripheral blood mononuclear cells (PBMCs) derived from healthy dnors or kidney transplant patients. Cell proliferation was induced with phytohemagglutinin (PHA), in the presence or absence of SLPI for 5 days. Then cells were labeled with 3H-thymidine, harvested and counted in a beta-counter. Cells derived from either healthy donors or transplanted patients, treated with PHA + SLPI showed lower proliferation index compared with those treated with PHA alone.
These results prove that SLPI has a protective role on epithelial kidney cells and favors the immunossupresive activity by decreasing lymphocyte cell proliferation. Furthermore, we can speculate that the objective of high level of SLPI observed in transplant patients tend to both protect the kidney cells from the noxa and control the immune response in order to reduce the kidney injury by the allogeneic immune response.





CONICET; Fundación 3er Milenio

References:

[1] Oberbauer R. Molecular pathogenesis of post-transplant acute kidney injury: assessment of whole-genome mRNA and miRNA profiles. PLoS One. 2014 Aug 5;9(8):e104164

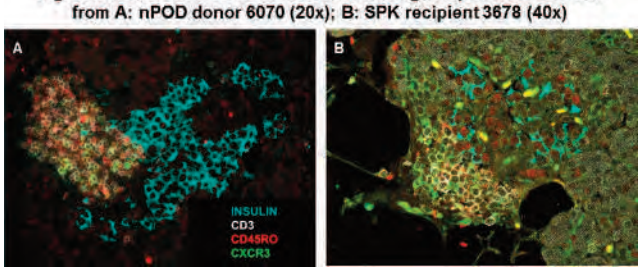
373.6

Expression of CXCR3 by islet-infiltrating memory T cells in the pancreas transplant with recurrent type 1

Francesco Vendrame[2], Ivan Gerling[3], Alberto Pugliese[1,3,5], Linda Chen[4], George Burke[4].
[1]Diabetes Research Institute, University of Miami, Miami, United States; [2]La Jolla Institute of Allergy and Immunology, La Jolla Institute of Allergy and Immunology, La Jolla, United States; [3]Medicine, Division of Diabetes, Endocrinology and Metabolisim, University of Tennessee Health Science Center, Memphis, United States; [4]Surgery/Miami Transplant Institute, University of Miami Miller School of Medicine, Miami, United States; [5]Medicine, Division of Endocrinology and Metabolism, University of Miami Miller School of Medicine, Miami, United States.

Clinical studies link memory T cells with type 1 diabetes (T1D). A recent trial in new onset patients has shown preserved insulin secretion in those treated with a drug that targets memory T cells. In initial studies we have shown a considerable frequency of memory T cells in the insulitis lesions of pancreata from SPK (simultaneous-pancreas-kidney) transplant recipients (79-83%), nPOD organ donors with T1D (23-54%) and autoantibody-positive, non-diabetic donors (10-60%). Gene expression studies of laser-captured islets showed increased expression of the chemokines CXCL9 and CXCL10. Therefore, we have begun to investigate whether islet infiltrating memory T cells expressed CXCR3 receptors, which bind these chemokines. For this proof of concept study we examined pancreas sections from nPOD donors 3678 (SPK, recurrent T1D) and 6070 (T1D, 7 years duration). We stained sections by multiple immunofluorescence for insulin, CD3, CD45RO and CXCR3. Insulitis lesions (CD3+ cells >15/islet) were identified in both the SPK and nPOD cases, which contained CD3+CD45RO+ memory T cells; a significant proportion of the memory T cells stained for CXCR3. Fig. 1 shows an example of an infiltrated pancreatic islet from T1D donor 6070 (panel A, 52.5% of the CD45RO+ CD3 T cells stained for CXCR3) and T1DR SPK recipient 3678 (panel B, 88% of the CD45RO+ CD3 T cells stained for CXCR3). In this preliminary study we show memory T cells infiltrating the islets expressed CXCR3. This finding supports the concept that the expression of CXCL9 and CXCL10 ligands by the islets, as shown previously, leads to the recruitment of CXCR3 positive memory T cells. We are currently completing studies on additional 3 SPK and 6 nPOD (T1D or autoantibody-positive donors) for an initial quantifying of CXCR3 expression. Of note, in related studies of SPK recipient with T1D recurrence, we find increased peripheral blood frequency of autoreactive memory CD4 T cell expressing CXCR3. Thus, if confirmed in additional studies, CXCR3 may become a target for immune intervention to antagonize islet autoimmunity.

Fig. 1: INSULIN-CD3-CD45RO-CXCR3 staining in a pancreas section from A: nPOD donor 6070 (20x); B: SPK recipient 3678 (40x)



374.1

Knowledge and attitude of people towards end-stage kidney disease, kidney transplantation and kidney donation: Opinion from a developing country

Palash Mitra[1,2], Muhammad A.Rahim[1], Mohammad A.Mansur[1,2].
[1]Nephrology and Dialysis, BIRDEM General Hospital, Dhaka, Bangladesh; [2]Transplant Unit, BADAS, Dhaka, Bangladesh.

Introduction: Organ transplantation is the most preferred treatment modality for end-stage kidney diseases (ESKD). The success of transplantation program depends on knowledge, awareness and positive attitudes. Our aims of this study were to assess the knowledge and attitudes of the people including physicians and other health care related professionals (HCPs) about ESKD, it's treatment options and their views towards kidney transplantation.
Methodology: This cross-sectional survey was conducted from July to December 2015, among trainee physicians and other HCPs of four tertiary care hospitals and adult people seeking general health care service as outpatients in two rural areas of Bangladesh. Data were recorded in preformed semi-structured forms. Data was analyzed using SPSS version 20.
Results: Among the 1312 participants, physicians were 17.7%, other HCPs 22% and general people 60.3%. Male: female was 1.1:1. Participants were from urban (74.4%) and rural (25.6%) areas with an educational background of higher secondary (HSC) or less in 36% and above HSC in 64%. Overall 65.8% had knowledge about ESKD and its' treatment options. About 90% thought that kidney transplantation is the best treatment option and live related donor renal transplant (LRDT) is better (58%). Chance of better function and survival of donated kidney (48.8%) and easy to convince both the recipient and donor (50%) was the benefit of LRDT. Unavailability of suitable donor was marked (42.7%) by the participants as the main disadvantage of LRDT. Only 43.3% participants were agreed to donate kidney and the rest had fear of developing renal failure (40.2%) after donation as well as non-cooperation of the other family members (33.7%). They were positive (53%) for cadaveric or deceased donor kidney transplant, as a brain dead person can donate two kidneys (72%). But 50% thought that the concept of cadaveric kidney donation is still not well accepted and 45% believe that there is religious barrier. There is no significant difference of opinions between the physicians or other HPCs or general people except there is significant difference regarding motivation of kidney donation (p=0.001 and p=0.000). The rural people's knowledge regarding ESKD and treatment options are significantly (p=0.000) less than the urban people and also significantly (p=0.000) less motivated for kidney donation and deceased donor kidney transplantation. Though the awareness about ESKD, its treatment and motivation for kidney donation is significantly less (p=0.000) among the respondents with educational background of HSC or less but there is no significant difference (p=0.126) of opinion regarding deceased donor kidney transplantation among the persons with higher educational background.
Conclusion: In spite of high level of awareness about ESKD, kidney transplantation and donation, a high proportion of the participants did not have positive attitudes towards kidney donation. We will have to address their misconceptions and fear about kidney transplantation and donation to continue a successful kidney transplant program in a developing country like ours.
Keywords: Cadaveric kidney donor, end-stage kidney disease, kidney transplantation, living related donor.

374.2

Development of a deceased donor program in New Caledonia through an international hybrid model

Nicolas Quirin[1], Fadi Haidar[1], Caroline Mesguen[1], Jean-Michel Tivollier[1], Jean-François Cantin[1], Cedric Formet[1], Yves Doussy[1], Frederic Touzain[2], Veronique Biche[5], Hervé Lecoq[4], Charbel Sandroussi[3], Richard Allen [3], Josette Eris[3].
[1]Nephrology, CHT de Noumea, Noumea, New Caledonia; [2]HLA laboratory , CT de Noumea, Noumea, New Caledonia;
[3]Transplantation, Royal Prince Albert Hospital, Sydney, Australia;
[4]Intensive Care, CHT de Noumea, Noumea, New Caledonia;
[5]Transplant coordination, CHT de Noumea, Noumea, New Caledonia.

Introduction: New Caledonia (NC) is a French Territory located 2000 Km from Sydney and 17000 km from France and has 266767 inhabitants. It has the fifth highest worldwide prevalence of ESRD at 2407 pmp. There are 514 patients on dialysis. Living donor transplantation is available both in France and Sydney for NC patients. In the absence of a living donor, transplantation access had until 2013 been dependent on relocation of the individual to France to be waitlisted. This was not a feasible option for many. Thus to enable transplantation access for all suitable recipients the implementation of a deceased donation program in New-Caledonia was required.
Aims and Methods: The aim was to establish a deceased donation and retrieval program in NC with subsequent transplantation of NC kidneys to NC recipients being undertaken in Sydney.
Firstly, brain death legislation was enacted in NC in accordance with French and New Caledonian laws. The French "Agence de la Biomedecine" standards of organ retrieval and organ allocation were implemented in NC. A tissue typing laboratory was established in NC in 2010. Transplant clinicians from Royal Prince Alfred Hospital (RPAH) in Sydney assisted in recipient assessments in NC and with initial retrieval surgery. Collaboration at all levels (clinical, administrative, government) addressed issues of customs, logistics of air transportation and surgery in order to deliver an acceptable cold ischemic time. Two matched recipients were transported with each set of donor kidneys to Sydney for transplantation. The results of this international collaborative model (2013-2015) are reported.
Results: 40 patients were initially waitlisted in November 2012 and assessment clinics are undertaken twice a year. The first retrieval occurred in April 2013. 39 brain dead patients have been evaluated to date leading to 9 procedures and 8 retrievals (1 in 2013, 5 in 2014 and 2 in 2015). Donation rates were 3,72 pmp, 18,6 pmp and 7,4 pmp in 2013, 2014, 2015 , respectively. Consent refusal rate was 50%, medical and administrative reasons accounted for 29 % not proceeding; resulting in a mean retrieval rate of 21%.
16 recipients received kidney transplants in Sydney with a mean cold ischemic time of 15 hours [11h – 22h]. Time for return trip for the RPAH retrieval team was a mean of 10 hours. Immediate graft function was observed in 78 % of cohort. Recipients returned to NC after 8 weeks. Patient and graft survival to date is 94 % (one death with a functioning graft from infection at 12 months).
Conclusion: This novel international medical collaboration model of transplantation has delivered access to deceased donor transplantation for New Caledonians unable to relocate to France. Similar collaborations should be explored to enable access to high standard ethical transplantation programs for isolated communities.

374.3

Success transformation of organ donation system brings positive feedback to donation manner of medical frontiers in China

Nga-Lei Tam[1,2], Wen-Feng Xie[2], Xiao-Sun He[2].
[1]Department of surgery, The Hong Kong University- Shenzhen Hosptial, Shenzhen, People's Republic of China; [2]Organ Transplantation Center, The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou,, People's Republic of China.

Introduction: China has initiated the donation after cardiac death (DCD) pioneer programme since 2010 and nationally enforced on 1st January 2015. Organs from DCD donors has thus officially become the only deceased organ for transplantation. The donation manner of ICU staff, the frontier to approach the potential DCD donors, reflects the success transformation of organ donation.
Method: A questionnaire survey was designed for the attendees in both ICU and OPO (Organ procurement organization) conferences in July to December, 2014 and 2015. A 12 multiple self choice questionnaires were facilitated and immediately collected. Demographic data and the participants' recognition, participation, as well as their acquaintance of organ donation procedures were recruited.
Results: In 2014 and 2015, a total of 1099 and 734 questionnaires were collected and 1079 and 675 were available, respectively. The awareness of DCD was 88.5% in 2014 and 96.0% in 2015. There were 13.5% attendees have been participated in DCD activity in 2014 whereas 61.3% in 2015. Among these 2 years, 8.8% and 48.0% of them owned correct DCD criteria, policies and procedures, while 76.6% and 86.1% expressed willingness of future DCD activities participation. In 2014, unfamiliar to the donation policies and procedures obstructed most of the donations. In 2015, the main barrier of DCD work was selected to the fierce conflict between medical workers and patients. The enforcement of DCD population and organ donation law were most recommended to improve donation success in whole survey.
Conclusion: It has been a half decade since the launch of DCD programme and full enforcement last year. The ratio of DCD awareness and participation, as well as the correct DCD concept has obviously improved by the organ donation system enforcement. The establishment of organ donation law is still to ensure the transformation of organ donation system.
References:
[1] Tam NL et at. The attitude of ICU staff towards organ donation in China. *China Medical Engineering*.2015.04:112-113
[2] Huang J, Millis JM, Mao Y et al. Voluntary organ donation system adapted to Chinese cultural values and social reality.*Liver Transpl.* 2015 Apr;21(4):419-22

374.4

Organ donation and transplantation in Montenegrin population

Marina Ratkovic, Branka Gledovic , Danilo Radunovic , Vladimir Prelevic , Batric Babovic , Elvir Mucic .
Nephrology and Hemodialysis Department , Clinical Center of Montenegro , Podgorica, Montenegro.

Introduction: In Montenegro there was no transplantation program until 2012. The transplantations were performed abroad; 15% in areas of black organ markets. The first kidney transplantations from living donors were performed in September 2012. Until now 25 kidney transplantations from living related donor were performed. Legislation was finalized in 2012. Montenegro is young country with many traditional attitudes. Death in is followed by numerous customs and represents one of the greatest sociological phenomenon in our society. Until now only one deceased donor has been realized. The attempt to legally provide presumed consent was not adopted by the Parliament in 2014. Less than 300 citizens took donor card.
Materials and Methods: We organized a public survey, about the attitudes of population on organ donation. It was conducted by team of physicians, renal nurses and medical students. It was anonymous and conducted in public places and in different institutions like University, medical institutions and other organizations. This was the biggest survey in this topic ever done in Montenegro.
Results and Discussion: The survey covered 6000 respondents, 48% male, 52% female, with the highest number in the age group between 30 – 50 years. The largest number of respondents was with higher education (58%), 36 % with secondary and 6% with elementary education. The highest proportion of respondents belongs to the Orthodox religion, 63%, 20% belong to the Islamic, 10 % to Catholic, and 2% were atheists and others. 45% of respondents said they would like to be deceased donors, but 52% had negative attitude. Among respondents with positive attitude, 80% would donate any organ, and 20 % answered that they would donate only few organs. 70% of respondents were only partially informed about the legal regulations. Only 20% of respondents had some experience in this area. 96% of respondents said that there are no enough information about organ donation in public media. In the last question respondents wrote the main problems they recognized: lack of information, lack of interest, religious dogma, fear and distrust. We made a stratification of respondents by age, gender, religious affiliation and level of education, but we found no statistically significant differences in answers in any group.
Conclusion: Despite great effort of activities, discrepancies still exist counted prejudices and open issues that need to be systematically considered in order to raise the number of deceased donors and deceased transplantation rate.

374.5

Comparison of cost and quality of life outcomes (qol) in hemodialysis patients and renal transplant recipients

M. M. Iqbal[1], S. Iqbal[2], R. M. Hossain[3], S. K. Saha[2], S. F. Islam[3], A. A. Chowdhury[4], M. A.W. Khan[5].
[1]Nephrology, SSMC&MH, Dhaka, Bangladesh; [2]Nephrology, BIRDEM, Dhaka, Bangladesh; [3]Nephrology, BSMMU, Dhaka, Bangladesh; [4]Nephrology, NIKDU, Dhaka, Bangladesh; [5]Nephrology, Square Hospital, Dhaka, Bangladesh.

Introduction: Outcome and patient benefits in different types of renal replacement therapies (RRT) are often debated and compared on the basis of cost involvement and finally improvement in quality of life (QoL) parameters. In this work cost and QoL of maintenance hemodialysis (MHD) and the renal transplant recipient (RTx) is compared.
Materials and Methods: The MHD and RTx subjects were selected from a number of tertiary renal care facilities where patients were initially on hemodialysis and later some opted for transplantation. The quality of life was assessed by KDQOL-SF 36 v1.3. The MHD patients were on bicarbonate dialysis and majority getting 3 sessions/week. Renal function was stable at the time of evaluation in RTx subjects and they were taking prednisolone, cyclosporine/tacrolimus and mycophenolate mofetil/azathioprine.
Results and Discussion: The RTx recipients (n=40) and MHD subjects (n=80) were matched for age as 36±8 vs.39±8, years, (p=NS).The score of major items in general health related scale evaluated by short form (SF36) among these 2 groups respectively showed that general health score 62±20 vs. 39±16; physical functioning 90±17 vs. 57±25; role physical 62±54 vs. 24±43; emotional well being 73±35 vs. 46±12; social function 91±16 vs. 42±29 and energy/fatigue 55±15 vs. 42±12; ($p<0.001$).The physical component aggregate was 75±22 vs. 48±21 and for mental component 68±20 vs. 51±16, ($P<0.001$). These results showing a persistently higher score in RTx subjects than those on MHD. On the other hand the estimated annual maintenance cost of 3 drug regimen in RTx was approximately US$ 2000 where as for MHD the standard cost for only dialysis service was around US$5000.
Conclusion: It is concluded that renal transplantation is by far the best option of renal replacement therapies. It is cost effective and undoubtedly provides superior quality of life over hemodialysis which as is evident from higher score in all items of QoL parameters in this study. Therefore transplantation should be emphasized in the economically disadvantaged less developed nations having the advantage of larger family structure.

401.1

Are women in transplant leadership roles judged more harshly than their male colleagues?

Nithya Krishnan[1], Deborah Biggerstaff[2], Neil Raymond[1], Monica Dolton[3], Hillary Braun[4], Sondra Livingston[5], John Hattersley[1], Josette Eris[6], Nancy Ascher[4], Robert Higgins[1], Kathryn Wood[3], Ala Szczepura[7]. [1]Renal Unit, University Hospitals Coventry & Warwickshire NHS Trust, Coventry, United Kingdom; [2]Psychology, University of Warwick, Coventry, United Kingdom; [3]Transplantation Research Immunology Group, University of Oxford, Oxford, United Kingdom; [4]Transplantation , University College San Francisco, San Francisco, CA, United States; [5]The Transplantation Society, Montreal, QC, Canada; [6]Transplantation , University of Sydney, Sydney, Australia; [7]Health Technology Assessment, University of Coventry, Coventry, United Kingdom.

Introduction: Despite the introduction of many positive changes aimed at helping women who work in medicine to flourish, the number of women holding senior leadership roles are a minority across many specialities. Research from other professions has found that when women do reach senior positions they appear to be judged more harshly and are more likely to fall from their position of seniority, the 'glass cliff effect'.
Aim: To explore any engendered differences in participants' perceptions and their views on potential wrong decisions by senior medical transplant staff, using hypothetical clinical scenarios in an online survey.
Methodology: The study was a prospective web-based survey involving five clinical scenarios, with either a male or female protagonist (Set1 and Set2). Each scenario was followed by two questions regarding the clinical performance. Respondents were invited to comment on their reasoning. Data were analysed using thematic analysis. To ensure elimination of any inherent biases in responses the survey was advertised as a study exploring whether adequate actions are taken following adverse clinical incidents in transplantation. Participants were recruited by advertising through the websites of various professional bodies. Individuals were randomly assigned to either Set1 or Set2 questionnaires, with each Set receiving the same scenarios in the same order, differing only in whether a male or female protagonist was mentioned.
Results: 102 participants provided further explanation and comments on the clinical performance. Themes identified included: issues of informed consent and acceptable risk; clinical judgement; systems errors; consensus among clinical teams; training issues; mentoring; and judgements as to whether appropriate actions had been taken at the time. Participants' described male protagonists as "forceful individual"; "maverick who ignores his colleagues" or, "one who has "cautious colleagues". Meanwhile, female protagonists were described using slightly different, more nuanced characteristics like competency; mentoring; training needs; need for supportive senior colleagues; or as having 'saved the day'. However, when things went wrong, participants were more likely to opine that female protagonists should not have gone ahead. Risk was perceived by respondents to be a more male leadership trait.
Conclusions: The quantitative survey showed no significant differences between the sets but the thematic analysis of participants' comments provided evidence of a subtle and nuanced use of language; where those traits identified in male leadership being viewed and described differently to those applied to women leaders. While gender may no longer be such an issue as in the past, respondents' use of language and their choice of words display elements of an engendered description. These findings suggest that the 'glass cliff" effect may still be a problem in transplantation but has become subtle and therefore, may not be so easily identified in the workplace.

The Transplantation Society, The British Transplantation Society, National Health Service Blood & Transplant, UK, Royal College of Paediatrics and Child Health, UK, National Kidney Federation, UK and Warwick Medical School, UK

420.1

The impact of ritonavir-boosted protease inhibitors and non-depleting antibody induction on outcomes in HIV-infected kidney transplant recipients

Brett Rollins, Susan Lerner, Meenakshi Rana, Shirish Huprikar, Leandra Miko, Vinay Nair, Sander Florman, Ron Shapiro.
Mount Sinai Hospital, New York, United States.

Introduction: One-year rejection rates in HIV-infected kidney transplant recipients range from 15-40%, compared to overall rejection rates of 10% in HIV-negative patients. Protocols for immunosuppression and highly active antiretroviral therapy (HAART) regimens in this population vary substantially among transplant programs. The potential for significant drug-drug interactions, specifically between ritonavir-boosted protease inhibitors (rtv+ PI) and calcineurin inhibitors, and the choice of induction therapy may influence outcomes.
Materials and Methods: This is an IRB-approved, single center, retrospective study of adult HIV-infected patients with a kidney transplant performed between 5/2009 to 8/2014 with one-year follow up for each patient.
Results and Discussion: 36 patients were identified with a median age of 52 (interquartile range [IQR] 46, 57) years. 78% were male, 53% were African American, 19% were Caucasian, and 17% were Hispanic. The most common cause of renal failure was hypertensive nephrosclerosis (51%) followed by HIV-associated nephropathy (17%), and the median duration of pre-transplant dialysis was 6.2 (3.2, 8.9) years.
All patients received IL-2 receptor antagonist (IL-2 RA): 81% with basiliximab and 19% with daclizumab induction. One patient also received thymoglobulin. Calcineurin inhibitor therapy included tacrolimus (75%), cyclosporine (19%), or transitions between these two (6%). 44% of patients received a rtv+ PI-based HAART regimen.
Overall one-year patient and graft survival was 94% and 92%, respectively, and the mean serum creatinine was 1.35 (1.21, 1.80). Treated biopsy-proven rejection within one year was 33% for the overall cohort; 44% for patients on rtv+ PI and 25% for patients on other HAART regimens (p=0.29).
Conclusion: Despite high rates of acute rejection, HIV+ kidney transplant recipients have excellent outcomes. Though not statistically significant (likely related to the small number of patients), higher rejection rates were observed in the rtv+ PI group. Future studies should evaluate whether thymoglobulin induction is associated with lower rejection rates compared to IL-2 RA induction.

420.2

Trends in immunosuppressive medication use among hemodialysis patients with failed kidney transplants in Europe, Australia, New Zealand and North America: results from the dialysis outcomes and practice patterns study

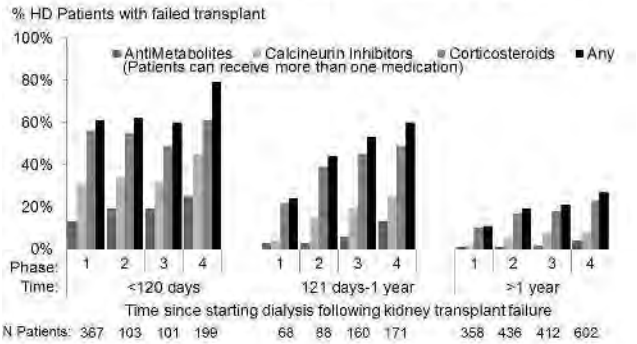
Jarcy Zee[1], Maria Larkina[1], Bruce M. Robinson[1], Francesca Tentori[1], Joan Fort[2], Richard J. Fluck[3], Hal Morgenstern[4], Alan B. Leichtman[1]. [1]Arbor Research, Ann Arbor, MI, United States; [2]Hospital Vall d'Hebron, Barcelona, Spain; [3]Royal Derby Hospital, Derby, United Kingdom; [4]University of Michigan, Ann Arbor, MI, United States.

Kidney transplant recipients who experience transplant failure and transition to dialysis typically are discontinued from their immunosuppressive medications (IM). However, the optimal timing of discontinuation is unknown and practices vary. Early IM discontinuation is undertaken with the intention of avoiding infections and other adverse effects from IM use; whereas, later IM discontinuation may prevent human leukocyte antigen antibody sensitization and need for graft nephrectomy. We used data from the Dialysis Outcomes and Practice Patterns Study (DOPPS) to investigate trends in the timing of IM withdrawal among hemodialysis (HD) patients following kidney transplant failure.

DOPPS is a multinational prospective cohort study of HD patients ≥18 years of age. For the current study, we assessed DOPPS enrollees in Australia, Belgium, Canada, France, Germany, Italy, New Zealand, Spain, Sweden, United Kingdom, and United States in four study phases: Phase 1 (1996-2001), Phase 2 (2002-04), Phase 3 (2005-08) and Phase 4 (2009-2012). A total of 3065 patients with a history of a failed kidney transplant and information about medication prescriptions were included. The percent of patients at time of enrollment receiving antimetabolites (azathioprine, mycophenolate mofetil, or mycophenolate sodium), calcineurin inhibitors (cyclosporin or tacrolimus), and oral corticosteroids (prednisone, prednisolone or methylprednisolone) were calculated by study phase and the time since initiation of dialysis after transplant failure.

From DOPPS Phase 1 to Phase 4, the percent receiving any IM increased from 61% to 79% for those patients within 120 days after transplant failure, from 24% to 60% for patients 121 days to 1 year after transplant failure, and from 11% to 27% for patients >1 year after transplant failure. The percentage of patients maintained on each class of IM also increased from DOPPS Phase 1 to Phase 4 (Fig). Corticosteroids were the most commonly used IM across phases and at any time after transplant failure, followed by calcineurin inhibitors and antimetabolites. IM prescription also varied across countries, 2 fold among patients <1 year since transplant failure, and 5 fold among patients >1 year since transplant failure.

Increasing use over time of immunosuppressive medications in dialysis patients with failed kidney transplants



In Europe, Australia, New Zealand and North America, there has been a temporal trend towards longer use of IM in patients with failed kidney transplants who have transitioned to dialysis. Corticosteroids remain the most commonly used IM, but prolonged calcineurin inhibitor and antimetabolite use are also more common. Studies are needed to determine whether longer periods of immunosuppression following kidney transplant failure result in lesser degrees of sensitization and fewer graft nephrectomies, or alternatively in higher rates of infections or other complications, and whether outcomes are dependent on the choice of maintained IM.

420.3

Long term outcomes of highly sensitized kidney transplant recipients

Jude Yagan[1], Anil Chandraker[2], Colm Magee[2].
[1]Nephrology, Organ Transplant Centre, Kuwait, Kuwait;
[2]Nephrology, Brigham and Women's hospital, Boston, MA, United States.

Aim: To follow the clinical outcomes of 45 highly sensitized patients who had undergone a desensitization protocol prior to kidney transplantation, and report the incidence of complications, allograft survival, and patient survival.
Methods: We conducted a retrospective review of 45 kidney transplant recipients transplanted between 9/2002 and10/2011, who had a positive T or B cell complement dependent cytotoxic (CDC) crossmatch assay. B cell CDC crossmatches were confirmed with a solid-phase assay to determine presence of class II anti-HLA antibodies.
Results: All subjects completed a desensitization protocol of plasmapheresis, intravenous immunoglobulin, +/- rituximab to render a negative T cell crossmatch or a negative or weak titer B cell crossmatch 24 hours prior to transplantation. Post-transplant all recipients received antibacterial and antiviral prophylaxis; allograft biopsies were performed when clinically indicated. The mean and median follow-up was 5 years. Thirty-three subjects (73%) suffered acute rejection of the allograft, 30 (67%) occurred in the first year post-transplant, and 27 (60%) occurred in the first month post-transplant. There was 1 case of hyperacute rejection necessitating transplant nephrectomy. Twenty-nine of the 33 (88%) were cases of acute antibody mediated rejection. BK viremia occurred in 7 patients (15.5%), leading to graft loss in 3. There were 5 patients that suffered multiple pneumonias, 5 cases (11%) of bacteremia, 1 case of fungemia, and 4 patients (8.8%) with cytomegalovirus infection. There were no cases of lymphoproliferative disease, although 1 patient developed an aggressive cutaneous angiosarcoma and died. There was also one case of renal cell carcinoma, and 4 cases (9%) of skin malignancies. The 1, 3, and 5 year allograft survival was 87%, 76%, and 68% respectively. The 1, 3, and 5 year patient survival was 93%, 91%, and 84% respectively.
Conclusion: Patients with a positive CDC crossmatch that are transplanted after a plasmapheresis-based desensitization protocol have high rates of acute rejection and infectious complications. Despite increased rate of rejection and over-immunosuppression, patient and graft survival in the desensitized group is comparable to the 1, 3, 5 year survival (graft: 89%, 78%, 67% respectively; patient: 95%, 90%, 85% respectively) of recipients of repeat transplants from living donors.

420.4

Use of mobile internet application to improve adherence to immunosuppressive medication adherence in kidney transplant recipients: preliminary results of the PRIMA (ImPRoving Adherence to Immunosuppressive Therapy by Mobile Internet Application) study

Ahram Han, Min-Ji Cho, Sungshin Cho, Chang Hwan Lee, Chanjoong Choi, Song-Yi Kim, Hyejin Hong, Sanghyun Ahn, Seung-Kee Min, Sang-Il Min, Jongwon Ha, Sang Joon Kim.
Department of Surgery, Seoul National University College of Medicine, Seoul, Korea.

Background: Nonadherence to immunosuppressive medication after renal transplantation is critical in terms of graft and patient outcome. The goal of this study was to determine whether usage of mobile application can improve adherence to calcineurin inhibitors.
Methods: Renal allograft recipients aged 15-70 years old, who were at least 12 month post-transplant, and on tacrolimus or cyclosporine based immunosuppressant regimen were enrolled. The internet based mobile phone application for androids providing alarm reminders at the time of dosing, data logs, and medication information (e.g., dosages, adverse effects, toxicities) was developed for this study. Patients were randomly assigned either to the mobile application (MA) group or the control group. The MA group were educated to use mobile phone application. Adherence were assessed with medication event monitoring system (MEMS), and self-reported surveys [Basel assessment of adherence to immunosuppressive medications scale (BASIS), visual analog scale (VAS)] in both group. The primary outcome was proportion of participants with adequate adherence (≥ 80% of prescribed doses taken by MEMS) during the 6 month trial. The study is ongoing and we hereby report the preliminary results of 124 participants; the complete data of 138 patients will be available at the meeting.
Results: Among 124 participants, 45 (36.2%) were male, 70 (56.4%) received a kidney from a living donor, and 103 (83%) had high education (more than high school education). The proportion of patients with adequate adherence did not differ between the MA group and the control group (74.1% vs. 66.1%, P=0.360). Similarly, there was no between-group difference in the adherence by MEMS as continuous variable (median 96.2%, IQR 20.8 vs. median 92.5% IQR 29.0, P=0.243) or adherence measured using the BASIS (at 6 month, 56.8% vs. 43.8%, P=0.192) or VAS scale (at 6 month, 41.2% vs. 50.0%, P=0.378). Proportion of adherent patient by self-reported surveys tend to increase at 1 month after study participation, but the improvement was not sustained at 3 or 6 months.
Conclusion: Six month intervention with mobile phone application was not associated with significant difference in adherence to immunosuppressant in patients with renal transplantation.

420.5

Characterization of genetic variation influencing pharmacokinetics of once-daily tacrolimus formulation by next generation sequencing technologies in Korean

Song-Yi Kim[1], Min-Ji Cho[1], Sungsin Cho[1], Chang Hwan Lee[1], Seonghae Yoon[2], Ahram Han[1], Chanjoong Choi[1], Sanghyun Ahn[1], Sang-il Min[1], Seung-Kee Min[1], Injin Jang[2], Sang Joon Kim[1], Jongwon Ha[1].
[1]Surgery, Seoul National University College of Medicine, Seoul, Korea; [2]Clinical Pharmacology and Therapeutics, Seoul National University College of Medicine, Seoul, Korea.

Therapeutic drug monitoring (TDM) is highly recommended for Tacrolimus (Tac) formulation and is currently baased on the measurement of blood trough concentrations after dosing. Pharmacogenomics has rapidly emerged as an additional tool to refine dose selection and to select the first dose to administer. CYP3A5*3 (rs776746) has been clearly demonstrated as a main genetic factor influencing Tac dose-adjusted trough blood concentrations. CYP3A4*22 (rs35599367), although to a lesser extent, has been also shown to influence Tac dose-adjusted trough blood concentration. However, a significant amount of variation still exists. In an effort to identify genetic variants associated with pharmacokinetic variation in once-daily tacrolimus (Advagraf®), we evaluated genotypic effect using DMETTM Plus Array.
The genotypic effect of the drug metabolism/transporter on the pharmacokinetic characteristics of Advagraf® was explored in 25 Korean de novo kidney transplant patients. The target genes for analysis was the drug absorption, distribution, metabolism and elimination related enzymes (ADME) and transporters. The 1,836 single nucleotide polymorphism (SNP) and 5 copy number variations (CNV) were evaluable after removing the variants, which were homozygous in (nearly) all samples. The Full AUC data were obtained around 10 days after transplantation. We tested for possible associations between ADME and transporter variants and tacrolimus trough level, concentration/dose (C/D) ratio, dose/weight-normalized AUClast and AUCinf.
Three novel genetic variants (CYP3A4 (rs3735451), CYP3A4 (rs4646440), and CYP3A4 (rs2242480) as well as CYP3A5*3 (rs776746) were identified. rs3735451 (CYP3A4) T-carriers, rs4646440 (CYP3A) G carriers and rs22422480 (CYP3A4) C-carriers were associated with higher C/D ratio and dose/weight-normalized AUCinf, with similar trough concentrations.
This research suggests that next generation sequencing allow us to detect new population-based pharmacogenetic biomarkers. Further larger investigation is needed to determine whether these associations have clinical utility beyond TDM.

420.6

Adverse events of maintenance immunosuppression following kidney transplantation reported in randomized controlled trials: A systematic review.

Martin Howell[1,2], Reichelle Yeo[1,2], Allison Tong[1,2], Jonathan C.Craig[1,2], Kirsten Howard[2], Germaine Wong[1,2,3].
[1]Centre for Kidney Research, The Children's Hospital at Westmead, Sydney, Australia; [2]School of Public Health, University of Sydney, Sydney, Australia; [3]Centre for Transplant and Renal Research, Westmead Hospital, Sydney, Australia.

Background: Side effects of maintenance immunosuppression have a major effect on quality of life and non-adherence of kidney transplant recipients. It is known that patients are willing to accept side effects as a necessary part of treatment after kidney transplantation, however it is unclear whether clinical trials inform patients of what side effects to expect in terms of both frequency and severity. The aim of this review was to evaluate the completeness of reporting of adverse events in randomized controlled trials (RCTs) of maintenance immunosuppression in kidney transplantation.
Methods: The Cochrane Kidney and Transplant Group Register was searched (January 2003 to December 2015) to identify RCTs reporting adverse events specific to drug related side effects. Reporting was appraised using the harms-extension of the CONSORT checklist. Data extracted included the characteristics of trials reporting and not reporting adverse events, and the adverse events reported.
Results: Of the 233 trials, 151 (68%) reported adverse events, and 99 (65%) of these trials complied with fewer than 11 of the 23 items in the harms-extension CONSORT checklist. Identification and justification of adverse events were reported in only 28 (19%) and 19 (13%) trials respectively, and assessment of severity in 34 (23%) trials. In 98 (67%) trials, adverse events were used to support either positively or negatively the conclusions. Compliance with the CONSORT checklist was positively associated with the year of publication (P=0.01), industry funding (P=0.02), and the number of adverse events reported (P<0.001) and negatively associated with trial duration (P=0.003). In total, 40 types of adverse events were reported in the 151 trials. The three most commonly reported adverse events were gastrointestinal (70%), peripheral oedema (31%), and undefined "adverse events" (22%). In 107 reports involving calcineurin inhibitors, 22% and 3% reported the common side effects of tremors and paraesthesia respectively and none reported anxiety, aggression or mood swings. For mTOR inhibitors (n=27 trials), 41% and 19% reported the common side effects of skin complaints and headaches respectively. All of the 27 mycophenolic acid trials reported gastrointestinal events, with only three other adverse events reported in more than 10% of the trials namely, abdominal pain (15%), headache (11%), and undefined "adverse event" (11%).
Conclusions: Reporting of adverse events in RCTs of maintenance immunosuppression in kidney transplantation is inadequate and poorly defined. Common side effects are rarely reported. Despite this, adverse events influenced conclusions in over half of the trials. Reporting of adverse events in RCTs should adhere to CONSORT and reflect adverse events that are commonly experienced or important to patients. This can inform patient-centred clinical decision-making.

420.7

A high intra-patient variability in tacrolimus exposure is associated with poor long-term outcome of kidney transplantation

Nauras Shuker[1,2], Lamis Shuker[1], Joost van Rosmalen [3], Joke I. Roodnat[1], Lennaert C.P. Borra[2], Willem Weimar [1], Dennis A. Hesselink [1], Teun van Gelder [1,2].
[1]Internal Medicine , Erasmus MC , Rotterdam , Netherlands; [2]Hospital Pharmacy , Erasmus MC, Rotterdam , Netherlands; [3]Biostatistics, Erasmus MC, Rotterdam , Netherlands.

Background: Tacrolimus (Tac) is a critical dose drug with a considerable intra-patient variability (IPV) in its pharmacokinetics[1]. We investigated whether a high IPV in Tac exposure is associated with adverse long-term renal transplantation outcomes.
Methods: Tac IPV was calculated from pre-dose concentrations measured between 6 and 12 months post-transplantation of 808 renal transplant recipients (RTRs) transplanted between 2000 and 2010. As a primary outcome, a composite end-point consisting of graft loss, biopsy-proven rejection, transplant glomerulopathy, or 'doubling of serum creatinine concentration between month 12 and last follow-up' was used.
Results: One hundred eighty-eight (23.3%) patients reached the composite end-point. The cumulative incidence of the composite endpoint was significantly higher in patients with high Tac IPV than in patients with low IPV (hazard ratio 1.41, 95%-CI: 1.06 – 1.89; p = 0.019). After adjustment for several recipient and transplantation factors the higher incidence of the composite endpoint for RTRs with a high IPV remained statistically significant, with a hazard ratio of 1.37 (95%-CI: 1.02 – 1.84; p = 0.035). Younger recipient age at transplantation, worse graft function (eGFR) and low mean Tac concentration at 1-year post-transplantation were additional predictors for worse long-term transplant outcome.
Conclusion: A high Tac IPV is an independent risk factor for adverse kidney transplant outcomes which has potential as an easy monitoring tool to help identify high-risk RTRs.
References:
[1] Shuker N. Intra-patient variability in tacrolimus exposure: Causes, consequences for clinical management. *Transplant Rev*.2015;29: 78-84.

420.8

Does post-transplant adherence come at a cost?

Kunal Yadav[1], Thomas Nevins[2], William Thomas[2], William N.Robiner[2], Arthur J.Matas[1].
[1]Transplant Surgery, University of Minnesota, Minneapolis, MN, United States; [2]Pediatric Nephrology, University of Minnesota, Minneapolis, MN, United States.

Background: It is well documented that organ transplant (tx)recipients have an increased incidence of cancer including virus associated cancers like lymphoma. Our objective was to assess the correlation between immunosuppression adherence, patient's cumulative immunosuppression exposure, and incidence of cancer.
Methods: Between 1998 and 2006, 195 kidney tx recipients agreed to prospective electronic medication monitoring. Quantitative adherence to a single immunosuppressant drug (azathioprine, n=17; sirolimus, n=28; mycophenolate, n=150), averaged over 6 months was used to divide the patients into 3 equal groups (Best adherence ≥ 97.8%; Middle 91-97.7%; Worst adherence ≤ 90%). We retrospectively examined the incidence of cancer in these patients.
Results: The mean duration of follow-up (7.6 yrs) was similar in all groups. The cumulative cancer incidence was highest in the best adherent group, and lowest in the worst adherent group, p=0.029 (figure1, table1).

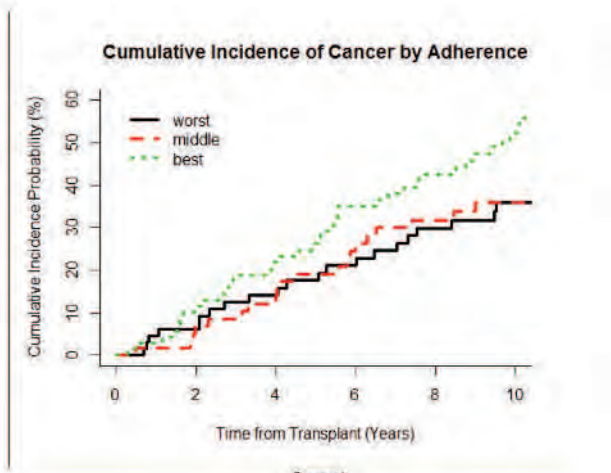


figure1

Table -1, Distribution of cancer by adherence, p=.019 (*Chi* square test)

Cancer	Best	Middle	Worst
Carcinoma	13	8	3
Hematologic (non-PTLD)	1	1	0
PTLD	3	1	1
Skin cancer	33	17	22
No Cancer	29	40	43
Total patients	**69**	**61**	**65**

Those with the best adherence had an absolute cancer rate of 6.2cases (per 100 pt-yrs at risk) during the first 5 yrs; , and 9.9 cases/100 pt-yrs) in yrs 5-10. For the group with least adherence, cancer rate was 4.1/100 pt yrs for the first 5 yrs; 6.0 cases/100 pt-yrs in yrs 5-10. If non-melanocytic skin cancers are excluded, the cancer incidence was significantly higher in the best adherent group (p=0.011). After adjusting for age and treating death as a competing risk, adherence grouping remained a significant factor in the incidence of cancer [p = 0.035], with the cancer hazard (vs best adherence group) 35% lower in the worst adherence group, and 49% lower in the midrange adherence group.

Conclusion: Medication adherence, although lowering AR rates and increasing long-term graft survival, is associated with increased cancer risk. Individualizing treatment to decrease the risk of either graft loss or other adverse outcomes, including malignancy, is necessary. Trials are needed to determine how to achieve this balance.

420.9

Low prevalence of post post-transplant anti-HLA Class 2 antibodies in living donor renal transplant recipients treated with ATG induction therapy – a single centre experience

Galina Severova[1], Aleksandar Sikole[1], Trajceska Lada[1], Ilina Danilovska[1], Zivko Popov[2], Ninoslav Ivanovski[1].
[1]University Clinic of Nephrology, Medical Faculty, Skopje, Macedonia, The Former Republic of Yugoslav; [2]Macedonian Academy of Sciences and Arts, Department od Medical Sciences, Skopje, Macedonia, The Former Republic of Yugoslav.

Background: The mono or polyclonal antibodies induction therapy became a standard treatment after solid organ transplantation. However, the long term effect of induction regarding anti –HLA antibodies is not yet very well known. The aim of our cross-sectional study is to investigate the prevalence of anti- HLA Class 1 and 2 antibodies in living donor kidney recipients treated with different induction therapy (ATG or Simulect) approximately 60 months after the surgery.
Methods: The serum samples from 46 stable living donor transplant recipients 61 months after the surgery (range 12 to 150) were investigated using a single antigen Luminex assay. Mean fluorescence intensity (MFI) more then 800 has taken as a relevant amount of antibodies. All patients were under standard immunosuppression including induction therapy (ATG or Basiliximab) and triple drug maintenance therapy (Tacrolimus or Cyclosporine, MMF and steroids).The pretransplant screening for DSA by CDC cross match was negative in all patients. Living donor renal transplantation was performed using predominantly HLA haploidenthical parents (n=38 or 84.4%) and non-related (spousal) donors (n=7 or 15.6%) with a minimum 1 HLA compatibility. According to the induction therapy, the patients were divided in ATG treated group (Group 1) and Basiliximab group (Group 2).There were no statistical difference in both Groups regarding recipient's age (41 vs. 42.8) gender (male 76% vs. 72%), primary renal diseases and the average time of follow up (61.16 vs. 61.22 months). The renal function (GFR-MDRD), Serum Creatinine, proteinuria and rejection episodes were analyzed
Results: A relevant amount of anti-HLA Class 2 were detected in 13 recipients, 3 in ATG group (15%) and 10 in SIM group (38%) with an average MFI of 980 compared vs. 4630, respectively ($p<0.01$). Regarding anti-HLA class 1 antibodies the differences are not significant (20% vs. 26.6%). The most frequent anti-HLA antibodies identified were against A1, A2, A3, A68, B44, B45, DR18, DR7, DR17, DQ 7, DQ8 and DQ9. The actual serum creatinine was 120 vs. 132 μmol/L, while GFR was 55.5 vs.56.6 ml/min in Group 1 and 2, respectively. Both differences are not statistically significant. In the same time pts in Group I showed significantly less proteinuria than pts in Group 2 (0.44 vs. 0.75 gr/24h, respectively). There was no statistical difference between the rejection episodes (17.6 % vs. 14.2 %) and DSA (one patient in Group 1 and two pts in group 2).
Conclusion: The authors emphasize the positive role of ATG induction therapy on incidence and prevalence of anti HLA-Class 2 antibodies which could be of importance for long term graft and patients survival. More careful investigation including protocol biopsies are needed for more relevant data regarding the long term clinical impact of anti-HLA Class 2 antibodies over the graft and patient survival rate and possible therapeutic approach.

420.10

Simultaneous limited blood sampling models for tacrolimus and mycophenolic acid provide an accurate area under the curve prediction for kidney transplant recipients

Kouhei Nishikawa[1], Manabu Kato[1], Yuko Yoshio[1], Yoshihiro Hasegawa[1], Hideki Kanda[1], Masahiro Kanai[1], Kiminobu Arima[1], Tomoyuki Enokiya[2], Yuichi Muraki[2], Takuya Iwamoto[2], Masahiro Okuda[2], Yoshiki Sugimura[1].
[1]Department of Nephro-Urologic Surgery and Andrology, Mie University School of Medicine, Tsu, Mie, Japan; [2]Department of Pharmacy, Mie University Hospital, Tsu, Mie, Japan.

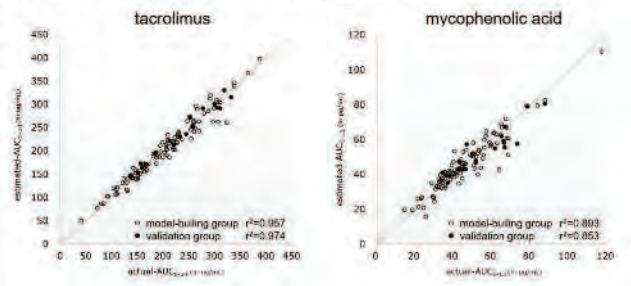
Introduction: Immunosuppressive therapy consisting of extended-release tacrolimus (TAC-ER) and mycophenolate mofetil (MMF) has been widely used for kidney transplant recipients in Japan. Although the area under the curve (AUC) is considered to be the best marker of overall exposure to tacrolimus (TAC) and mycophenolic acid (MPA), multiple blood concentration measurements obtained during the full dosing duration can be invasive for patients and increase the cost. Therefore, limited sampling strategies, where the AUC is estimated from less measurements using multiple linear regression analysis, could be very useful in clinical practice. To date, there have been no reports describing limited sampling models for TAC-ER and MPA to predict the corresponding AUCs using samples from the same time points.
Objectives: The purpose of this study was to build and validate limited sampling models for TAC-ER and MPA to predict the corresponding AUCs using samples from the same time points among kidney transplant patients.
Methods: We retrospectively analyzed 120 full sets of TAC and MPA blood concentration measurements from 46 kidney transplant recipients at our institution from August 2010 to April 2015. The immunosuppressive regimen used for participants was the combination of TAC-ER, MMF and methylprednisolone. Blood samples were taken to determine the blood concentrations of TAC at 0, 1, 2, 3, 4, 6, 8, 12, 16, and 24 hours and MPA at 0, 0.5, 1, 2, 3, 4, 6, 8, and 12 hours after oral administration of TAC-ER or MMF, respectively. The blood concentrations of TAC and MPA were measured using an affinity column mediated immunoassay and enzyme multiplied immunoassay technique, respectively. The actual-$AUC_{0\text{-}24}$ of TAC and actual-$AUC_{0\text{-}12}$ of MPA were calculated by the trapezoidal method. Patients were randomly divided into 2 groups; one group was for model-building (n=100) and the other group was for validation (n=20). In the model-building group, stepwise multiple linear regression models for estimating the AUCs of TAC and MPA were applied based on 4 sampling time points after drug administration in order to make this approach acceptable in clinical practice. In both groups, a correlation coefficientswas calculated between the actual- and estimated-AUCs.
Results: The best sampling points to estimate the actual-AUCs of both TAC and MPA were 1, 3, 8 and 12 hours after oral administration (Table 1).

Table1. Estimating equation for AUC of tacrolimus and mycophenolic acid

Immunosuppressive agents	Regression formula	r^2
tacrolimus ($AUC_{0\text{-}24}$)	14.92+1.88×C1+2.48×C3+5.29×C8+11.58×C12	0.957
mycophenolic acid ($AUC_{0\text{-}12}$)	6.72+1.06×C1+2.81×C3+4.49×C8+2.53×C12	0.893

The correlation coefficients (r^2) between the actual- and estimated-AUCs of TAC in the model-building and validation groups were 0.957 and 0.974, respectively. The correlation coefficients between the actual- and estimated-AUCs of MPA in the model-building and validation groups were 0.893 and 0.853, respectively (Figure 1).

Figure1. Correlation between actual-AUC and estimated-AUC



Conclusion: We demonstrated that it was possible to precisely estimate the AUCs for both TAC and MPA by taking blood samples at only 4 time points, which could be more clinically beneficial for kidney transplant patients.

420.11

A comparative study between low dose anti-thymocyte globulin versus basiliximab as induction therapy for standard immunologic risk kidney transplant patients

Paolo Miguel David[1], Glenda Eleanor P.Pamugas[1].
[1]Adult Nephrology, National Kidney and Transplant Institute, Quezon, Philippines.

Introduction: Induction therapy with immunosuppressive medications has changed the world of solid transplants today. rATG, a lymphocyte depleting polyclonal antibody, is typically used in either medium-risk or high-risk kidney transplants. Basiliximab, a monoclonal antibody, is often used in patients who are at low or standard immunologic risk. Based on results of previous studies done using low dose rATG , our center has been using either basiliximab or rATG at 1.0-1.5 mg/kg/day for 3 days for standard immunologic risk patients, but no comparison of clinical outcomes has ever been done.
Methods: A retrospective cohort study with 165 patients who underwent kidney transplant at NKTI from June 2012 until December 2014 and given either Basiliximab or rabbit ATG until 1 year post transplant. The following were collected: age, gender, native kidney disease, induction therapy used, maintenance immunosuppression used, number of HLA mismatches, episodes of acute rejection, delayed graft function, infections and incidence of malignancies. Statistical treatment of data was done using SPSS version 22. Log rank test and Cox-regression were used to analyze graft and patient survival. Mann Whitney and Fischer's Exact test were used to compare differences of outcomes between the groups.
Results: Among the 165 patients, there were no graft failures nor deaths within 1 year post transplant. There was no significant difference in terms of renal function and incidence of acute rejection but severity of acute rejection was lower in the ATG group. 7 patients had episodes of acute rejection with 6 of them belonging to the Basiliximab group. 1 patient had an acute antibody mediated rejection and treated with methylprednisolone pulsing combined with plasmapharesis and CYA was shifted to Tacrolimus. The others were acute cellular rejection and steroid responsive. There was no significant difference in terms of delayed graft function. 1 patient under the Basiliximab group had delayed graft function due to surgical complications and CNI toxicity. There was no significant difference in terms of infections within 1 year post transplant. 7 patients in the rATG group and 32 in the Basiliximab group had infections. Among the Basiliximab group, 1 presented with CMV infection while another presented with sepsis secondary to Bartholin cyst abscess. The most common type of infection was urinary tract infection. No patients developed malignancy within 1 year after transplant.
Conclusion: Based on this study, low dose rATG is effective and safe for induction therapy in standard risk patients. It also showed that low dose rATG when compared to basiliximab was as effective in preventing acute rejection and incidence of infection was not greater. Having the option of using low dose rATG has a huge impact on clinical management since this will be less costly. Further studies are needed to see the long term outcomes of low dose rATG in comparison to Basiliximab.
References:
[1] Brennan et al. *Rabbit Antithymocyte Globulin versus Basiliximab in Renal Transplantation*. New England Journal of Medicine 2006; 355
[2] Andress et al. *Rabbit Anti-Thymocyte Globulin induction i renal transplantation: review of the literature*. Transplant and Research Management 2014; 6
[3] Guodong Chen et al. *Efficacy and safety of Thyroglobulin and Basiliximab in Kidney Transplant for patients at high risk for acute rejection and delayed graft function*. Exp Clinical Transplant 2013; 11
[4] Patel et al. *Low-dose rabbit Anti-Thymoglobulin versus Basiliximab for induction therapy in kidney transplantation*. Saudi Journal of Kidney Disease and Transplantation 2014; 25
[5] Mothy et al. *New directions for rabbit Anti-Thymocyte Globulin in solid organ transplantation, stem cell transplants and autoimmunity*. Drugs 2014
[6] Waichi Wong et al. *Comparison of two dosages of thymoglobulin used as a short course for induction to kidney transplantation*. Transplant International 2006
[7] Atlani et al. *Basiliximab induction in renal transplantation: Long term outcome*. Saudi Journal of Kidney Diseases and Transplantation 2013; 24
[8] Hardinger et al. *Thymoglobulin induction dosing strategies in a low risk kidney transplant population: Three or four days?*. Journal of Transplantation 2010
[9] Grafals et al. *Immunophenotyping and efficacy of low dose ATG in non-sensitized kidney recipients undergoing early steroid withdrawal: A randomized pilot study*. PLOS One 2014
[10] Issa et al. *Infectious complications of anti lymphocyte therapies in solid organ transplantation*. Oxford Journals: Clinical Infectious Diseases 2009; 48
[11] Djamali et al. *Low dose antithymocyte globulins in renal transplantation* . Transplantation 2000; 69
[12] Kesiraju et al. *Anti-Thymocye Globulin versus Basiliximab induction in renal transplant patients: Long term outcome* . Saudi Journal of Kidney Diseases and Transplantation 2014; 25
[13] Philippine Health Insurance Corporation. *Circular 30, s-2012*. Philhealth.gov.ph

420.12

Depression of complement regulatory factors in rat and human renal grafts is associated with the progress of acute T-cell mediated rejection

Kazuaki Yamanaka[1], Yoichi Kakuta[1], Shuji Miyagawa[2], Shigeaki Nakazawa[1], Toyofumi Abe[1], Ryoichi Imamura[1], Akira Maeda[2], Hiroomi Okuyama[2], Norio Nonomura[1].
[1]Urology, Osaka University Graduate School of Medicine, Suita, Japan; [2]Surgery, Osaka University Graduate School of Medicine, Suita, Japan.

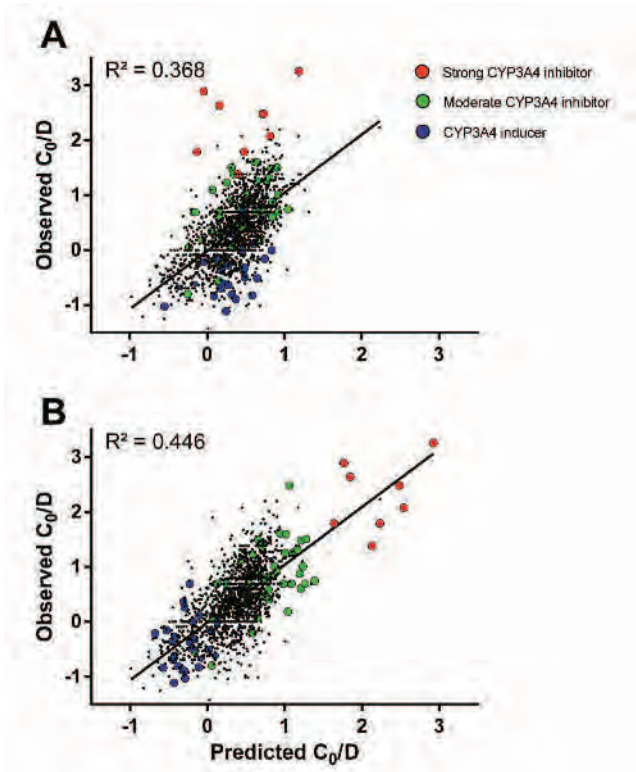
Introduction: It is commonly known that complements play important roles in antibody-mediated rejection in renal transplantation and drugs that control complements are sometimes used for treating affected patients. However, the association of complements with progression of acute T cell mediated rejection (ATCMR) has not been fully elucidated. We investigated the production of complement components and complement regulatory proteins (Cregs) in ATCMR using rat and human renal allografts.
Methods and Methods: We prepared rat allograft (n=6) and syngeneic graft (n=3) models of renal transplantation. The expressions of C1q, C3, C3aR, C4, Factor B, C5, C5aR, C9, CD59, and the rodent-specific complement regulator complement receptor 1-related gene/protein-y (Crry) over time were assessed in the rat grafts using quantitative real-time PCR (qRT-PCR) and immunofluorescent staining. We also administered anti-Crry and anti-CD59 antibodies to the rat allograft model, and assessed overall survival. Further, we obtained data from 67 human renal transplant recipients diagnosed with ATCMR, and divided them into high and low membrane cofactor protein (MCP) expression groups by immunohistochemical staining, and assessed the relationship between the expression of MCP and their clinical course.
Results and Discussion: qRT-PCR results showed that the expression of Cregs, CD59 and Crry, was diminished in the rat allograft model especially on day 5 after transplantation in comparison with the syngeneic model ($p < 0.01$). In contrast, the expression of complement components and receptors: C1q, C3, C3a receptor, Factor B, C5a receptor, C9 was increased, but not the expression of C4 and C5, indicating a possible activation of the alternative pathway. Compared to the IgG control group, the group that was administered the anti-Crry mAb had a significantly shortened median survival period from 8.60 (1.03) days to 4.80 (0.49) days ($p<0.01$). Delivering anti-CD59 mAb also reduced the survival duration to 5.86 (0.70) days, but this value did not reach the level of significance ($p=0.08$). In the human ATCMR cases, the group with higher MCP expression in the grafts showed improved serum creatinine levels after the ATCMR treatment as well as a better 5-year graft survival rate ($p<0.05$ for each).
Conclusion: We conclude that the expression of Cregs in allografts is connected with ATCMR. Our results suggest that controlling complement activation in renal grafts can be a new strategy for the treatment of ATCMR.

420.13

Effect of MDR1 diplotype on tacrolimus disposition in renal recipients depends on CYP3A5 and CYP3A4 genotype

Thomas Vanhove[1], Pieter Annaert[2], Dirk R.J.Kuypers[1].
[1]Nephrology and Renal Transplantation, University Hospitals Leuven, Leuven, Belgium; [2]Pharmaceutical and Pharmacological Sciences, KU Leuven – University of Leuven, Leuven, Belgium.

Introduction: Several clinical and genetic determinants of tacrolimus disposition have been identified. Tacrolimus exposure is decreased in patients possessing the CYP3A5*1 allele (CYP3A5 expressers) and increased in patients possessing the CYP3A4*22 polymorphism. The effect of polymorphisms in other genes such as MDR1 (coding for P-glycoprotein, P-gp), PPARA and NR1I2 has varied between studies.
Methods: We constructed a predictive linear mixed model for tacrolimus dose-corrected trough level (C/D ratio) at months 3, 12 and 24 after transplantation in a retrospective cohort of 766 predominantly Causasian adult renal recipients (n=2042 trough levels). All patients were genotyped for 32 single nucleotide polymorphisms with a proven or possible relevance to tacrolimus disposition. ABCB1, MRP2, OATP1B1, COMT, FMO, PPARA and APOA5 were analyzed as (functional) diplotype groups.



Results: Predictors of C/D ratio were presence of the CYP3A5*1 allele (n=118), hematocrit, age, presence of CYP3A4*22 (n=83), use of a CYP3A4 inhibitor or inducer, serum ALT and albumin, eGFR, tacrolimus formulation (once vs. twice daily), ABCB1 haplotype and time after transplantation. The final model explained 42.9% of interindividual variability in C/D. MDR1 diplotype had no effect in CYP3A5 expressers. In CYP3A4 wild type patients, model predicted C/D ratio was 1.46 (1.14-1.87), 1.47 (1.15-1.87) and 1.62 (1.28-2.07) ng/ml/mg for MDR1 CGC-CGC, CGC-TTT and TTT-TTT diplotypes, respectively, compared with 1.57 (1.13-2.17), 1.83 (1.35-2.49) and 2.55 (1.85-3.52) ng/ml/mg in CYP3A4*22 carriers (p=0.017). Overall model fit was significantly improved by correcting for whether or not patients

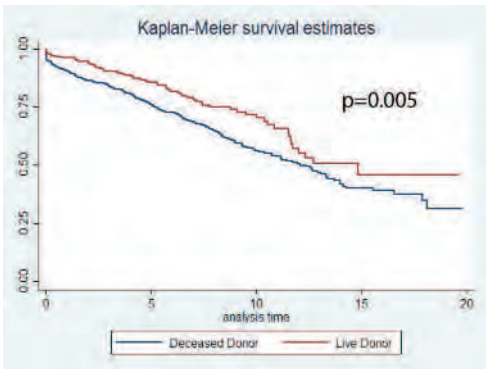
used concomitant CYP3A4 inhibitors or inducers, see Figure (A depicts model fit without correction for concomitant medication, R^2=0.368; B depicts model fit with correction, R^2=0.446).
Conclusion: Most genetic polymorphisms beyond CYP3A5*1 and CYP3A4*22 seem of limited use for tacrolimus modeling on a population level in Caucasians, after correction for clinical confounders. However, the effect of loss-of-function diplotypes in P-gp on tacrolimus disposition was strongly accentuated in CYP3A4*22 carriers but non-existent in CYP3A5 expressers, likely because high CYP3A activity reduces the importance of the P-gp transporter. The combination of loss-of-function in CYP3A5, CYP3A4 and P-gp might have a multiplicative effect leading to strongly increased tacrolimus exposure in specific patients.

421.1

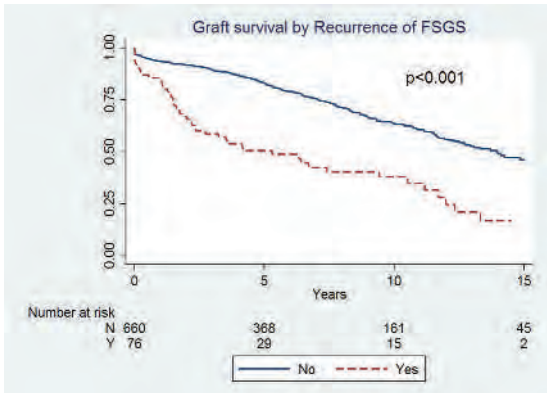
Outcomes of recurrent focal segmental glomerulosclerosis post kidney transplantation

Anna Francis, Peter Trnka, Steven McTaggart.
Paediatric Nephrology, Lady Cilento Children's Hospital, Brisbane, Australia.

Introduction: Recurrent focal segmental glomerulosclerosis (FSGS) after kidney transplantation contributes to poor graft outcomes[1]. There is uncertainty around using living donors for patients with FSGS because of concerns of an increased risk of disease recurrence in the transplanted kidney resulting in poorer transplant outcomes[2]. We aimed to determine the effect of donor source on recurrence and graft survival in patients with FSGS.
Materials and Methods: Using the Australian and New Zealand Dialysis and Transplant registry, we compared the risk of FSGS recurrence and graft loss between living donor (LD) and deceased donor (DD) kidney transplants in children and adults with biopsy proven FSGS over a twenty-year period. Statistical analysis included paired t test, chi squared and logistic regression analysis.
Results: Between 1992 and 2011, 736 first kidney transplants were performed in 666 adults and 70 children with primary FSGS. FSGS recurred in 76 (10.3%) patients and was more common in recipients of a LD transplant compared with DD (14% vs 8%, respectively, p=0.03). However, graft failure due to recurrent disease in the first 5 years after transplant (30 patients, 39%) was more common in DD recipients compared with LD recipients (20 vs 10, p=0.011) and median graft survival was significantly better for LD compared to DD grafts (14.8 years vs. 12.1 years; P=0.005).



On logistic regression, younger age and non-caucasian ethnicity were the only independent factors associated with increased risk of recurrence. Disease recurrence predicted poor graft outcomes with 51% (95% CI 38-62%) 5 year graft survival in the recurrence group compared to 83% (95% CI 79-86%) 5 year graft survival in the group without recurrent disease (p= <0.0001).



In those with disease recurrence, the majority of graft loss occurred in the first two years.

Discussion: Our study confirms that recurrent FSGS after kidney transplant predicts poor graft outcomes. Whilst FSGS more commonly recurred in LD grafts, graft survival in LD recipients was significantly better for both children and adults with FSGS. We postulate that the well described graft survival advantage of LD grafts may overcome the negative effect of increased risk of recurrence, with its attendant poor prognosis. Short term studies of outcomes of kidney transplantation for FSGS would only capture the adverse effects of early recurrence and graft loss, but long term follow up reveals the improved outcomes for LD grafts.

Conclusion: We propose live donor transplantation should not be avoided in patients with FSGS.

References:

[1] Hickson LJ, Gera M, Amer H, Iqbal CW, Moore TB, Milliner DS, Cosio FG, Larson TS, Stegall MD, Ishitani MB et al: Kidney transplantation for primary focal segmental glomerulosclerosis: outcomes and response to therapy for recurrence. Transplantation 2009;87:1232-1239.

[2] First MR: Living-related donor transplants should be performed with caution in patients with focal segmental glomerulosclerosis. Pediatric nephrology 1995, 9 Suppl:S40-42.

421.2

Age-independent influence of pre-transplant coronary artery disease and diabetes mellitus on patient survival after kidney transplantation

Fabian Halleck, Anna Kleinsteuber, Dmytro Khadzhynov, Lukas Lehner, Klemens Budde, Oliver Staeck.
Nephrology, Charite Universitaetsmedizin Berlin, Berlin, Germany.

Background: Pre-existing coronary artery disease (CAD) and diabetes mellitus (DM) are frequent comorbidities in kidney transplant candidates. Only limited data exists examining the influence of pre-existing CAD and DM on the long-term outcomes of kidney transplant recipients (KTR) in different age categories.

Methods: This long-term retrospective single center analysis included 839 deceased donor KTR transplanted 1999-2014. Prevalence and history of CAD and DM at time of transplantation were determined. Follow-up analysis over a maximal period of 15 years included patient and graft survival.

Results: 212 KTR (24.8%) with CAD and 138 KTR (16%) with DM were identified among all included patients. Mean follow-up in the cohort was 5.9±3.7 years. KTR with CAD or KTR with DM had a significantly worse 7-year survival compared to recipients without CAD or DM (after 7 years 58.7% vs. 84.0 %, p<0.0001; and 58.6% vs. 81.6%, p<0.0001, respectively).

KTR were further categorized according to age at transplantation. Due to the nature of the Eurotransplant senior program KTR ≥65 years of age were significantly more likely to have older donors, shorter median waiting time on dialysis, shorter cold ischemia time, more HLA-mismatches and a higher proportion of delayed graft function. In addition, these patients were less likely to have prior kidney transplantations and had higher creatinine levels after transplantation. CAD and DM were more frequently observed in the group of elderly KTR compared with the group of young KTR: 47% vs. 16% (p<0.001) for CAD, and 32% vs. 10% (p<0.001) for DM. Comparing elderly (≥65 years) and younger (<65 years) KTR with and without CAD, we found survival rates of 44.8% vs. 69.5% in elderly KTR, and 74.2% vs. 87.9% in younger KTR at 7-years post-transplant (Fig. 1a). Similar survival was observed for DM (Fig. 1b) in old (45.9% vs. 64.2%) and young recipients (74.6% vs. 87.1%). Graft survival rates, however, were not significantly influenced by these pre-existing conditions.

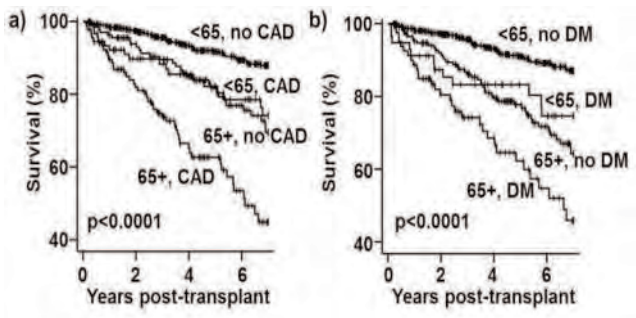


FIGURE 1. Patient survival depending on preexisting CAD (a) and DM (b) categorized by age at transplantation

Conclusion: Both, pre-existing CAD and DM significantly worsen patient survival in younger and elderly KTR. Our findings emphasize the importance of careful pretransplant evaluation as well as thorough monitoring and consequent treatment of these crucial comorbidities.

421.3

Frailty in kidney transplant recipients

Mara McAdams-DeMarco, Hao Ying, Israel Olorundare, Elizabeth King, Dorry Segev.
Surgery, Johns Hopkins, Baltimore, United States.

Background: Among kidney transplant (KT) recipients, frailty increases the risk of DGF, early hospital readmission, MMF intolerance and mortality. While it is clear that frail KT recipients represent a high risk group, the frailty correlates and high-risk patterns of the frailty components are unclear. Furthermore, it is unclear the best way to measure frailty in KT recipients of all ages.
Materials and Methods: 663 KT recipients were enrolled in a prospective cohort study (12/2008-8/2015) at Johns Hopkins Hospital. The Fried frailty phenotype (5 components: weight loss, poor grip strength, low activity, exhaustion and slowed walk speed), activities of daily living (ADL)/ instrumental activities of daily living (IADL) disability, Centers for Epidemiologic Studied-Depression Scale (CESD), education and health related quality of life (HRQOL) were measured at evaluation and KT admission. Additionally, age, sex, race, obesity status, smoking status, time on dialysis, cause of ESRD and donor type were also ascertained from the medical record. We used adjusted multinomial regression to identify frailty correlates. We identified which pattern of the 5 components was associated with mortality using adjusted Cox proportional hazards models.

Table: Correlates of Frailty Status Among Kidney Transplant (KT) Recipients (n=663).

Correlate	Intermediately frail		Frail	
	PR (95% CI)	P value	PR (95% CI)	P value
Age ≥ 65 years	1.83 (1.14, 2.98)	0.01	2.11 (1.16, 3.85)	0.015
Female sex	1.17 (0.79, 1.74)	0.42	0.94 (0.58, 1.54)	0.82
Black race	0.69 (0.45, 1.07)	0.10	0.97 (0.56, 1.68)	0.92
Obesity				
Normal weight (BMI<25)	Ref		Ref	
Overweight (BMI 25-29)	0.85 (0.54, 1.35)	0.49	1.06 (0.60, 1.87)	0.85
Obesity (BMI ≥30)	1.11 (0.69, 1.80)	0.67	1.10 (0.61, 2.01)	0.75
IADL disability	1.35 (0.74, 2.49)	0.33	3.21 (1.66, 6.22)	0.001
CESD depression	4.16 (1.51, 11.47)	0.006	11.87 (4.33, 32.58)	<0.001
< High school education	1.63 (0.70, 3.81)	0.25	3.66 (1.54, 8.73)	0.004
Current smoker	0.69 (0.28, 1.72)	0.42	0.40 (0.13, 1.26)	0.12
No residual kidney function	0.74 (0.46, 1.18)	0.21	1.67 (0.80, 3.46)	0.17
Time and type of dialysis				
Not on dialysis	Ref		Ref	
Hemodialysis; 0-2 y	2.38 (1.26, 4.49)	0.008	1.21 (0.48, 3.05)	0.68
Hemodialysis; >2 y	1.22 (0.67, 2.20)	0.52	0.83 (0.38, 1.80)	0.64
Peritoneal dialysis; 0-2 y	1.14 (0.46, 2.84)	0.77	0.61 (0.12, 3.12)	0.53
Peritoneal dialysis; >2 y	2.20 (0.55, 2.61)	0.65	0.92 (0.28, 3.01)	0.89
Cause of ESRD				
Hypertension	Ref		Ref	
Diabetes	1.09 (0.63, 1.88)	0.76	1.23 (0.61, 2.45)	0.57
Glomerulonephritis	0.48 (0.14, 1.59)	0.23	1.31 (0.41, 4.17)	0.64
Other	0.94 (0.59, 1.50)	0.81	0.93 (0.52, 1.67)	0.80
Self-rated HRQOL				
Excellent/Very good	Ref		Ref	
Good	1.50 (0.90, 2.48)	0.12	2.95 (1.36, 6.43)	0.007
Fair/Poor	1.69 (1.00, 2.84)	0.049	3.62 (1.66, 7.88)	0.001
Donor type				
Live	Ref		Ref	
Deceased	1.50 (0.98, 2.29)	0.06	1.13 (0.65, 1.97)	0.68

Results: Among KT recipients 19.1% were frail. The only factors associated with frailty status were older age (≥65) (2.11, 95%CI:1.16-3.85), IADL disability (3.21, 95%CI:1.66-6.22), CESD depressive symptoms (11.87, 95%CI:4.33-32.58), less than a high school education (3.66, 95% CI:1.54-8.73) and HRQOL (Good: 2.95, 95%CI:1.36-6.43 and Fair/Poor: 3.62, 95%CI:1.66-7.88). The most common pattern of the 5 components was poor grip strength, low physical activity and slowed walk speed (19.4%) among KT recipients. KT recipients with exhaustion and slowed walking speed (HR=2.41, 95%CI:1.17-5.01, P=0.02) and poor grip strength, exhaustion and slowed walking speed (HR=2.48, 95%CI:1.09-5.64, P=0.02) were at increased mortality risk.
Discussion: Frailty is common among KT recipients of all ages. Age was the only conventional factor associated with frailty; however, factors rarely measured as part of clinical practice, namely HRQOL, IADL disability and depressive symptoms, were significant correlates of frailty among KT recipients. Only 2 patterns of the 5 components emerged as having the strongest association with post-KT mortality.
Conclusion: Frailty is a novel risk factor among KT recipients and not all of the 5 components may be relevant for risk prediction in the population.

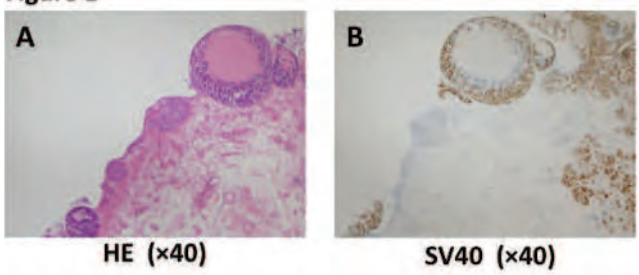
421.4

BK virus infection is a high risk factor for urothelial carcinoma in renal transplant recipients

Masashi Takenaka[1], Mamoru Kusaka[1], Naohiko Fukami[1], Taihei Ito[2], Hitomi Sasaki[1], Takashi Kenmochi[2], Ryoichi Shiroki[1], Kiyotaka Hoshinaga[1].
[1]Urology, Fujita Health University School of Medicine, Toyoake, Japan; [2]Transplantation and Regenerative Medicine, Fujita Health University School of Medicine, Toyoake, Japan.

Introduction: BK virus (BKV) latently infects 70-100% of the human population during childhood and subsequently remains lifelong latent in the epithelium of the urinary tract. Urinary reactivation of the BKV occurs in approximately one-third of renal transplant (RTx) recipients under the current immunosuppression protocols. BKV infection in immunodeficient patients may have a pathogenic role in the development of urothelial carcinoma (UC), but the role of BKV in UC is a controversial topic and is difficult to determine. Several reports have demonstrated BKV associated UC diagnosed in the native urinary bladder.
In this study, we report the cases of high-grade UC in RTx recipients, and investigate the potential role of BKV in post-transplant UC by immunostaining tumor tissue with simian vacuolating virus-40 (SV-40) and p53.
Case 1: A 36-year-old woman underwent RTx from donation after cardiac death (DCD) donor after developing end-stage renal disease (ESRD). The immunosuppressive regimen included ciclosporin, mycophenolate mofetil (MMF), and prednisolone. The patient developed BKV reactivation 1 month post-transplantation, diagnosed on urine cytology and polymerase chain reaction (PCR). Seven years later, the patient presented with intermittent asymptomatic gross hematuria with preserved renal function. The urine cytology was negative, but Computerized tomography showed a cT3a transplant renal pelvis carcinoma. The patient subsequently underwent a transplant nephroureterectomy. Histopathologic findings revealed a high-grade UC, pT3, in the renal pelvis carcinoma with SV40-positive staining.
Case 2: A 63-year-old man underwent RTx from DCD donor after developing ESRD. The immunosuppressive regimen included tacrolimus, MMF, and prednisolone. The patient developed BKV reactivation 4 months post-transplantation, diagnosed on urine cytology and PCR. The patient developed BKV-associated nephropathy 2 years post-transplantation. Five years later, cytologic diagnosis of the urine revealed a high-grade UC, and cystoscopy revealed papillary lesions. A transurethral resection of the bladder tumor was performed, and the pathology result confirmed existence of high-grade UC, pT1 with SV-40 and p53-positive staining.
Result: We experienced two cases of high-grade UC in RTx recipients with BKV reactivation. In both cases, polyomavirus immunohistochemistry performed on the tumor specimens were strongly positive, whereas the surrounding normal tissue was negative (Figure 1). And positive staining for p53 is consistent with an abnormal state of the UC.

Figure 1



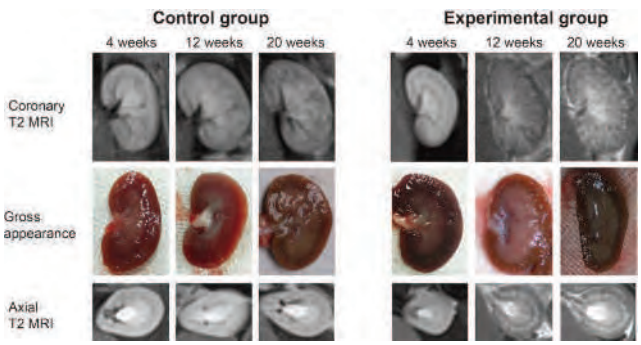
Conclusion: An association between BKV and UC has been reported in a small number of case reports involving RTx recipients. These cases are an addition to accumulating body of evidence implicating BKV in the development of UC.
There is evidence suggesting that BKV infection may be a high risk factor for UC in RTx recipients.
References:
[1] Papadimitriou JC. Am J Transplant. 2016; 16(2):398-406
[2] Yin WY. J Formos Med Assoc. 2015;114(4):373-374
[3] Aalderen MC. Neth J Med. 2013;71(1):26-28
[4] Oikawa M. Transplant Proc. 2014;46:616-619
[5] Borislav A. human Pathology. 2013;44:908-917

421.5

Intravoxel incoherent motion imaging is an effective way to monitor chronic allograft dysfunction in rat models

Qiang Zhang[1], Lu Liang[2], Song Zeng[1], Yue Xu[1], Wenrui Xue[3], Zijian Zhang[1], Wei Wang[1], Xiaodong Zhang[1], Xiaopeng Hu[1].
[1]Department of Urology, Beijing Chao-Yang Hospital, Beijing, People's Republic of China; [2]Department of Radiology, Beijing Chao-Yang Hospital, Beijing, People's Republic of China; [3]Department of Urology, Beijing YouAn Hospital, Beijing, People's Republic of China.

Objectives: To evaluate effect of intravoxel incoherent motion (IVIM) imaging MRI on monitoring chronic allograft Dysfunction (CAD) in rat models.
Materials and Methods: For experimental group, twenty Fisher donor kidneys were transplanted into Lewis recipients. For control group, twenty Lewis kidneys were transplanted into Lewis recipients. Serum creatinine (sCr) was monitored every two weeks. All the rats underwent IVIM examination at 4, 12, 20 weeks after modeling. IVIM parameters were obtained using region of interests (ROI) drawn over the renal parenchyma. Five rats from both groups were chosen randomly for histological analysis at different time points. Histological changes in the kidney were evaluated by chronic allograft damage index (CADI) scores. Differences between groups were analyzed by Mann-Whitney U test, and the correlation of IVIM parameters with CADI was determined by Spearman correlation analysis.
Results: All the rats in the experimental group developed into CAD which was confirmed by histological analysis at 4 weeks after transplantation. However, sCr showed no significant difference between two groups until 10 weeks after modeling. IVIM revealed impairment of renal perfusion and diffusion in experimental group at 4 weeks after transplantation compared with the control group. And these changes became severe over time. IVIM parameters were significantly correlated with CADI ($P<0.05$)
Conclusions: Intravoxel incoherent motion imaging MRI is an effective technique for detecting early signs of CAD and monitoring progress of CAD in rat model.



National Natural Science Foundation of China (No.81270837); Beijing Natural Science Foundation (No. 7132107); Beijing Municipal Administration of Hospitals Clinical Medicine Development of Special Funding Support (ZYLX201408); Beijing Municipal health system high level health technology personnel training program (2013-3-015)

421.6

The efficacy of sonoelastography method for the diagnosis of fibrosis in renal transplant patients

Arash Soudmand[1], Nihal Uslu[1], Nihan Haberal[2], Funda Ulu Ozturk[1], Fatih Boyvat[1], Gokhan Moray[3], Mehmet Haberal[3].
[1]Radiology, Baskent University, Ankara, Turkey; [2]Pathology, Baskent University, Ankara, Turkey; [3]Transplantation Center, Baskent University, Ankara, Turkey.

Introduction: Allograft biopsy is the most important method in the diagnoses of ethological factors of dysfunction of renal allografts. Sonoelastography is a new ultrasound method which is used to distinguish the soft or hard nature of the lesions and this can be determined numerically by this method. The aim of this study is to investigate the efficacy of sonoelastography method for the diagnosis of fibrosis in renal transplant patients.
Material and Methods: This study was performed prospectively in the Department of Radiology of Baskent University. Patients over the age of 18, to whom clinical biopsies were recommended were included in this study. Sonoelastography measurements for the renal transplant patients, who were admitted to the clinic for biopsy, were performed just before the biopsy procedure. Sonoelastography measurements were not used for final decision of biopsy. In all cases Siemens S 3000 Ultrasound was used for examination. Measurements were made by two experienced radiologists. Comparison of sonoelastographic values with biopsy results was made by SPSS 20 Statistics program.
Results and Discussion: Total of 65 transplant patients, 15 (23, 1 %) were female and 50 (76.9 %) were male. The average age of the patients was 38.84 ± 14. Of all patient, 51 (78.5 %) had live donors and 14 (21.5 %) had cadaveric donors. Pathology has approved chronic active humoral rejection in 11 patients (18.3%), acute T-cell mediated rejection in 37 patients (61.7 %), acute humoral rejection in 2 patients (3.3 %) , acute tubulointerstitial nephritis in 1 patient (1.7 %), inhibitor toxicity of Chronic calcineurin in 8 patients (11.7%) and thrombotic microangiopathy in 1 patient (1.7 %)
Renal parenchymal echogenicity of patients differed by gender which was an average grade 1 renal echogenicity in women and grade 0 in men, T-test results showed that this difference was statistically significant (P=,009<0,05). Also, statistically significant difference was found between the age and the kidney volume of the patients as well as the age and resistive index (RI) values. Both the kidney volume and the resistive index (RI) values elevated as the age increased. T test results showed that there was a statistically significant directional correlation between the serum creatinine values and Banfii degrees of the patients (r = 362; P=003<0, 05). Besides, there was a strong (r=, 382) correlation (r=, 382) between the degree of Banfi and ARFI values (P=002<0, 05). It was seen that as the means values of the degree of Banfi increased the means values of the ARFI increased. Finally, this showed that ARFI values increased in kidney transplants as the rate of interstitial fibrosis increased.
Conclusions: Our study reveals that there is a statistically significant correlation between the Banfi degrees and ARFI values of renal transplant patients. It is shown that sonoelastography is a method which can be beneficial for the diagnosis of fibrosis in renal transplant patients besides biopsy.

421.7

Prevalence and characteristics of duodenal villous atrophy in renal transplant patients presenting with persistent diarrhea

Farina Hanif[1], Nasir Hassan Luck[1], Tahir Aziz[2], S. Mujahid Hassan[1], Zaigham Abbas[1], Muhammad Mubarak[3], Anwar Naqvi[4].
[1]Hepatogastroenterology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [2]Nephrology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [3]Pathology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [4]Urology and Transplantation, Sindh Institute of Urology and Transplantation, Karachi, Pakistan.

Introduction: Persistent diarrhea (PD) is a common complication after solid organ transplantation (SOT), including kidney transplantation. Duodenal villous atrophy (DVA) as a cause of PD has not been investigated exclusively in renal transplant recipients (RTRs).
Methods: Data of 207 RTRs with PD who underwent upper gastrointestinal (GI) endoscopy and duodenal biopsies was collected from 2009 to 2012. The duodenal biopsies were examined for DVA. Age, gender, time taken for onset of symptoms after transplant and drugs used were compared between patients with and without DVA. After exclusion of known causes of DVA, a three-month course of antibiotics was given and the outcome analyzed.
Results: Out of 207 patients, 104 (49.8 %) were diagnosed as DVA. Of these, 92 (88.5%) were males. The mean age of patients with DVA was 34.9 ± 10.3 years. The mean post-transplant duration of DVA positive patients was 2.16 ± 0.8 years. Celiac disease (CD) serology was positive in 18 (%) patients. Giardiasis was demonstrated in 11 (10.7 %) patients, while immunoproliferative small intestinal disease (IPSID) in 7 (6.8 %) biopsies. Remaining 68 patients received antibiotics. Of the latter, 50 (74.6%) recipients showed complete response while 13 patients relapsed after initial response. Among the remaining 18 patients, other causes were found in nine cases and in nine no cause could be found. Isoniazid (INH) prophylaxis showed statistically significant negative association with DVA.
Conclusion: Duodenaol villous atrophy is common in RTRs irrespective of age, gender and duration of transplant. Tropical sprue, giardiasis, immunoploferative small intestinal disease and celiac disease are important causes of DVA in our set-up. Therefore, duodenal biopsy is recommended in all renal transplant recipients with persistent diarrhoea.

421.8

Different patterns of risk factors for late graft loss according recipient age after renal transplantation in the real medicine. An observational, multicenter and prospective study at ten years

Jose Morales, Roberto Marcen.
Research Institute, Hospital 12 de Octubre, Madrid, Spain.

The purpose of the present work was to define the most important risk factors (RF) for late graft loss(GL) after renal transplantation (RT) in three different subgroups of patients: young (under forty), midle aged (40-60 yr) and old (more than 60 yr) in the real medicine in our country with universal national health.
We study 2592 patients who received a cadaveric renal transplant between 2000-2002 prospectively followed for ten years, representing the clinical practice. According recipient age, 672 were under forty, 1320 among 40-60 yr and 600 were older than 60 yr. They received steroids, tacrolimus preferently than Cyclosporine and MMF with or without induction.
Censored death graft survival (CDGS) (K-M) was 71%, 76% and 65% while non-censoring death GS (NCGS) was 68%, 65% and 38% respectively at ten years., Recurrent/de novo glomerulonephritis and Chronic allograft nephropaty in young patients were the most frequent causes of GLwhile death with function was the the main one the other groups. In the multivariate analysis including 2259 patients with more than 12 monts of functioning graft, especially serum creatinine (SCr) at 6 mo. (HR 4.1, 5.7 and 5.3 p <0.001 respectively) and then proteinuria > 1gm at the same time .were the most important significant RF for late GL in all groups, in CDGS and in NCGS.
In addition, pretransplant diabetes (HR 3.2 p <0.001) and smoking (HR 3.4 p <0.001) in youngs, previous transplant (HR 3,3 p <0.001) in middle age and hepatitis C virus infection (HR 3,3 p<0.001) in older patients were also clearly significant RF for GL especially in CDGS.
Patient survival at 10 yr was 94%, 84% and 65 % respectively. As expected cardiovascular diseases and infections were the most frequent causes of death in all groups. In the multivariate analysis we identify recipient age in all groups, pre-transplant diabetes in patients under forty and among 40-60 yr while SCr at 6mo and pretransplant cardiovascular disease in older patients as the most significant RF for mortality.
Conclusions: Our results strongly suggest that although renal function and proteinuria at 6 mo are the most universal RF for late GL other ones in relation with recipient age such as pretransplant diabetes and smoking in youngs, previous transplants in midle age and HCV infection in old people can be also considered as significant RF for late GL after renal transplantation.

421.9

Age adapted immunosuppression for elderly kidney allograft recipients: balancing risks for cancer versus rejection

Francesca Jackson-Spence[1], Holly Gillott[1], Sanna Tahir[1], Jay Nath[3], Felicity Evison[2], Adnan Sharif[3].
[1]Medical School, University of Birmingham, Birmingham, United Kingdom; [2]Department of Medical Informatics, Queen Elizabeth Hospital, Birmingham, United Kingdom; [3]Department of Nephrology and Transplantation, Queen Elizabeth Hospital, Birmingham, United Kingdom.

Introduction: Cancer is a significant cause of morbidity and mortality after kidney transplantation, driven by the mileau of immunosuppression. Age is one of the strongest risk factors for developing cancer after transplantation and, with immunosenescence in the elderly well documented, age-adapted immunosuppression may be warranted for older adults to reduce overall immunosuppression burden but this requires further investigation.
Methods: Data was extracted by the University Hospitals Birmingham informatics team for all kidney allograft recipients transplanted at the Queen Elizabeth Hospital between 2007 and 2015. Electronic patient records were then manually searched to facilitate data linkage between various sources to create a comprehensive database of baseline demographics, donor details, clinical/biochemical parameters, histology and clinical events. SPSS version 22 was utilised for all statistical analysis.
Results: Data was extracted for 1,140 patients who received a kidney allograft, with median follow up 4.4 years post-transplantation. Median age for the study cohort was 47 and we classified older at this dichotomised age of 47 and over compared to younger recipients aged under 47. Older versus younger recipients had increased risk for death post-transplantation (10.6% versus 3.3% respectively, p<0.001) but not death-censored graft losses (9.1% versus 11.3% respectively, p=0.130). Cancer-related mortality for older versus younger recipients was 12.9% versus 5.9% respectively. Cancer was more common in older versus younger recipients (9.0% versus 3.1% respectively, p<0.001), with increased risk among older versus younger adults for both skin cancer (3.0% versus 0.9%) and non-skin cancers (6.1% versus 2.2%). Cancer-related mortality occurred in 12.9% of patients who developed cancer post-transplant (all non-skin cancer related). As a further sign of increased immunosuppression-related complications, older versus younger recipients were more likely to have cardiac events (9.6% versus 2.2% respectively, p<0.001) and cerebrovascular events (3.2% versus 1.5% respectively, p=0.039) post-transplant. However, older versus younger recipients had the same risk for cellular rejection (13.7% versus 12.6% respectively, p=0.324) but reduced risk for either antibody-mediated rejection (2.7% versus 4.7% respectively, p=0.047) or mixed rejection (1.4% versus 3.5% respectively, p=0.015). Of note, older recipients were more likely to be receiving their first kidney allograft versus younger recipients (94.6%versus 85.6% respectively, P<0.001), which confounds the risk for rejection among this cohort.
Conclusion: Older kidney allograft recipients have increased risk for death and immunosuppression-related complications including cancer, cardiac and cerebrovascular events but reduced risk for rejection. Our data supports the rationale that older recipients may benefit from tailored immunosuppression to reduce risk from immunosuppression-related complications but this will require targeted clinical trials to investigate further.
Kidney Research UK

421.10

Cancer-related outcomes in kidney allograft recipients in England versus New York State: a comparative population-cohort analysis between 2003 and 2013

Francesca Jackson-Spence[1], Holly Gillott[1], Sanna Tahir[1], Jay Nath[3], Felicity Evison[2], Jemma Mytton[2], Adnan Sharif[3].
[1]Medical School, University of Birmingham, Birmingham, United Kingdom; [2]Department of Medical Informatics, Queen Elizabeth Hospital, Birmingham, United Kingdom; [3]Department of Nephrology and Transplantation, Queen Elizabeth Hospital, Birmingham, United Kingdom.

Background: Global studies have confirmed higher cancer-related incidence and mortality for kidney allograft recipients versus the general population. However, no study has compared cancer-related epidemiology between different population cohorts and it is unclear whether country-specific data is translatable across countries. In this population-cohort study, we compared cancer-related incidence and mortality in kidney allograft recipients in England versus New York State.
Methods: We obtained data from every kidney-alone transplant procedure performed in England and New York State between 2003 and 2013, collecting patient demographics that included age, gender, donor type (living or deceased), transplant year, medical co-morbidities and ethnicity. Cancer data was derived from ICD-10 and ICD-9 codes from English and New York State cohorts respectively. English data was extracted from Hospital Episode Statistics, with linkage to the Office for National Statistics for mortality data. New York State data was extracted from Statewide Planning and Research Cooperative System (SPARCS).
Results: Data was analysed for 18,493 and 11,602 patients from England and New York State respectively, with median follow up in England and New York State 6.3 years and 5.5 years respectively. Baseline demographics revealed English patients were younger and more likely to be of White ethnicity compared to New York State patients, but no difference was observed in gender between the cohorts. English patients were significantly less likely to have a history of pre-transplant cancer compared to New York State patients (0.8% versus 3.1%, p<0.001). Admissions to hospital post kidney transplantation with a cancer diagnosis was more common in England versus New York State (12.3% versus 5.9%, p<0.001). Cancer-incidence was disparate between the two different cohorts; English compared to New York State patients were more likely to develop cancers of the skin (5.3% versus 0.3%, p<0.001), breast (0.5% versus 0.2%, p=0.001), bladder (0.3% versus 0.2%, p=0.01) and post-transplant lymphoproliferative disease (1.6% versus 1.1%, p=0.001), but less likely to get cancer of the lung (0.4% versus 0.7%, p<0.001). Kidney allograft recipients in England had lower mortality compared to their New York State counterparts during the immediate post-operative stay (0.7% versus 1.0%, p=0.011), after 30-days (0.9% versus 1.8%, p<0.001) and after 1-year post-transplantation (3.0% versus 5.1%, p<0.001). Logistic regression analysis demonstrated patients admitted with cancer within the first year post-transplant were more likely to die within the first 12 months in England (Odds Ratio 4.28 [95% CI 3.09 - 5.93], p-value <0.001) and New York State (Odds Ration 2.88 [95% CI 1.70 – 4.89], p<0.001).
Conclusion: Kidney allograft recipients in England compared to New York State have different cancer-related epidemiology and outcomes. Our analysis demonstrates significant differences between kidney allograft recipients in England versus New York States and suggests caution in translating post-transplant cancer-related epidemiology between different countries. However, in both countries we observed admission with cancer within the first year post kidney transplantation is associated with increased risk for mortality.
Kidney Research UK

421.11

Effect of ureteral stents on BK virus activation in kidney transplant recipients

Rachid Akiki[1,2], Ronald Akiki[1], Linda Pelletier[2], Jean Francis[1,2], Amitabh Gautam[1,2].
[1]Transplant Surgery, Boston University School of Medicine, Boston, United States; [2]Medicine, Boston Medical Center, Boston, United States.

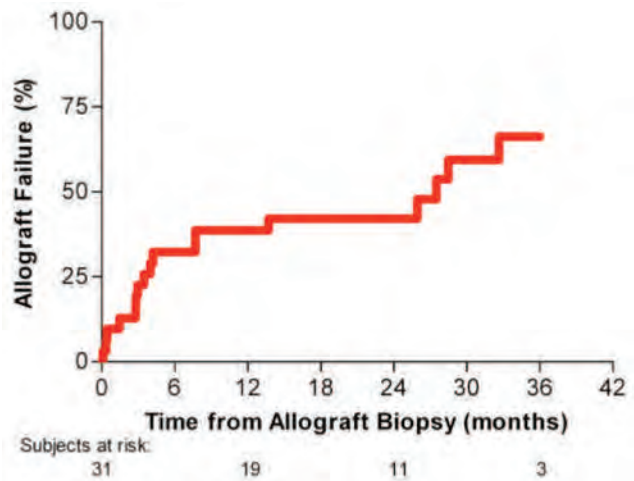
Introduction: Ureteral stents have been used, in kidney transplantation surgery, to minimize the risk of urinary leakage in the early post-operative period. Whether these stents predispose to, and are a risk factor favoring, BK virus replication has been controversial[1],[2]. We have two kidney transplant surgeons in our program, with one routinely using ureteric stents and the other not, and we analyzed our patients to see if there is a difference in the incidence of BK Virus activation in these two cohorts of patients.
Methods: Retrospective chart review of all kidney transplants done in our center, over a 8 years period from January 1, 2007 to December 31, 2014 was done and all patients had at least one year of follow up.. Whole blood and urine BK virus PCR, were done, as part of routine transplant follow up starting at 4 weeks post-transplant and repeated at least monthly for the first three months and then with all subsequent clinic visits. This study was approved by the IRB of Boston Univeristy.
Results: A total of 261 kidney transplant were done in 257 patients. Four patients had an early graft loss and were re-transplanted successfully during the study period. Nine kidney recipients died, within the first year, after transplantation and 9 other patients had an early graft loss and were all excluded from the study analysis and thus a total of 239 kidney transplants were included in our study and their data was analyzed. Of these, 119 had a ureteric stent placement and 120 did not. A total of 90 (37.6%) cases of BK viruria were detected, 41 (34.4%) in those with ureteric stent, and 49 (40.8%) in those without ureteric stent. Fifty patients (20.9% of total) with viruria went on to develop viremia, 25 (20.8%) in those with ureteric stent and 25(20.8%) in those without ureteric stents. The differences are not statistically significant. Although a majority of cases with BK virus activation (69-77%) occurred within 12 months of transplantation, a significant number (21-23%) occurred after the first year after transplantation.
Conclusion: There is no difference in the incidence of BK Virus activation in kidney transplant recipients, whether or not they had ureteric stent placement at the time of surgery. Other risk factors such as immunosuppression intensity, and rejection should be explored to reduce the incidence of BK Viremia.
References:
[1] Thomas A et al, Uretral Stents: a novel risk factor for polyomavirus nephropathy. Transplantation 84:433-6 (2007)
[2] Dadhania D et al, Epidemiology of BK Virus in renal allograft recipients. Transplantation 86:521-8 (2008)

421.12

Clinical profile and messenger RNA transcriptome sequencing of kidney allograft recipients with plasma cell rich acute rejection (PCAR)

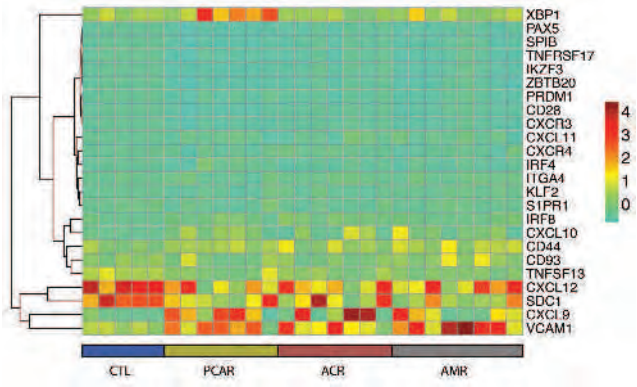
Laurent Mesnard[1], Fabien Campagne[1], Hua Yang[2], Carol Li[2], Catherine Snopkowski[2], Liana Perry[2], Elly Varma[2], John Lee[2], Darshana Dadhania[2], Ruchuang Ding[2], Manikkam Suthanthiran[2], Thangamani Muthukumar[2].
[1]Department of Physiology and Biophysics, Weill Cornell Medicine, New York, United States; [2]Department of Medicine and Transplantation Medicine, Weill Cornell Medicine, New York, United States.

Introduction: PCAR portends a poor prognosis. Better understanding of its mechanistic basis may pave way for development of biomarkers and targeted therapies.
Methods: We studied 31 consecutive kidney allograft recipients with PCAR (plasma cells constituting >20% of the interstitial infiltrating cells) at our center from 7/2007 to 6/2013. We obtained demographic and follow up information on these patients. We did 100bp single-end messenger RNA sequencing of kidney allograft biopsies with PCAR (n=7), acute cellular rejection (ACR, n=7), acute antibody mediated rejection (AMR, n=8) and normal (Normal, n=5) on a HiSeq 2000 sequencer. Reads were aligned against the reference genome (Ensembl 75 Genome, hg19). Differential expression analysis was done on R language (3.1) and Bioconductor. Low count tags were filtered out (threshold: 0.5 cpm/sample for a given gene) and significantly regulated genes were identified (FDR<0.05 and log fold change >1.5). For each subset build by the Venn analysis, genes list were sorted and was used as input for pathway analysis using DAVID.
Results: Mean (SD) age: 43 (16) years. Women: 14 (45%). Time from transplant to biopsy: 41 months. Documented non-adherence of immunosuppressive medicines: 9 (29%). Serum creatinine at biopsy (median): 3.9 mg/dl. Tacrolimus trough level (median): 3.5 ng/ml. Seventeen (55%) patients developed graft failure within 4 months (median) from the diagnosis (Figure-1). Time from transplantation to biopsy (HR: 1.21, 95%CI: 1.03-1.41, P<0.02) and creatinine at biopsy (HR: 1.11, 95%CI: 1.05-1.17, P<0.01) were independently associated with allograft failure.



Total RNA yield (mean[SD]): 1927ng[784ng]; RNA integrity number: 7.4[0.6]. There were 1190 gene transcripts significantly regulated between PCAR and Normal, 969 between AMR and Normal and 751 between ACR and Normal.
Next we did pathway profiling limited to renal expressed transcripts that appeared specific for each condition after Venn diagram analysis. We identified pathways specific for the rejection type that included

tryptophan metabolism and PPAR signaling pathways (PCAR), pyruvate metabolism (AMR) and ECM interaction (ACR). We also analyzed 24 genes that have been documented as associated with plasma cells biology (Nutt SL et al, Nature Review Immunology 2015) to seek for a specific signature associated with renal plasma cell infiltration.



Among the 24 genes that have been documented as associated with plasma cells biology, except XBP1, the rest were minimally expressed in the renal tissue during PCAR (Figure 2). Thus, XBP1 could represent a potential factor in the crosstalk between plasma cells and renal cells. Moreover, XBP1 interacts with PPAR pathway as well.

Conclusion: In this first characterization of intragraft mRNA transcriptome by sequencing of human allograft biopsies, we have discovered mRNA expression pattern unique to PCAR. Modulating XBP1 associated response or PPAR/tryptophan pathways may improve allograft survival in patients with PCAR.

421.13

Treatments against renin-angiotensin-aldosterone system could influence FGF23 and Klotho levels in renal transplant recipients

Siren Sezer[1], Emre Tutal[1], Zeynep Bal[1], Mehtap Erkmen[1], Gokhan Moray[2], Turan Colak[1], Mehmet Haberal[2].
[1]Department of Nephrology, Baskent University Faculty of Medicine, Ankara, Turkey; [2]Department of General Surgery, Baskent University Faculty of Medicine, Ankara, Turkey.

Introduction: Angiotensin-converting enzyme inhibitor (ACEI) and angiotensin II receptor blocker (ARB) are frequently used in post transplant hypertension[1]. Recent trials reported that renin-angiotensin-aldosterone system (RAAS) activation could trigger FGF23 and download Klotho synthesis , a mechanism that plays a role in their adverse influence on cardiovascular system[2]. We cross-sectionally analysed the FGF23 and Klotho levels in a group of stable renal transplant patients and searched for the influence of antihypertensive drug use on FGF23 and Klotho levels.

Materials and Methods: A group of 160 RT recipients were included (median age 52 years old, 67 female, post transplantation duration median 58 months). Patients with stable creatinine levels and no history of acute rejection episodes in last 12 months were included. Serum samples for FGF 23 and Klotho were obtained during routine follow-up controls. Subjects were grouped according to their antihypertensive use: ACEI /ARB administered (n:78) and not using patients(none or any antihypertensive apart from ACEI/ARB (n: 82).

Results and Discussion: Two groups were similar in means of demographic characteristics, medications and creatinine and creatinine clearance levels. Calcium, phosphorus, CaxP and PTH levels were similar. Patients using ACEI/ARB had significantly lower FGF-23 levels (49.5 vs 91.9 pg/mL, p: 0.049) and Klotho levels were significantly higher in this group (14.2 vs 9.2 ng/mL, p: 0.04). FGF-23 levels were also positively correlated with PTH (r:0.221, p:0.049), creatinine (r:0.522, p:0.0001) and negatively correlated with calcium (r:-0.238, p:0.034) levels. Patients under ACEI/ARB treatment had significantly lower proteinuria (p: 0.03). Posttransplant ACEI and ARB use is significantly associated with lower FGF23 and higher Klotho levels.

Conclusion: We suggest that the beneficial influence of ACEI and ARB on long term graft function could take place through lowering these adverse prognostic markers.

References:

[1] Heinze G et al. Angiotensinconverting enzyme inhibitor or angiotensin II type 1 receptor antagonist therapy is associated with prolonged patient and graft survival after renal transplantation. J Am Soc Nephrol. 2006;17: 889 –999.
[2] Orlić L et al. Use of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers in the early period afterkidney transplantation. Coll Antropol. 2013;37:809-14.

422.1

Defining the tipping point in surgical performance in laparoscopic donor nephrectomy during abdominal transplant surgery fellowship: a learning curve analysis.

Oscar Serrano[1], Ananta Bangdiwala[2], David Vock[3], Varvara Kirchner[1], Danielle Berglund[1], Ty B.Dunn[1], Erik B.Finger[1], Timothy L.Pruett[1], Arthur J.Matas[1], Raja Kandaswamy[1].
[1]Surgery, University of Minnesota, Minneapolis, MN, United States; [2]Biostatistics and Bioinformatics Core, University of Minnesota, Minneapolis, MN, United States; [3]Biostatistics, University of Minnesota, Minneapolis, MN, United States.

Purpose: Laparoscopic donor nephrectomy (LDN) requires a high degree of surgical competence and skill. UNOS recommends that fellowship-trained surgeons participate in 15 LDN to be considered proficient. However, evaluating proficiency among transplant surgery fellows (TSF) in the modern training paradigm has yet to be defined.
Methodology: A retrospective, single center case analysis was performed of all LDN procedures between January 2000 and December 2014 to construct a learning curve model. Measures of surgical performance included 3 dichotomized outcomes: high operating time (ORT) defined as >4.6 hours, high estimated blood loss (EBL) defined as >50 cc, and the presence of a complication graded on an ordinal scale. Case complexity was defined by clinical metrics obtained from donors with BMI>30, prior abdominal surgeries, renal vasculature multiplicity, and those in whom a right nephrectomy was performed. Outcomes and case complexity were compared between junior transplant surgery fellows (jrTSF) and senior transplant surgery fellows (srTSF) using chi-square tests of association. Plots of cumulative risk-adjusted residual error were used to analyze the learning curve in non-parametric fashion. Logistic regression was used to model outcomes, adjusting for fellow experience, case complexity, and institutional experience.

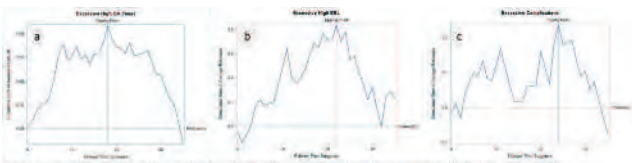


Figure 1. Risk-adjusted cumulative sum plots for ORT (a), EBL (b), and intraoperative complications (c) for jrTSF learning LDN.

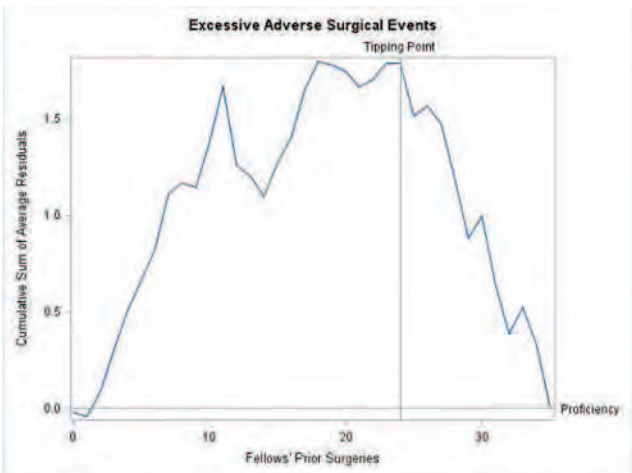


Figure 2. Learning curve for laparoscopic donor nephrectomy during abdominal transplant surgery fellowship.

Results: During our study time period, 970 LDN procedures were performed (791 performed by jrTSF; 179 performed by srTSF). The proportions of adverse surgical events were higher for jrTSF than for srTSF: high EBL (56% vs 43%; p=0.01), incidence of complication (6% vs 4.5%; p=0.54), and high ORT (58% vs 39%; p=0.0004). Incidence of high risk donor characteristics did not differ among patients in the jrTSF or srTSF cohorts: BMI>30, p=0.82; right nephrectomy, p=0.70; multiple renal arteries, p=0.73; prior abdominal surgery, p=0.43. Based on the risk-adjusted non-parametric analysis of the learning curves, there is a great deal of variability among trainees with a trend of improving OR time after 18 LDN procedures (Fig. 1a), EBL after 22 LDN procedures (Fig. 1b) and incidence of intraoperative complications after 24 LDN procedures (Fig. 1c). The comprehensive learning curve model for LDN during abdominal transplant surgery fellowship demonstrates a decrease in adverse surgical events after approximately 24 procedures, with proficiency achieved after 35 procedures (Figure 2).
Conclusion: Defining the learning curve of LDN during fellowship training has critical implications for surgical education, patient safety, and program credentialing. In the current era, where fundamental laparoscopy skills are mandated during residency training, TSF seem to acquire peak LDN performance in about 24 cases, with proficiency achieved after 35 procedures.

422.2

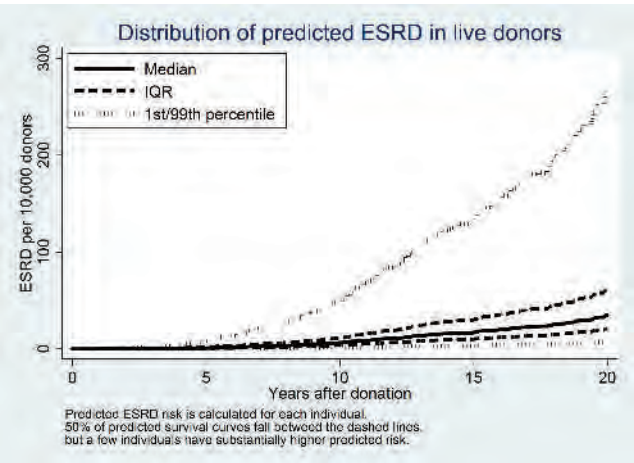
A prediction model for long-term risk of ESRD in living kidney donors based on individual characteristics

Allan B. Massie, Abimereki D. Muzaale, Xun Luo, Eric K.H. Chow, Dorry L. Segev.
Surgery, Johns Hopkins, Baltimore, United States.

In the face of recent evidence has suggested increased risk of ESRD in living kidney donors compared with healthy nondonors[1],[2], accurate communication of donor risk is required for informed consent. However, individual risk prediction is unavailable.

Materials and Methods: We modeled risk of ESRD in living donors in a cohort of all living donors in the United States 1987-2014 (N=128303) based on donor characteristics using Cox regression. ESRD outcomes were ascertained using linkage to CMS (national registry with mandatory reporting), with multiple imputation to account for missing BMI and eGFR. We used this model to produce individual predicted ESRD risk curves for each donor in the cohort.

Characteristic	Hazard ratio	p
Male sex	1.58 **2.02** 2.58	<0.001
Black race (at age 40)	2.32 **3.20** 4.41	<0.001
Age per 10y (non-black donors)	1.21 **1.42** 1.67	<0.001
Age per 10y (black donors)	0.61 **0.77** 0.98	0.03
BMI per 5 units	1.20 **1.56** 2.03	<0.001
Biologically related to recipient	0.43 **0.65** 0.97	0.03
eGFR per 10 mL/min/1.73 m^2	0.85 **0.97** 1.10	0.6



Results: Male sex was associated with twofold higher risk of ESRD in living kidney donors (HR=$_{1.58}$ 2.02 $_{2.58}$, p<0.001, Table). Older age was associated with increased risk (p<0.001), except among black donors, for whom age was associated with decreased risk (p=0.03). Lack of biological relationship to recipient was associated with decreased risk (HR=$_{0.43}$ 0.65 $_{0.97}$, p=0.03). Higher BMI was associated with increased risk (HR per 5 units = $_{1.20}$ 1.56 $_{2.03}$, p<0.001). There was no evidence of association between eGFR and risk after adjusting for other factors (p=0.6).

Median (IQR) predicted risk of ESRD was 6.0 (3.5-10.8) per 10,000 at 10 years post-donation, and 33.3 (19.5-59.2) per 10,000 at 20 years. However, the 99th percentile of risk was 49 per 10,000 at 10 years post-donation and 270 per 10,000 at 20 years post-donation. The C-statistic of the model was 0.704, indicating good predictive discrimination. The model can be found online at www.transplantmodels.com/donesrd/.

Discussion: The increased ESRD risk in black donors may reflect genetic risk due to high-risk alleles of APOL1, and/or discrimination and decreased access to health care in the US-based setting. The inverse association between age and ESRD risk in black donors may reflect selection, in that older potential donors with high-risk APOL1 alleles may be excluded from donation due to decreased kidney function.

Conclusion: In this US-based cohort, most individuals cleared for donation had very low long-term risk of ESRD. However, a few individuals had dramatically higher risk and should be counseled appropriately. Adequate lifetime access to health care should be ensured for all living kidney donors.

Jon Snyder, SRTR

References:

[1] Mjøen G, Hallan S et al. Long-term risks for kidney donors. Kidney Int. 2014 Jul;86(1):162-7.
[2] Muzaale AD, Massie AB et al. Risk of end-stage renal disease following live kidney donation. JAMA. 2014 Feb 12;311(6):579-86.

422.3

Where do living donors come from? A comparison of potential living liver and kidney donors at a high-volume U.S. center

Hillary Braun[1], John Feiner[3], Garrett RRoll[2].
[1]School of Medicine, University of California, San Francisco, San Francisco, CA, United States; [2]Department of Surgery, University of California, San Francisco, San Francisco, CA, United States; [3]Department of Anesthesia, University of California, San Francisco, San Francisco, CA, United States.

Introduction: Organs from living donors are an important component of the solution for the ongoing organ shortage. Understanding the potential donor pool is critical to recruitment efforts, and to date it is poorly defined. In 2013, UCSF implemented an online donor screening system to simplify the initial evaluation process for potential living kidney donors (BREEZE TRANSPLANT, MedSleuth, Inc.)[1],[2], and then expanded to include potential liver donors in 2015. Here we compare the demographics between potential kidney and potential liver donors who passed the initial screening process to identify characteristics that may help guide future efforts for recruiting living donors.

Methods: Using the web-based portal, potential living kidney and liver donors initiated the process of donation by completing an online questionnaire. This questionnaire gathers demographic information including gender, age, ethnicity, relationship to recipient, BMI, and health related behaviors including alcohol use, tobacco use, drug use, and exercise frequency. Potential donors are screened out for medical reasons such as current tobacco use without the desire to quit, diabetes, age, or simply failure to make it to the end of the survey. We included only those potential liver and kidney donors who passed the initial online screen in this analysis. Next we compared potential liver donors with potential kidney donors.

Results: 2306 potential living kidney donors passed the initial screen between 2013-2015, and 464 potential liver donors passed the initial screen in 2015. Comparison of basic demographic information is shown in (Figure 1- table). A greater percentage of potential kidney donors were female, older in age, related to their recipient, and had a higher BMI. Interestingly, a greater percentage of liver donors were unrelated to their intended recipient (24.1% vs. 10.6%). Among kidney donors alone, 11.9% (275/2306) went on to successfully donate. Interesting, only 1% of donors met through social media actually went on to organ donation.

	Kidney	Liver
N	2306	464
% Female	64.4%	62.7%
Age	41.1	38.5
BMI	26.3	25.2
Relationship to Recipient		
Parent	13.4%	12.3%
Child	5.3%	8.6%
Full Sibling	13.5%	9.3%
Unrelated	10.6%	24.1%
Other Relationships		
Community Member	4%	5.2%
Social Media	3.9%	2.8%
Acquaintance	5.1%	7.1%
Other	10.6%	7.8%
Ethnicity		
Asian	11.9%	6.9%
African American	6.5%	3.9%
Caucasian	52.5%	63.1%
Other	13.3%	1.7%
Alcohol Use		
Never	21.8%	24.6%
Occasional	25.2%	23.9%
Daily	6.7%	6.3%
Smoking Frequency		
Denies	69.3%	69.6%
Remote	19.5%	17.5%
Current	10.8%	7.1%
Exercise Frequency		
Infrequent	19.9%	16.4%
3x/week	27.8%	20%
>3x/week	36%	30%

Discussion and Conclusion: Understanding living donor demographics is essential to refining effective and efficient recruitment of donors. Our study of a large cohort of liver and kidney donors demonstrates subtle differences. Notably, unrelated donors were more prevalent in the population of potential liver donors. This study also demonstrates that many individuals who are recruited via social media fail to become successful donors even if they pass initial screening. Social media appears to be effective in generating interest but ineffective at attracting donor candidates who successfully donate.

References:

[1] A851, ASA 2011, USA
[2] S104, IARS 2011, Canada

422.4

Psychosocial evaluation of living organ donors (PELOD): an international professional survey of current practices

Lucinda Wynter[1], Dominique E.Martin[3], Katrina Bramstedt[5], Ian Dittmer[6], Beatriz Dominguez-Gil[9], Rudolf Garcia-Gallont[7], Elizabeth Pomfret[4], Kristof Van Assche[8], Haibo Wang[10], Riadh Fadhil[2].
[1]Sydney University , Sydney, Australia; [2]Hamad Medical Corporation, Doha, Qatar; [3]Deakin University, Geelong, Australia; [4]Tufts University, Medford, MA, United States; [5]Bond University, Melbourne, Australia; [6]Auckland District Health Board, Auckland, New Zealand; [7]Hospital General San Juan de Dios, Guatemala City, Guatemala; [8]University of Antwerp, Antwerp, Belgium; [9]Organización Nacional de Transplantes, Madrid, Spain; [10]Quantitative Medicine Research Institute, Shenzen, People's Republic of China.

Introduction: Transplantation from living donors (LD) comprises an increasing proportion of kidney(42%) and liver(18%) transplants worldwide. LD may suffer a range of psychosocial harms including coercion and impaired mental health and socioeconomic wellbeing. Global consensus recommends psychosocial evaluation (PE) of all prospective living liver donors (LLD) and living kidney donors (LKD) to identify and address risk factors. The Ethics Committee of The Transplantation Society(TTS) conducted this study to assess the extent to which PE is performed internationally and the features and goals of PE programs, and to evaluate needs for PE guidelines and educational resources.

Table 1: Information reportedly collected or verified during PE of LD

PE of LKD		PE of LLD	
Socioeconomic	**n (%)**	**Socioeconomic**	**n (%)**
Age	289/290 (99.7)	Age	33/33 (100)
Sex	287/290 (99)	Sex	33/33 (100)
Marital Status	282/286 (98.6)	Marital Status	31/33 (93.9)
Education Level	240/278 (86)	Education Level	23/31 (74.2)
No. Dependents	265/284 (93.3)	No. Dependents	29/31 (93.5)
Financial Burden/Debt	136/255 (53.3)	Financial Burdens/Debt	14/29 (48.3)
Employment Status	268/283 (94.7)	Employment Status	3232 (100)
Nationality	265/285 (93)	Nationality	31/32 (96.9)
Immigration Status	186/257 (72.4)	Immigration Status	27/32 (84.4)
History of Criminal Problems	142/258 (55)	History of Criminal Problems	16/28 (57)
Health Information	**n (%)**	**Health Information**	**n (%)**
Medical History	284/289 (98.3)	Medical History	33/33 (100)
History non-compliance	234/282 (83)	History non-compliance	26/31 (83.9)
Health Behaviours *(smoking, exercise)*	286/289 (99)	Health Behaviors *(smoking, exercise)*	32/32 (100)
High Risk Behaviours *(drugs, alcohol)*	283/288 (98.3)	High Risk Behaviours *(drugs, alcohol)*	32/32 (100)
Psychiatric History	283/290 (97.6)	Psychiatric History	31/33 (93.9)
Current Psychological Status	280/289 (96.9)	Current Psychological Status	31/33 (93.9)
Cognitive Capacity	246/276 (89.1)	Cognitive Capacity	28/32 (87.5)
History of Abuse *(physical, sexual)*	191/ 273 (70)	History of Abuse *(physical, sexual)*	28/31 (90.3)
Identity Verification	**n (%)**	**Identity Verification**	**n (%)**
Identity Card	216/284 (76.1)	Identity Card	29/32 (90.6)
Passport or Visa	111/255 (43.5)	Passport or Visa	16/32 (50)
Either of the above	223/284 (78.5)	Either of the above	29/32 (90.6)
Donor Decision Making	**n (%)**	**Donor Decision Making**	**n (%)**
Attitude of Significant Others (e.g *spouse)*	244/281 (86.7)	Attitude of Significant Others (e.g *spouse)*	32/32 (100)
Expectations of Donation	244/281 (86.7)	Expectations of Donation	28/31 (90.3)
If Donor Feel Pressure to Donate	263/282 (93.3)	If Donor Feel Pressure to Donate	30/32 (93.8)
Reasons For Donating	280/289 (96.9)	Reasons For Donating	32/32 (97)
Relationship to Recipient	289/290 (99.7)	Relationship to Recipient	33/33 (100)
Promise of Financial Reward	203/276 (73.6)	Promise of Financial Reward	21/27 (77.8)

Methods: Participants were recruited via email using TTS membership lists and other professional society networks. Professionals involved in LD transplant programs were invited to complete an anonymous online survey hosted on SurveyMonkey. The questionnaire was available in English, Spanish, Arabic and Chinese and included items concerning LD transplant activity and PE practices (e.g., goals; frequency; professionals involved and responsibilities for decision-making; information collected) at the participant's centre, as well as the duration of psychosocial follow-up after donation.
Results: 417 professionals from 83 countries completed the survey: 348 from centres performing LKD; 167 from centres performing LLD. Respondents were from Europe(31%), North America(24%), Latin America(14%), Western Pacific(10%), South East Asia(8%), Eastern Mediterranean(7%) and Africa(6%), and were mostly nephrologists (42%) but also surgeons, nurses, social workers, psychologists, psychiatrists and hepatologists.
Most participants reported PE was 'always' performed for LKD(82% n=293) and LLD(91% n=34), but national and regional differences were observed. Professionals involved in PE reportedly varied, however most respondents reported involvement of a mental health professional (85.2% for LKD; 90.9% for LLD). Participants identified goals of PE as: ensuring voluntariness of donation (97%); minimizing the risk of psychological (92% LKD; 100% LLD), physical (87%LKD; 91% LLD), or economic harm (50% LKD; 61% LLD); and ensuring altruistic donor motivations (85% LKD; 67% LLD). A range of information was solicited during PE (Table 1).
83% of respondents reported providing years or lifelong follow up of LKD physical health compared with 68% for LLD. Follow up of both LKD and LLD mental health, social wellbeing and financial wellbeing were less commonly reported, and was of shorter duration where provided. Guidelines for PE and more staff trained in PE were identified as resources that would improve PE by 58% and 59% of respondents respectively.
Conclusion: Despite international recommendations, PE of LKD and LLD is not consistently performed in many centres, possibly reflecting the lack of evidence-based standards for PE internationally and of resources required for PE in some countries.

422.5

Living kidney donor priorities for outcomes: a nominal group technique study

Camilla Hanson[1,2], John Kanellis[3,4], Steven J.Chadban[5,6], Jeremy R.Chapman[7], Jonathan C.Craig[1,2], Germaine Wong[1,2], Jule Pinter[2], Amit X.Garg[8], John S.Gill[9], Joshua R.Lewis[1,2], Allison Tong[1,2].
[1]Sydney School of Public Health, University of Sydney, Sydney, Australia; [2]Centre for Kidney Research, The Children's Hospital at Westmead, Sydney, Australia; [3]Department of Medicine, Monash University, Clayton, Australia; [4]Department of Nephrology, Monash Medical Centre, Clayton, Australia; [5]Department of Renal Medicine, Royal Prince Alfred Hospital, Sydney, Australia; [6]Sydney Medical School, University of Sydney, Sydney, Australia; [7]Centre for Transplant and Renal Research, Westmead Institute for Medical Research, The University of Sydney , Sydney, Australia; [8]Division of Nephrology, Department of Medicine, Western University , London , Canada; [9]Division of Nephrology, University of British Columbia, Vancouver, Canada.

Background: Living kidney donors must accept risks associated with nephrectomy. While this is ethically justified with informed consent, screening and access to follow up, the outcomes that are most important to donors are yet to be established. We aimed to identify living kidney donor's priorities for outcomes and describe the reasons for their choices.
Methods: Living kidney donors were purposively sampled from two Australian transplant centres. Participants identified important outcomes of kidney donation, ranked the importance of the outcomes, and discussed reasons for their priorities. For each outcome, we calculated a mean rank score from zero (least important) to 10 (most important) and analysed the transcripts thematically.
Results: Across eight nominal groups, 67 participants aged 27-78 years identified 32 outcomes. The highest ranked outcomes were: time to recovery (mean rank score 5.39, SD =3.77), family life (5.24, SD = 4.02), donor-recipient relationship (4.25, SD = 4.07), diet and lifestyle restrictions (3.90, SD = 3.55), and kidney function (3.76, SD = 3.50). Kidney failure and mortality ranked 10th and 13th respectively. Women ranked the donor-recipient relationship, life satisfaction, and family life higher than men; whilst men ranked kidney failure, physical function (fitness), mortality and kidney function higher. The themes underpinning participants' priorities included: overriding concern for recipient wellbeing, undeterred by low risks, heightened susceptibility and unfulfilled expectations.
Conclusions: Living kidney donors prioritised outcomes that could potentially disrupt their lifestyle and relationships, were unexpected, or caused fear and anxiety about their health. Donor assessment and follow up should address expectations regarding recovery time, relationship challenges and possible constraints on vocational and recreational activities; and donors may be more empowered with advice about diet, exercise and other healthy lifestyle practices to prevent long-term health problems. This may increase donor satisfaction and improve outcomes that matter to donors.

422.6

Peri-operative events and complications in minimally-invasive live donor nephrectomy: What should we tell potential donors? A systematic review and meta-analysis

Kirsten Kortram, Jan N.M.Ijzermans, Frank J.M.F.Dor.
Surgery, Erasmus MC University Medical Center, Rotterdam, Netherlands.

Background: There are many different techniques for live donor nephrectomy, preferences vary per center. A small number of randomized controlled trials have been performed comparing these different techniques with regard to complications and adverse events. Donors have to be educated about all risks and details during the informed consent process. For this to be successful, more information regarding short-term outcome is necessary.
Methods: A literature search was performed; all studies discussing short-term complications after minimally-invasive live donor nephrectomy were included. Outcomes evaluated were intra- and postoperative complications, conversions, operative and warm ischemia times, blood loss, length of hospital stay, pain score, convalescence, quality of life and costs. Overall results of the minimally-invasive live donor nephrectomy were described in a systematic review. A meta-analysis was performed comparing the individual techniques currently employed, with regards to conversion rate, intra-operative events and post-operative complications.
Results: 174 Articles were included in the systematic review, 37 in the meta-analysis. Mortality was reported in 128 articles (74%), and three fatalities were described (0.02%). Mean operative time was 197.4 minutes (range 78-320), warm ischemia time was 3.6 minutes (0.7-8.7) and average blood loss was 148.9 milliliters (15-545). Overall conversion rate was 1.3% (n=306), of which 181 emergency (0.8%). Intraoperative complication rate was 2.4%, most frequently bleeding (1.6%). Postoperative complications occurred in 6.8% of donors. Ileus (6.3%), infectious complications (2.6%), wound infection (1.6%), and bleeding (1.1%) were encountered most. The average length of stay was 4.2 days (range 0.6-13), the average pain score on discharge was 2.7 (2-5). Mean duration of convalescence was 23.2 days (range 4-60), and the average quality of life as measured by the Short-Form 36 questionnaire was 66.3 (34.5-88). None of the minimally-invasive techniques was superior to another with regard to complication or conversion rate. However, intra-operative bleeding was more often encountered in hand-assisted procedures (3.9 vs 3.7%, p=0.04), as was postoperative ileus (1.7 vs 0.3%, p=0.06). When compared to mini-open procedures, intra-operative events occurred more often in laparoscopic procedures: 10.2 vs 4.2%, p=0.02, but postoperative infections were more common after mini-open procedures; 18% versus 7.9%, p=0.03. This was mainly attributed to an increased incidence of pneumonia after mini-open procedures: 7.9 versus 4.3%, p=0.04.
Conclusions: Minimally-invasive live donor nephrectomy is safe, and associated with low complication rates and an even lower risk of mortality. These data, combined with further analysis of donor comprehension and satisfaction will assist us in developing a standardized, donor-tailored informed consent procedure for live donor nephrectomy.

422.7

Evolution of living donor nephrectomy at a single center: long-term outcomes with 4 different techniques in > 4,000 donors for over half-century

Oscar Serrano[1], Varvara Kirchner[1], Ananta Bangdiwala[2], David Vock[3], Ty B.Dunn[1], Erik B.Finger[1], William D.Payne[1], Timothy L.Pruett[1], David E.R.Sutherland[1], John S.Najarian[1], Arthur J.Matas[1], Raja Kandaswamy[1].
[1]Surgery, University of Minnesota, Minneapolis, MN, United States; [2]Biostatistics and Bioinformatics Core, University of Minnesota, Minneapolis, MN, United States; [3]Biostatistics, University of Minnesota, Minneapolis, MN, United States.

Introduction: The development of minimally invasive approaches to donor nephrectomy (DN) has been primarily driven by the potential advantages for the donor. Questions remain about long-term outcomes, for the donor as well as the recipient, with the newer minimally invasive techniques versus traditional open DN.
Methods: In this study, we reviewed all donor and recipient records related to DNs performed from June 1963 through December 2014 at our center. We analyzed donor and recipient demographic characteristics, intraoperative parameters, complications, and short- and long-term outcomes.
Results: During our 51.5-year study period, we performed 4,296 DNs: 2,638 open (ODNs), 1,183 hand-assisted laparoscopic DNs (HA-LDNs), 200 pure LDNs (P-LDNs), and 94 robot-assisted LDNs (RA-LDNs). For donors, we found that LDNs (versus ODNs) made donation more accessible to an older and heavier ($p<0.001$) population. LDNs were associated with a higher probability of left kidney procurement ($p<0.001$). All 3 LDN modalities required a longer operative time ($p<0.001$); RA-LDNs took significantly longer than HA-LDNs or P-LDNs ($p<0.001$). LDNs decreased the need for intraoperative blood transfusion ($p<0.001$) and reduced both the incidence of intraoperative complications ($p<0.001$) and hospital length of stay ($p<0.001$). However, LDNs (versus ODNs) led to a significantly higher rate of short-term ($p<0.001$) and long-term ($p<0.001$) readmissions. Undergoing HA-LDNs (versus all other modalities) was an independent risk factor for an incisional hernia ($p<0.001$). For recipients, LDNs (versus ODNs) seemed to be associated with a lower rate of graft failure at 1 year ($p<0.001$). However, the rate of delayed graft function was unaffected.
Conclusion: LDNs have made kidney donation more accessible to a greater number of donors, decreasing their intraoperative complications and hospital length of stay. But LDNs were also associated with a higher readmission rate and long-term complications.

Table 1. Outcomes Between ODN, HA-LDN, P-LDN, and RA-LDN

	ODN	HA-LDN	P-LDN	RA-LDN
Age (yrs)	37±12	42±11 (p<0.001)	41±10 (p<0.001)	40±11 (p<0.001)
BMI (kg/m^2)	25±5	27±4 (p<0.001)	26±4 (p<0.001)	25±4 (p=0.70)
Left Kidney (%)	68%	78% (p<0.001)	87% (p<0.001)	94% (p<0.001)
OR time (hrs)	4.4	4.6 (p<0.001)	4.9 (p<0.001)	5.0 (p<0.001)
Blood transfusion (%)	3%	0.3% (p<0.001)	1% (p<0.001)	0 (p<0.001)
Intraoperative complications (%)	6%	3% (p<0.001)	5% (p=0.54)	3% (p=0.25)
LOS (days)	5±2	3.1±1 (p<0.001)	3.4±2 (p<0.001)	3.0±1 (p<0.001)
Readmission				
Short-term (%)	1%	4% (p<0.001)	2% (p=0.30)	4% (p=0.03)
Long-term (%)	1%	5% (p<0.001)	3% (p=0.19)	4% (p=0.04)
Incisional hernia (%)	1%	4% (p<0.001)	3% (p=0.19)	1% (p=1)
DGF (%)	4%	5% (p=0.12)	6% (p=0.19)	4% (p=0.80)
Graft failure, 1-year (%)	17%	20% (p=0.03)	17% (p=0.99)	22% (p=0.18)

422.8

Psychosocial long impact of living kidney donation: Evaluation of donors satisfaction

Ana Menjivar[1], Josep M.Peri[1], Xavier Torres[1], Eva Oliver[2], Núria Masnou[3], Teresa Rangil[4], Maribel Delgado[5], Anton Fernandez[6], Montserrat Martinez[7], Anna Vila[8], Ignacio Revuelta[1], Fritz Diekmann[1], Entela Kondo[9], Nuria Avinyo[1], Chloë Ballesté-Delpierre[9], Jaume Grau[1], Laura Cañas[4], Ricardo Lauzurica[4], Amado Andres[5], Marti Manyalich[1].
[1]Hospital Clinic of Barcelona, Barcelona, Spain; [2]Bellvitge Hospital, Barcelona, Spain; [3]Hospital Universitari Josep Trueta, Girona, Spain; [4]Hospital Universitari Germans Trias i Pujol, Badalona, Spain; [5]Hospital Universitario 12 de Octubre, Madrid, Spain; [6]Complexo Hospitalio Universitario A Coruña, A Coruña, Spain; [7]Fundació Puigvert, Barcelona, Spain; [8]Hospital Sant Joan de Deú, Barcelona, Spain; [9]Universitat de Barcelona, Barcelona, Spain.

Introduction: Living Donation has a positive impact on Kidney Living Donors (KLDs) in terms of their self-estimation and social value. However, an evaluated risk on their physical and psychosocial outcome is presented. Such risk appears to be linearly increased for a longer post donation time. This study, co-founded by European Regional Development Fund (FEDER) 2011-14, intend to assess some aspects of the presumed risk.
Objective: To analyze the long term impact of donation process on KLDs and to evaluate the level of satisfaction they received.
Methodology: The participants of the study were KLDs who donated in nine Spanish transplant centers during the year 2000 till 2010. The satisfaction level the donors received was assessed retrospectively by means of a satisfaction survey; a new version of the one designed during EULID (European Living Donation and Public Health) project. An analogical- visual scale was used to improve the survey. The centers adapted the methodology to their characteristics and resources.
The questions were addressed to explore perception and acceptance of the donation process in terms of information received, decision making and impact of donation on economics, life opportunities, job and donor recipient relationship.
Results: 240 KLDs were included in the study. Socio demographically described the most frequent donor is female, 51 years-old, genetically related with the recipient, full-time employed. The mean time between the donation process and the survey was 5.2 years. A Pearson correlation was calculated to verify that the KLDs opinions do not depend of the donors' age neither of the elapsed time form donation. The donors perception about the recipient status was good, pointed 8.1 in a scale from zero to10. Pre-donation KLDs experiences more uncertainty than fear ($p<0.0005$) and more happiness than responsibility ($p<0.0005$). Information given: KLDs feel that they have been informed in details about the medical assessment before donation 9.5 (scale from 0 to 10). Donation impact: median time to return to their normal life was 58 days. KLDs reports full recovery 9.4 (scale from 0 to 10). The Donor-recipient relationship was rated with 7.4 (scale from 0-worse to 10-better).
Conclusion: The Living Donors satisfaction is key point to ensure the overall quality and security of donation procedures and the key to detect the potential negative consequences of becoming Living Organ Donor.

422.9

A 5-year analysis after live donor nephrectomy within the era of extended criteria donors – identifying factors for decline of kidney function

Shiromani Janki[1], Leonienke F.C. Dols[1], Evalyn E.A.P. Mulder[1], Khe T.C. Tran[1], Michiel G.H. Betjes[2], Ine M.M.Dooper[3], Jan N.M. IJzermans[1].
[1]Department of Surgery, Division of HPB and Transplant Surgery, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [2]Department of Nephrology, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [3]Department of Nephrology, Radboud University Medical Center, Nijmegen, Netherlands.

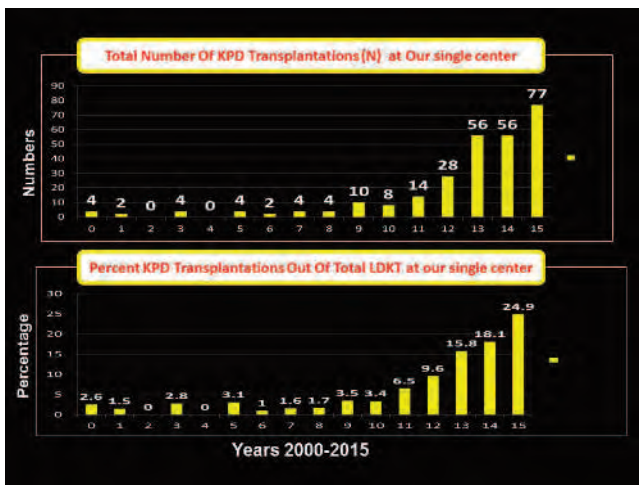
Background: Recently some studies have reported less favorable results after live kidney donation. We investigated therefore which donors will possibly be at risk for progressive kidney function decline and chronic kidney disease.
Methods: A 5-year follow-up on renal function, hypertension, quality of life, and survival was performed of a prospective cohort of 190 donors who were part of a randomized controlled trial comparing lapascopic versus hand-assisted donor nephrectomy. A creatinine based estimated glomerular filtration rate (eGFR) was measured with the CKD-EPI formula. Quality of life was assessed by the physical (PCS) and mental component score (MCS) of the Short Form 12.
Results: Data was available for 94% of the donors. The mean age at baseline was 52.8 (range 21-77) years, of whom 15.7% had pre-existent hypertension. The mean follow-up of the population was 5.1±0.9 years. Eight donors died during follow-up due to causes unrelated to donation, and four donors were lost to follow-up.
There was a mean decrease of 33.7% in kidney function as compared with predonation levels from a mean of 90.1 to 60.2 ml/min/1.73m^2 ($p<0.001$) after 5-years of follow-up; at least 93 donors (48.9%) had an eGFR of <60 ml/min/1.73m^2. The 5-year follow-up measurements of the eGFR of all donors were also different compared to the 1-year measurements, mean 60.1±12.1 and 58.5±11.8 ml/min/1.73m^2 ($p<0.001$) respectively.
Donors with a current eGFR of <60 ml/min/1.73m^2 were older at the time of donation (58.3 versus 47.5 years, $p<0.001$) and had a lower eGFR (82.3 versus 101.0 ml/min/1.73m^2, $p<0.001$) compared with donors with a current eGFR of ≥60 ml/min/1.73m^2. In addition, their eGFR decline was higher (mean 36.9±8.6 versus 30.0±7.8 percent, $p<0.001$ respectively). However, there was no difference in gender ($p=0.152$), BMI ($p=0.920$), proteinuria in urine samples ($p=0.745$), or PCS and MCS scores ($p=0.993$ and $p=0.754$). No donors were found at risk for end-stage renal disease or renal replacement therapy.
Thirty donors (16.9%) developed new-onset hypertension. Their mean eGFR at follow-up was 55.0±10.5 ml/min/1.73m^2. New-onset hypertensive donors were older at the time of donation (58.9 versus 50.4 years, $p<0.001$) with a higher BMI (27.7 versus 25.9, $p=0.036$), and had a higher systolic (144.1 versus 130.9 mmHg, $p<0.001$) and diastolic (85.2 versus 79.2 mmHg, $p<0.001$) blood pressure at follow-up compared to non-hypertensive donors. Furthermore, donors with an eGFR <60 ml/min/1.73m^2 had a higher incidence of new-onset hypertension (44.9 versus 22.1%, $p=0.004$) compared with donors with a current eGFR of ≥60 ml/min/1.73m2. However, there was no significant difference in the percentage of eGFR decline between these groups, 34.5 versus 33.5 % ($p=0.465$), respectively. Also, there was no significant difference in the use of diuretics between donors with different eGFR classifications ($p=0.288$).
Conclusion: These results indicate a plea for surveillance of new-onset high blood pressure after kidney donation and yearly monitoring of renal function. Future studies are indicated to identify those individuals at risk for a progressive loss of renal function after kidney donation.
Anneke van Duuren

422.10

impact of single center kidney paired donation transplantation to increase donor pool in India

Vivek B. Kute, H.V. Patel, P.R. Shah, A.V. Vanikar, P.R. Modi, V.R. Shah, S.J. Rizvi, B.C. Pal, P.S. Shah, U.T. Varyani, P.S. Wakhare, V.A. Ghodela, S.G. Shinde, V.B. Trivedi, G.S. Kasat, M.V. Patil, J.C. Patel, D.P. Kumar, H.L. Trivedi.
IKDRCITS, Ahmedabad, India.

Background: As per Indian chronic kidney disease (CKD) registry only 2% CKD patients received kidney transplantation. The majority of kidney donors in India are living donors (90%). ABO incompatibility is most common reason (40%) to reject the healthy willing living donors. The deceased donor kidney transplantation (DDKT) is in infantile stage and living donor kidney transplantation (LDKT) with desensitization protocols and ABO incompatible transplantation could not be expanded due to high costs and increased risk of infections from more intense immunosuppression and inferior long term survival in India. Kidney paired donation (KPD) transplantation can be done without additional cost and infrastructure at any center that does LDKT. KPD outcomes are just like any other ABO compatible transplants and long-term management also just like any other transplant.
Material and Methods: We present a government and institutional ethical review board approved study of 275 end stage renal disease patients who consented to participate in KPD transplantation at our single center between 2000 to 2016. All patients had anatomic, functional, and immunologically comparable donors. KPD was performed to avoid blood group incompatibility (n = 201) or positive cross-match (n = 59) and for getting better HLA matching/donor age (n=15). Mean age of patients (230 males, 45 females) and donors (60 males, 215 females) were 39.3 and 42.9 years. Patient ABO type was A (n=101), B (n=113), AB (n=11) and O (n=50). Donor ABO blood group type was A (n=98), B (n=113), AB (n=3) and O (n=61).
Results: Out of 275 patients, 230 patients had two-way exchange and 39 patients had three-way exchange and one domino chain of 6 pairs. 179 patients were from Gujarat and 96 from other states. The majority of the donors were spouse (n=177) and parents (n=81).The waiting time in KPD was short as compared to DDKT. Laparoscopic donor nephrectomy was performed in 89.7% donors. Graft survival was 95% and 15.2% had biopsy-proven acute rejection. Mean serum creatinine was 1.3 mg/dl at last follow-up. With dedicated team work, mandatory counseling and awareness about KPD, there is significant growth and progress in KPD transplantation in the last 4 years (Figure 1 and 2).



Conclusion: KPD is legal and rapidly growing modality for facilitating LDKT for patients who are incompatible with their healthy, willing living donor. KPD has increased LDKT rate by 25 % in the year 2015

in our single center. We have performed the largest number of KPD Transplantations in our single center in 2015 and to the best of our knowledge this is largest number for KPD transplants in single center in one year in the World. KPD has the potential to decrease commercial transplantation and it should be promoted in centers with low DDKT rates.

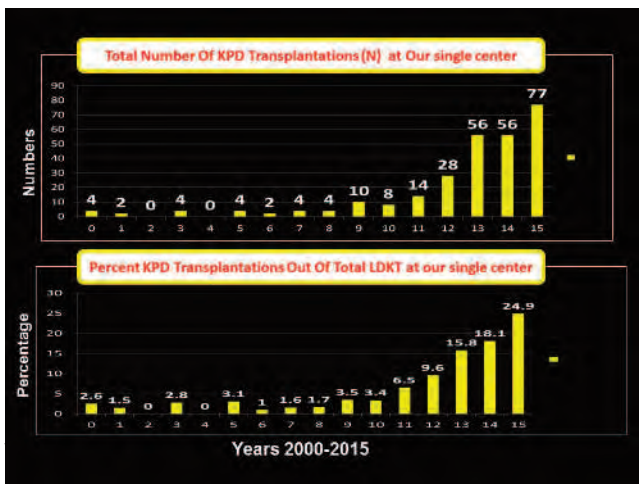
Milestones : Single center KPD transplantation in India	
1st June 2000	First two-way KPD transplantation
2000-2011	56 KPD transplantations (2.5 % of LDKT)
13th Feb 2013	First three-way KPD transplantation
14th March 2013	Ten KPD transplantations on world kidney day 2013
2013	56 KPD transplantations (15.8% of LDKT)
6th May 2014	First 3-way KPD transplantation in combination with desensitization protocol
2014	56 KPD transplantations (18.1 % of LDKT)
17th Feb 2015	First international 2-way KPD transplant between patients from Portugal and India
August 2015	First non-simultaneous domino chain transplant of 6 ESRD patients and 6 donors
2015	77 KPD transplantations (25 % of LDKT)

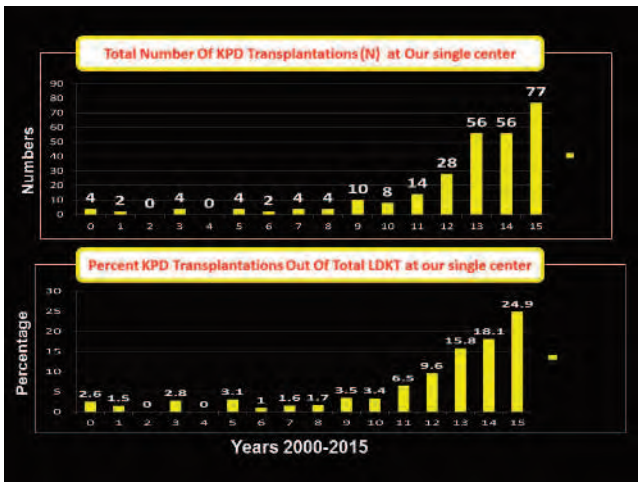
422.11

World record: 77 kidney paired donation transplantation in year 2015 at one transplant center in India: a model for the developing world to prevent commercial transplantation

VivekB. Kute, H. V.Patel, P. R.Shah, A. V.Vanikar, P. R.Modi, V. R.Shah, S. J.Rizvi, B. C.Pal, P. S.Shah, U. T.Varyani, P. S.Wakhare, V. A.Ghodela, S. G.Shinde, V. B.Trivedi, G. S.Kasat, M. V.Patil, J. C.Patel, D. P.Kumar, H. L.Trivedi.
IKDRCITS, Ahmedabad, India.

Introduction: Indian is having living donor kidney transplantation(LDKT) program(90%).Desensitization protocols, ABO incompatible kidney transplantation(KT),deceased donor kidney transplant(DDKT) could not be expanded.Kidney paired donation (KPD) promises hope to ESRD patients because of best outcome.
Methods: We have performed 380 KT from 1January 2015-1 January 2016(71 DDKT, 309 LDKT). 77 were KPD transplantation [25 two-way and 7 three-way pairs and one domino chain of 6 pairs]. This included first international KPD Transplantation and first non-simultaneous domino chain transplant(n=6), combination of KPD with desensitization in sensitized patient(n=1).
Patient ABO type was A(n=24), B(n=31), AB(n=4) and O(n=18). Donor ABO blood type was A(n=21), B(n=35), AB(n=0) and O(n=21). Number of transplants for each blood group were AtoA(n=21), BtoB(n=30), OtoO(n=18), OtoA(n=3), OtoB(n=1), AtoAB(n=1), BtoAB(n=3). Reason for joining KPD was ABO incompatibility(n = 45),sensitisation(n=26) and for better HLA matching(6 O group donor). Out of 26 sensitised patients 16 patients joined KPD without desensitization and 10 after failure of desensitization. All patients had anatomic, functional, and immunologically comparable donors.12 patients underwent non-simultaneous KT
Key to success of our single center KPD program are formation of registry to maintain database about ABO incompatible /sensitized patients, awareness and mandatory counseling about KPD, expert transplant coordinator ,dedicated KPD team . Figure 1 and 2 showed growth of KPD and KT in our single center.





Results: 158 pairs were registered and 77 transplants were completed. 13.2 %(n=21) are waiting for documents and permission from authorization committee(AC) despite having KPD donor (9 sensitized and 8 O group patients). Eight patients were not willing for KPD and underwent ABO incompatible KT due to low ABO titer . Four and 2 patients underwent LDKT with alternative donors and desensitization protocol respectively, 2 underwent DDKT. We Facilitated KPD transplant in 65.3%(98/150) and KPD Transplant completd in 51.3%(77 /150)

Waiting time in KPD was short as compared to DDKT(median 2 vs24 months). Donor relationships were spousal(n = 48), parental(n = 24), and others(n =5). Outcomes of KPD transplants are comparable to that of directed LDKT. Graft survival was 100%. Two patients died with functioning graft due to ischemic heart disease(n=1) and pneumonia(n=1). 14.2%(n=11) had biopsy-proven acute rejection. Mean serum creatinine was 1.1 mg/dl at last follow up. Donor survival is 100% and all underwent lapascopic donor nephrectomy. Transplant match rates among sensitized patients(n=60) was 58.3%(n=35) of which 43.3%(n=26) KT were completed and 15%(n=9) waiting for permission from AC. Transplant match rates among O group of patients(n=62) was 41.9%(n=26) of which 29%(n=18) KT were completed and 12.9%(n=8) waiting for permission from AC.

Conclusion: Our single center KPD has increased LDKT by 24.9% in 2015. This is largest number of KPD transplantations in single center in one year(2015) in World. IKPD should be promoted to overcome organ crisis and shortage of DDKT to prevent commercial KT.

422.12

Simulation of international kidney exchange between Saudi Arabia and the United States using actual incompatible pair data

Abdulnaser Alabadi[1], Khalid B.Akkari[1], Fahad Alotaibi[1], Khaled Hamawi[1], Meteb Albugami[1], Alvin E.Roth[2], Jonathan E.Kopke[3], Michael A.Rees[3,4].
[1]Multi Organ Transplant Center, King Fahad Specialist Hospital, Dammam, Saudi Arabia; [2]Economics, Stanford University, Palo Alto, United States; [3]Alliance for Paired Donation, Perrysburg, United States; [4]Urology, University of Toleod Medical Center, Toledo, United States.

Background: Kidney paired donation (KPD) has become a common method of increasing living kidney donation in many nations, but it has not become common practice in the Middle East. Recently, one Saudi transplant center began using KPD matching software from an American KPD non-profit organization.

Methods: Multiple transplant centers in the United States participated in a KPD program leading to a pool of 160 incompatible pairs for whom no opportunities for kidney exchange were identified. Using the same KPD matching software on a different server, a single Saudi transplant program enrolled 30 incompatible pairs and identified one 3-way exchange. The remaining 27 incompatible Saudi pairs were found no opportunity for kidney exchange. The 160 unmatched US pairs and the 27 unmatched Saudi pairs were placed into a common pool and a match run was performed to look for opportunities for international kidney exchange.

Results: From 187 incompatible pairs composed of 160 unmatched US pairs and 27 unmatched Saudi pairs, 25 potential kidney exchanges were identified. Of the 25 matched pairs, fourteen were from the USA (8.8% of 160) and 11 were from Saudi Arabia (40.7% of 27). More than 600 potential 1-way exchanges were evaluated by the matching software to identify the single, maximal cardinality optimized solution allowing 25 potential kidney transplants. If exchanges were limited to exchanges and chains of length ≤2 transplants, 11 transplants were possible; if limited to length ≤3 transplants, 15 transplants were possible; if limited to length ≤4 transplants, 19 transplants were possible; and when exchanges were allowed to be unlimited in length, 25 transplants were possible in a 12-way and 13-way chain. In these two chains, 17 patients were highly sensitized and 14 had blood type O. Mechanisms to overcome logistical, financial, legal, cultural, and governmental challenges are currently being explored to provide additional transplant opportunities for these highly sensitized, hard-to-match patients.

Conclusion: International kidney exchange is a promising strategy to increase the opportunity for living donor kidney transplantation for patients with willing, but immunologically incompatible living kidney donors. We hypothesize that differences in HLA antigen prevalence across different ethnicities may synergize KPD matching in pools that combine international incompatible pairs due to differential donor specific antibody specificities in candidates and available HLA antigens in donors.

422.13

Increasing deceased donor transplant activity in India: the Rajasthan story

Manoj Maloo, Dilip Jain, ML Swarankar, Christopher Taylor Barry.
Liver Transplant and hepatobiliary Surgery, Mahatma Gandhi University of Medical Sciences and Technology, Jaipur, India.

Deceased donor transplantation and organ donation in India have only recently become accepted by the Indian public and medical fraternity. Southern states have led the way to acceptance and sustainability of deceased donor transplant programs since 2008, but most other states in the nation have lagged behind in this regard. Rajasthan has only very recently begun to show success. The keys to Rajasthan's success involve strong political support from the State Government and cooperation between various NGOs, hospital administrators and dedicated transplant professionals.

In November 2014, a combination of State Government support, transplant NGOs, and transplant professionals trained in donor management and organ retrieval together helped to establish the first deceased donor transplant program in Rajasthan within 4 months. During this period, the Rajasthan Network for Organ Sharing (RNOS) was established, employing allocation rules agreed upon by the government and stakeholder institutions that would allow for equitable sharing of available donor organs.

On 6 February 2015, the first multiorgan recovery surgery from a brain dead declared, family consented organ donor was performed at Mahatma Gandhi University Hospital, Jaipur (MGH). Since then, a total of 8 multiorgan recoveries have been performed in Rajasthan, with 5 kidney transplants, 1 heart transplant and 2 liver transplants at MGH, all with successful outcomes to date. In addition, 8 deceased donor kidney transplants have been performed at Sawai Man Singh Government Hospital in Jaipur.

Conclusions: 1) The rapidity of establishing successful and sustainable deceased donor transplantation programs in Rajasthan was only possible because of the cooperation between government officials, NGOs, transplant professionals understanding donor management and organ recovery and interested nontransplant medical professionals, as well as hospital administrative support from existing transplant institutions, 2) well coordinated efforts have led to public acceptance of organ donation in Rajasthan at much higher than expected levels thanks to well trained transplant coordinators and dedicated multidisciplinary transplant teams, and 3) establishment of a fair and transparent allocation system (RNOS) has allowed for further increased trust among the public and the nontransplant medical fraternity that deceased donor transplantation in India can be conducted fairly, transparently and sustainably.

Date	Hospital	Organs Recovered	Organs Transplanted
5 February 2015	MGH	kidneys, liver	kidneys (pediatric en bloc) at MGH, liver to ILBS
25 February 2015	SMS	kidneys, liver	2 kidneys at SMS, liver to Army R&R
31 March 2015	SMS	liver	liver to Apollo Delhi
23 July 2015	MGH	kidneys	1 kidney at SMS, 1 kidney at MGH
25 July 2015	SMS	kidneys, liver	2 kidneys at SMS, liver to ILBS
2 August 2015	MGH	kidneys, liver, heart	1 kidney at MGH, 1 kidney at SMS, heart at MGH
28 November 2015	MGH	kidneys, liver	Liver and 1 kidney at MGH, 1 kidney at SMS
5 December 2015	MGH	Kidneys, liver	Liver and 1 kidney at MGH, 1 kidney at SMS

423.1

Efficient PRA monitoring strategy for cost reduction and improved waitlist management

Lourdes Robles[1], Yoshitsugu Obi[2], Jon Le[1], Eric Belen[1], Clarence Foster[1], Hirohito Ichii[1].
[1]Surgery, University of California Irvine, Orange, CA, United States; [2]Division of Nephrology and Hypertension, University of California Irvine, Orange, CA, United States.

Introduction: The kidney waiting list is increasing at a 20% per year rate. Donor-specific antibodies against human leukocyte antigen contribute to hyperacute rejection, accelerated acute rejection, and antibody-mediated rejection, and longer term complications. Periodical Panel Reactive Antibody (PRA) testing using the Luminex single antigen bead assay is one of the major expenses for pre-transplant evaluation.

The aim of this study is to develop and validate a novel PRA monitoring strategy for waitlisted renal transplant candidates to efficiently reduce the frequency of the PRA test without compromising the safety against all organ offers.

Methods: We retrospectively reviewed all 2,509 deceased kidney offers from 1/ 2011 to 3/2012. We concomitantly utilized the same approach for identifying those patients that would be most likely to receive an organ offer. Those patients were determined to require PRA testing. All other patients had annual status reviews.

Based on the reviewed data, we developed and started a new strategy where quarterly PRA testing was performed in those who met the following conditions:

1. Top 20% of blood group A, B and O - utilizing dialysis time.
2. 100% of blood group AB - which accounts for 1.8% of total waitlisted patients.
3. 100% of kidney/pancreas and pancreas waitlisted patients- less than 10 patients in our waitlist
4. Any patient with a CPRA of > of 10%

We then calculated the estimated annual savings comparing the periods on the old system.

Results: In retrospectively review, among a total of 2,509 organ offers during 1/2011 to 3/2012, 88 % (2,201) of offers were determined to be within the selected quarterly PRA testing group while the remaining 12 % (308) were either zero mismatch (0.08%, n=2) or organ offers which were turned down (99%, n=306). Blood groups A, B and O accounted for 96% of all patients in the waitlist. During this review period, all organ donations were offered to transplant candidates who had the AB blood group or who were wait-listed for the kidney/pancreas and pancreas transplantation were offered organs. Contrary, kidney transplant candidates with the other blood groups were offered only when they were within the top 20% of the waitlist.

We then started the PRA testing strategy from 4/2012. This new strategy excluded 77% of the waitlisted candidates from PRA testing and no organ donation was offered to those in the annual review period except zero mismatch and direct donation to the recipient from donor's family. Analysis of this new selective testing strategy contrasted to the old method resulted in a significant savings of $551,864/year. This model has been in use at our program from 4/2012 to 3/2014 with the benefits of annual cost savings and no reported detrimental effects.

Conclusion: More than 65% of waitlisted candidates did not require quarterly PRA testing. Our new PRA testing strategy saved substantial medical cost without compromising the safety against all organ offers.

423.2

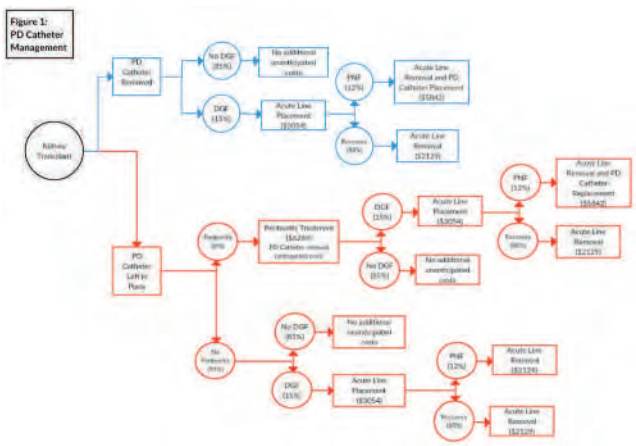
Financial implications of peritoneal dialysis catheter removal at the time of kidney transplantation

Meredith Barrett[1], Andrew M.Rizzi[1], Stephen D.Riutta[1], Joshua M.Peterson[1], Galina Gagin[1], Danielle M.Fritze[1], Kristen A.Schaedig[1,2], Randall S.Sung[1], Yee Lu[1], Kenneth J.Woodside[1]. [1]Transplant Center, University of Michigan, Ann Arbor, MI, United States; [2]Clinical Financial Planning and Analysis, University of Michigan, Ann Arbor, MI, United States.

Introduction: Peritoneal dialysis (PD) is increasingly used as a means for dialysis access for those with end stage renal disease. Management of PD catheters at time of kidney transplant has yet to be well standardized, although many centers are reluctant to utilize them for several weeks post-engraftment. Given this heterogeneity of practice, we sought to investigate the cost ramifications of PD catheter removal at time of transplant.

Methods: PD patients undergoing kidney transplantation at the University of Michigan from 2000-2015 were included for analysis (n=329). Outcomes data, with particular attention to management of PD catheters at time of transplant, was collected via chart review. Financial data was obtained from institutional finance officers and costs per procedure were established. Peritonitis treatment ranged from outpatient antibiotics to admission in a critical care unit, therefore an average cost of both inpatient and outpatient treatment was established and synthesized into a weighted average peritonitis cost. Differences in unanticipated costs—costs associated with delayed graft function (DGF), primary nonfunction (PNF), and peritonitis—were compared to assess which treatment practice was less costly at our institution.

Results: During the study period, 16 (5%) had their PD catheters removed at time of engraftment. The DGF rate was 15%, with 12% of DGF patients (1.2% of the total population) progressing to PNF. Of those with planned delayed PD catheter removal, 19 (6%) developed peritonitis. Treatment varied with the wide range of clinical presentation—from abdominal pain to ICU admission. Seven of 19 (37%) required outpatient oral or IV antibiotics, five (26%) had delayed discharge from transplant admission, and seven (37%) were readmitted (2-20 days) for treatment. With this wide range in treatment, outpatient and inpatient costs were quite variable therefore a conservative cost of $6269 per episode was used for analysis. To determine the cost of PD removal at transplant vs. post procedurally a model of 1000 patients per treatment arm was developed (Figure 1, Table 1). Utilizing the DGF, PNF, and peritonitis rates, we estimated unanticipated (excluding planned PD catheter removal) costs. Delayed PD catheter removal resulted in higher unanticipated costs ($1157/patient) when compared to PD catheter removal at kidney engraftment ($844/patient).



Conclusion: With our DGF and PNF rates, removal of the PD catheters at the time of kidney transplantation was cost effective. Institutions should take into account nephrology policy on PD catheter use immediately postoperatively, along with institutional DGF and PNF rates, when developing peri-transplant PD catheter management policies.

TABLE 1.				
Clinical Outcome	**Treatment**	**Percentage**	**Cost per procedure**	**Cost***
A. PD Catheter Removed at Transplant				
DGF	Acute line placement	15% (150)	$3,054	$458,100
-PNF	Acute line removal and PD cath replacement	12% of DGF (18)	$5,842	$105,156
-DGF Recovery	Acute line removal	88% of DGF (132)	$2,129	$281,028
No DGF	No further treatment	85% (850)	no additional unanticipated costs	
			Total:	**$844,284**
			Cost per patient:	**$844.28**
B. PD Catheter Removed after Transplant Recovery				
Peritonitis	PD removal and treatment of peritonitis	6% (60)	$6,269	$376,140
DGF	Acute line placement	15% of peritonitis (9)	$3,054	$27,486
-PNF	Acute line removal and PD cath placement	12% of DGF and peritonitis (1)	$5,842	$5,842
-DGF Recovery	Acute line removal	88% of DGF and peritonitis (8)	$2,129	$17,032
Recovery with DGF	No further treatment		no additional unanticipated costs	
No Peritonitis		94%(940)		
DGF	Acute line placement	15% of no peritonitis (141)	$3,054	$430,614
-PNF	Acute line removal	12% of no peritonitis and DGF (17)	$2,129	$36,193
-DGF Recovery	Acute line removal	88% of no peritonitis and DGF (124)	$2,129	$263,996
No Complications	Anticipated PD Cath Removal	85% of no peritonitis (799)	no additional unanticipated costs	
			Total:	**$1,157,303**
*Based on 1000pt/treatment arm			**Cost per patient:**	**$1,157.30**

Table 1: Unanticipated post transplant cost for 1000 patients undergoing PD catheter removal at transplant (A) versus after tranplant recovery (B). The percentages are derived from our intitution's clinical data and the numbers within parenthesis are the number of patients that would be in each subgroup based on a model of 1000 patients per treatment arm.

423.3

Low access of highly-sensitized patients to kidney transplantation associated with virtual crossmatch using standard MFI threshold: data from Northern Portugal

Jorge Malheiro[1], Sandra Tafulo[2], Leonidio Dias[1], La Salete Martins[1], Idalina Beirão[1], António Castro-Henriques[1].
[1]Nephrology & Kidney Transplantation, Centro Hospitalar do Porto, Porto, Portugal; [2]Histocompatibility Lab, Centro do Sangue e Transplantação do Porto, Porto, Portugal.

Introduction: In the Portuguese allocation system, at each deceased donor (DD) organ offer, points are attributed, considering candidate-donor age difference, HLA mismatches, and candidate time on dialysis, to all candidates AB0-identical with the donor. Candidates with cytotoxic PRA ≥50% receive extra-points, but no national prioritization program for highly-sensitized (HS) candidates exists. Since the first step in the allocation algorithm is to perform a virtual crossmatch that excludes every candidate with donor-specific anti-HLA antibodies (DSA) at a MFI threshold of ≥1000 automatically (before the cytotoxic crossmatch), HS candidates transplantability is curtailed. Previously, we published data showing that no antibody-mediated rejection occurred in non-desensitized patients with preformed DSA with a MFI below 3000[1]. In order to investigate the consequences of this allocation policy, we analyzed the access of HS candidates to kidney transplantation (KT).

Materials and Methods: Analysis of 987 adult candidates from North Portugal wait-listed with regular priority for DD KT between 2009 and 2011 was performed. The rate of DD organ offers and transplant cumulative incidence, considering candidates AB0 group and calculated PRA (cPRA), were assessed. For the analysis of cumulative incidence of KT, in order to control for competitive events, we excluded candidates that received a kidney graft from a living-donor (n=45), those removed from the waiting-list (n=136) or that died (n=30) during follow-up, with 776 candidates remaining for this analysis, with time being defined between the date of admission on the waiting-list and the date of DD transplantation or end of follow-up (31/December/2015).

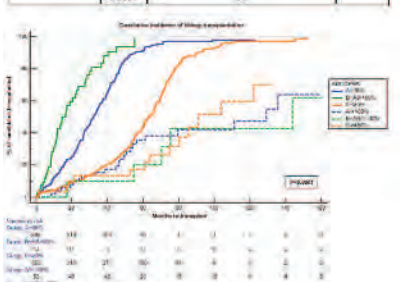
Results:

Candidates characteristics by cPRA

	cPRA<80% (n=863)	cPRA≥80% (n=124)	P
Age, mean	49	46	0.006
Female gender, %	33	62	<0.001
Previous KT, %	11	55	<0.001
AB0 blood group, %			<0.001
A	42	57	
B+AB	5	12	
0	53	32	
Cytotoxic PRA≥50%, %	2	35	<0.001
Years on dialysis, mean	2.9	6.5	<0.001
Years on the waiting-list, mean	2.4	5.7	<0.001

DD organ offer rate (Poisson regression)

Blood group	cPRA	Months until one DD offer	P
A	<80%	5	<0.001
	≥80%	16	
B+AB	<80%	4	<0.001
	≥80%	15	
0	<80%	10	<0.001
	≥80%	30	



Median waiting-time for DD kidney transplantation

Blood group	cPRA	% of candidates transplanted	Median waiting-time (months)	Log-rank P
A	<80%	91	32	<0.001
	≥80%	42	129	
B+AB	<80%	95	14	<0.001
	≥80%	50	144	
0	<80%	72	63	<0.001
	≥80%	43	91	

Conclusions: HS candidates had a reduced access to cytotoxic crossmatch during the allocation algorithm, since the time until one DD offer was 3 times higher than in non-HS candidates, independently from the AB0 group. Median waiting-time for a KT was also clearly higher in HS candidates, particularly in A and B+AB blood groups. Considering a higher DSA MFI threshold in the virtual crossmatch of HS candidates (particularly those wait-listed longer), would allow them to have a better chance of undergoing cytotoxic crossmatch with a potential donor. If negative and together with the eventual use of desensitization protocols according with the degree of immunological risk involved, this could allow for more HS candidates to be transplanted.

References:

[1] Malheiro J. Analysis of preformed donor-specific anti-HLA antibodies characteristics for prediction of antibody-mediated rejection in kidney transplantation. *Transpl Immunol*. 2015; 32(2): 66-71.

423.4

The new kidney allocation system: early impact at one of the most active transplant centers in the US

Franco Cabeza[1], Adriana Dejman[2], Aldo Torres[3], Jeffrey Gaynor[1], Analucia Schneegans[1], David Roth[1], Warren Kupin[1], Adela Mattiazzi[1], George Burke[1], Gaetano Ciancio[1], Linda Chen[1], Rodrigo Vianna[1], Giselle Guerra[1].
[1]Kidney Transplant, Miami Transplant Institute, Miami, United States; [2]Nephrology and Hypertension, University of Miami, Miami, United States; [3]Internal Medicine, Hospital San Jose Tec de Monterrey, Monterrey, Mexico.

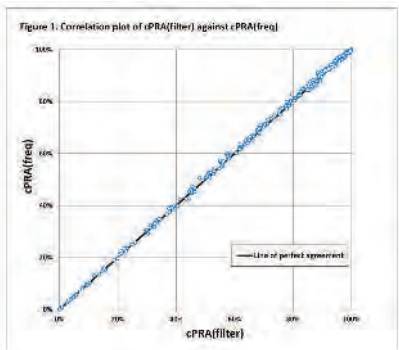
Background: The new kidney allocation system (KAS) was implemented in December 2014 to improve the graft-recipient longevity matching, to facilitate transplantation of harder to match recipients and to decrease discard rates. As one of the most actives centers in the US, we evaluated the early impact of the new KAS compared to recipients transplanted prior.
Methods: We performed a retrospective analysis of 269 kidney recipients transplanted between 1/1/2013-4/30/2015; 190 pre-KAS (control group= C) vs 79 recipients (new KAS group= KAS). Pearson (uncorrected) chi-squared tests of association were performed between selected baseline characteristics and group status (C vs. KAS) as well as between group status and selected outcome variables (%DGF, % biopsy proven acute rejection (BPAR)). Treatment regimens were similar for both groups with induction consisting in a combination of basiliximab, thymoglobulin and steroids. Maintenance immunosuppression was mainly based in tacrolimus and mychophenolic acid with the addition of steroids for the highly sensitized patients.
Results: *Baseline characteristics:* The mean age was 52.6 years for both groups, p=0.12; male gender was 60% in the KAS vs. 56% C; 71% (56/79) of KAS were African-Americans vs. 45.8% (87/190) in C (p=0.0002). Dialysis time ≥ 10 years was found in 41% (32/79) in KAS vs 12 % (23/190) in C (p< 0.01). There were no statistical differences on KDPI scores between both groups.
Outcomes: Under the new KAS, there was a higher trend to transplant very highly sensitized patients (cPRA ≥ 98%), 10.1% (8/79) compared to the 4.2% (8/190) in the C group (p= 0.07). Imported kidneys represented 32.9% of KAS (26/79) vs. 12.1% (23/190) C (P<0.001). Cold ischemia time (CIT) was higher in the C group with CIT > 30hs of 22.8% (18/79) in KAS vs 56.3% (107/190) in C, p<0.001.
There was also a higher rate of recipients receiving donation after cardiac death (DCD) kidneys, 16.5% (13/79) vs 3.2% (6/190) in the C group; that correlated with a significant increase in delayed graft function (DGF), 32.9% (26/79) vs. 11.1% (21/190), respectively, p<0.001.
Dialysis time ≥ 10 years was associated with a significantly higher readmission rate, p=0.04. The frequency of BPAR during the first 6 months after transplantation between both groups were similar, 20.5% (39/190) in KAS vs. 19.0% (15/79) in C (p =0.77).
Conclusion: The new KAS is effectively changing the allograft and recipient characteristics allowing transplant center to favor variables such as prolonged time on dialysis, and higher sensitization state. More frequent use of import and DCD kidneys may occur adversely impacting graft outcomes over time and thus requiring future long-term analysis.
We reported a three times increase in DGF leading to greater economic burden. Transplant centers may be unevenly affected by the new KAS policies with larger centers such as ours absorbing the bulk of higher risk patients.

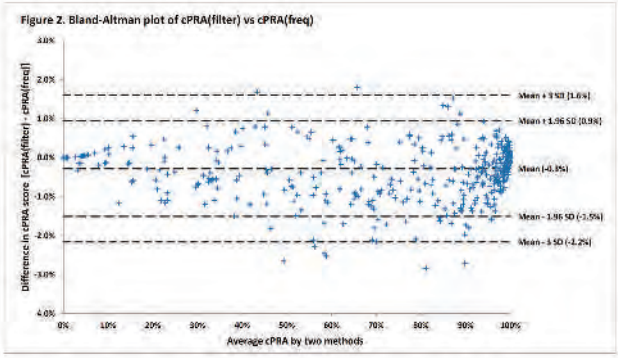
423.5

A simplified method of calculating cPRA for kidney allocation application in Hong Kong

Yuen Piu Chan[1], Monica Wing Kan Wong [1], Lydia Wing Mui Tang[1], Mengbiao Guo[2], Wanling Yang[2], Philip Kam Tao LI[3], Chi Bon Leung[3], Ka Foon Chau[4], Johnny Chi Kwong Lam[5], Nicholas Ka Ming Yeung[5], Janette Siu Yin Kwok[1].
[1]Division of Transplantation & Immunogenetics, Department of Pathology & Clinical Biochemistry, Queen Mary Hospital, Hong Kong, Hong Kong; [2]Department of Paediatrics and Adolescent Medicine, The University of Hong Kong, Hong Kong, Hong Kong; [3]Department of Medicine and Therapeutics, Prince of Wales Hospital, Hong Kong, Hong Kong; [4]Department of Medicine, Queen Elizabeth Hospital, Hong Kong, Hong Kong; [5]Information Technology and Health Informatics Division, Hospital Authority, Hong Kong, Hong Kong.

Introduction: Panel reactive antibody (PRA) has been used to assess the sensitisation level of renal patients in organ allocation program. In 2009 the Organ Procurement and Transplantation Network (OPTN) replaced PRA with Calculated PRA (cPRA) for US renal transplant candidates[1]. cPRA is defined as the percentage of donors expected to have HLA antigens that are unacceptable for a candidate. It is the probability for a patient to give a positive crossmatch and is based on Hardy-Weinberg equilibrium.
In view of no previous research of cPRA in Hong Kong, we embark on employing two methods of cPRA calculation on a renal transplant candidate cohort.
Materials and Methods: HLA-A, -B and -DR typing of 563 Chinese deceased donors in Hong Kong from February 1996 to July 2015 was included. Unacceptable antigens from 613 renal candidates were used for cPRA calculation.
Single locus HLA allele frequencies were counted from donor phenotype. Two and three loci haplotype frequencies were estimated from observed phenotype with Markov Chain Monte Carlo algorithm. cPRA(freq) was calculated based on the formula used by OPTN[2].
A computer script was written to compare listed unacceptable antigens of patients against HLA phenotype of each donor. Patients were filtered out once if they have at least one unacceptable antigen against a donor. cPRA(filter) was expressed as the percentage of filter out count over total number of donors.
cPRA(freq) and cPRA(filter) scores of 70 random selected renal candidates were compared with cPRAs obtained by OPTN and Eurotransplant PRA calculators[3],[4].
Agreement between cPRA calculation methods was analysed by Bland-Altman plot. Concordance correlation was assessed by Lin's correlation coefficient.
Results and Discussion: Both methods showed highly comparable cPRA scores. SD of bias is 0.6%. Limit of agreement is 0.9% to -1.5% difference between cPRA(filter) and cPRA(freq). Lin's correlation coefficient is 1.000, indicating an almost perfect agreement.





Lin's coefficients were 0.824 and 0.829 for cPRA(freq) against cPRA from OPTN, and cPRA(filter) against cPRA from Eurotransplant respectively. These reflected poor agreement and their calculators deemed not applicable in Hong Kong.

Conclusion: This is the first study on applying cPRA in Hong Kong. cPRA values calculated from allele frequencies and donor filtering method showed virtually perfect agreement.

The donor filtering method is more suitable for Hong Kong because the corresponding database and HLA matching function is already in place. cPRA(filter) incorporates existing and new donors in calculation instead of allele and haplotype frequencies captured at one time point. Real-time donor pool will reflect the population allele frequencies and also permits efficient periodic update for cPRA scores.

cPRA can be applied in allocation program for highly sensitised patients for renal and other organs such as heart or lung recipients. Inclusion of other HLA loci can be integrated easily in the future.

References:

[1] Cecka, J. M., Kucheryavaya, A. Y., Reinsmoen, N. L., & Leffell, M. S. (2010). Calculated PRA: Initial Results Show Benefits for Sensitized Patients and a Reduction in Positive Crossmatches. *American Journal of Transplantation*, 11(4), 719-724.

[2] Current CPRA calculation. (2013, December 5). Retrieved February 1, 2016, from http://transplantpro.org/wp-content/uploads/Current-CPRA-Calculation-2.ppt

[3] OPTN CPRA Calculator. Retrieved February 1, 2016, from http://optn.transplant.hrsa.gov/resources/allocation-calculators/cpra-calculator/

[4] Eurotransplant Reference Laboratory, Virtual PRA Calculator. Retrieved February 1, 2016, from https://www.etrl.org/VirtualPRA/Default.aspx

423.6

Comparison of Hong Kong cPRA results with results obtained from OPTN and Eurotransplant PRA online calculators

Yuen Piu Chan[1], Monica Wing Kan Wong[1], Lydia Wing Mui Tang[1], Mengbiao Guo[2], Wanling Yang[2], Philip Kam Tao LI[3], Chi Bon Leung[3], Ka Foon Chau[4], Johnny Chi Kwong Lam[5], Nicholas Ka Ming Yeung[5], Janette Siu Yin Kwok[1].

[1]Division of Transplantation & Immunogenetics, Department of Pathology & Clinical Biochemistry, Queen Mary Hospital, Hong Kong, Hong Kong; [2]Department of Paediatrics and Adolescent Medicine, The University of Hong Kong, Hong Kong, Hong Kong; [3]Department of Medicine and Therapeutics, Prince of Wales Hospital, Hong Kong, Hong Kong; [4]Department of Medicine, Queen Elizabeth Hospital, Hong Kong, Hong Kong; [5]Information Technology and Health Informatics Division, Hospital Authority, Hong Kong, Hong Kong.

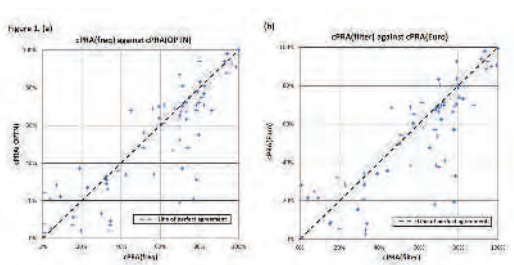
Introduction: Panel reactive antibody (PRA) has been used to assess the sensitisation level of renal patients in organ allocation program. In 2009 the Organ Procurement and Transplantation Network (OPTN) replaced PRA with Calculated PRA (cPRA) for US renal transplant candidates[1]. cPRA is defined as the percentage of donors expected to have HLA antigens that are unacceptable for a candidate. It is the probability for a patient to give a positive crossmatch. In view of no previous research of cPRA in Hong Kong, we set up the local cPRA calculation and compared them with OPTN and Eurotransplant PRA online calculators since HLA gene polymorphism is population specific.

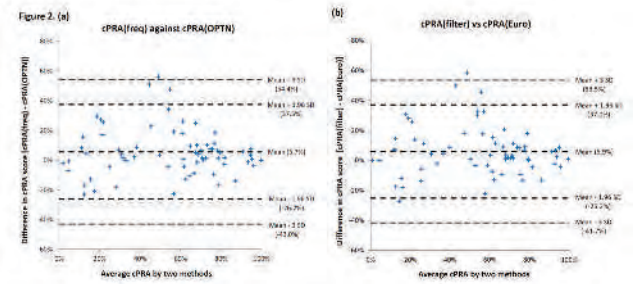
Methods: HLA-A, -B and -DR typing of 563 Chinese deceased donors in Hong Kong from February 1996 to July 2015 was included. Unacceptable antigens from 613 renal candidates were used for cPRA calculation. Single locus HLA allele frequencies were counted from donor phenotype. Two and three loci haplotype frequencies were estimated from observed phenotype with Markov Chain Monte Carlo algorithm. cPRA(freq) was calculated based on the formula used by OPTN[2]. A computer script was written to compare listed unacceptable antigens of patients against HLA phenotype of each donor. Patients were filtered out once if they have at least one unacceptable antigen against a donor. cPRA(filter) was expressed as the percentage of filter out count over total number of donors. cPRA(freq) and cPRA(filter) scores of 70 random selected renal candidates were compared with cPRAs obtained by OPTN cPRA(OPTN) and Eurotransplant PRA cPRA(Euro) calculators[3],[4].

Agreement between local cPRA results and results from overseas cPRA calculation were analysed by Bland-Altman plot. Concordance correlation was assessed by Lin's correlation coefficient.

Results and Discussion: For cPRA(freq) against cPRA(OPTN), SD of bias is 16.2%. Limit of agreement is 37.5% to -26.2%. For cPRA(filter) against cPRA(Euro), SD of bias is 15.9%. Limit of agreement is 37.0% to -25.2%.

Lin's coefficients were 0.824 and 0.829 for cPRA(freq) against cPRA(OPTN), and cPRA(filter) against cPRA(Euro) respectively. These reflected poor agreement and can be explained by the significant HLA allele frequency difference between Chinese and Caucasian population. The result indicates these calculators deemed not applicable in Hong Kong.





Conclusion: This is the first study on applying cPRA in Hong Kong. The poor agreement between our methods and online calculators indicates the necessity to use local population data for accurate cPRA calculation.

The donor filtering method is more suitable for Hong Kong because the corresponding database and HLA matching function is already in place. cPRA(filter) incorporates existing and new donors in calculation instead of allele and haplotype frequencies captured at one time point. Real-time donor pool will reflect the population allele frequencies and also permits efficient periodic update for cPRA scores.

cPRA can be applied in allocation program for highly sensitised patients for renal and other organs such as heart or lung recipients. Inclusion of other HLA loci can be integrated easily in future.

References:

[1] Cecka, J. M., Kucheryavaya, A. Y., Reinsmoen, N. L., & Leffell, M. S. (2010). Calculated PRA: Initial Results Show Benefits for Sensitized Patients and a Reduction in Positive Crossmatches. *American Journal of Transplantation*, 11(4), 719-724.

[2] Current CPRA calculation. (2013, December 5). Retrieved February 1, 2016, from http://transplantpro.org/wp-content/uploads/Current-CPRA-Calculation-2.ppt

[3] OPTN CPRA Calculator. Retrieved February 1, 2016, from http://optn.transplant.hrsa.gov/resources/allocation-calculators/cpra-calculator/

[4] Eurotransplant Reference Laboratory, Virtual PRA Calculator. Retrieved February 1, 2016, from https://www.etrl.org/VirtualPRA/Default.aspx

423.7

Access to kidney transplantation among HIV-infected waitlist candidates

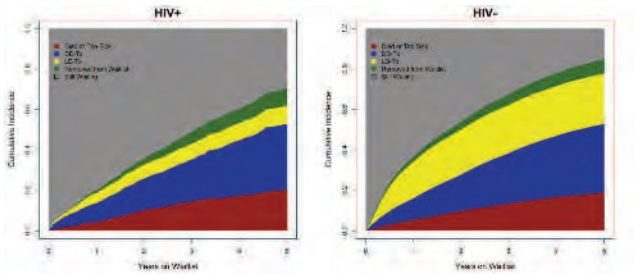
Jayme E. Locke[1], Shikha G.Mehta[1], Deirdre Sawinski[2], Sally Gustafson[3], Brittany Shelton[1], Rhiannon D. Reed[1], Paul A. MacLennan[1], Charolotte Bolch[3], Christine Durand[4], Allan Massie[4], Dorry L. Segev[4].

[1]Surgery, University of Alabama at Birmingham, Birmingham, AL, United States; [2]Medicine, University of Pennsylvania, Philadelphia, PA, United States; [3]Scientific Registry for Transplant Recipients, Minneapolis, MN, United States; [4]Johns Hopkins University, Baltimore, MD, United States.

Background: Kidney transplantation (KT) among HIV-infected ESRD patients confers a significant survival benefit over remaining on dialysis. Given the high mortality burden associated with dialysis, understanding access to KT after waitlisting among HIV+ candidates is warranted.

Methods: Data from SRTR were linked to IMS pharmacy fills (1/1/01-10/1/12) to identify and study 1,636 HIV+ and 72,297 HIV- KT candidates.

Results: Compared to HIV- waitlist candidates, HIV+ waitlist candidates were more often young (<50yrs: 37.5% vs. 62.7%, p<0.001), male (59.3% vs. 75.2%, p<0.001), African American (27.9% vs. 73.6%, p<0.001), > time on dialysis (Yrs: 2.3 vs. 3.8, p<0.001), co-infected with HCV (3.9% vs. 9.0%, p<0.001); and were less likely to remain active on the waiting list (always active: 49.4% vs 37.7%, p<0.001). Although likelihood of waitlist mortality among HIV+ candidates was similar compared to HIV- (aHR: 1.03, 95%CI: 0.89-1.20, p=0.67), likelihood of transplantation was 1.4-fold lower among HIV+ compared to HIV- candidates (aHR: 0.72, 95%CI: 0.64-0.82, p<0.001), and appeared related to an almost 2-fold lower likelihood of living donor kidney transplantation in HIV+ waitlist candidates (aHR: 0.53, 95%CI: 0.44-0.64, p<0.001).



Conclusions: While waitlist mortality has improved for HIV+ candidates, transplant rates remain lower than among HIV- candidates. These results suggest the need for further study to better understand disparities in access to transplantation among HIV+ kidney waitlist candidates, particularly access to living donor transplantation.

423.8

Prioritising renal transplantation based on clinical need: The role of an "urgent" kidney waiting list

Emma Aitken[1], Ann-Margaret Little[2], Marc Clancy[1].
[1]Department of Renal Surgery, Queen Elizabeth University Hospital, Glasgow, United Kingdom; [2]Histocompatability and Immunogenetics, Gartnavel General Hospital, Glasgow, United Kingdom.

Background: In the UK, cadaveric kidneys are allocated for transplantation according to the NHS-BT Deceased Donor Organ Allocation Policy. This complex matching algorithm attempts to provide equity of access by prioritising based on factors such as waiting time, HLA-match and age difference. Unlike liver transplantation however, the current system provides no opportunity to prioritise based on clinical need. There are a small, but significant, number of patients with end-stage vascular access (ESVA), who cannot wait 3 or 4 years for their "turn" and die from access loss on the waiting list for a cadaveric kidney.
Methodology: The current DCD donor sharing scheme permits local allocation of DCD kidneys. At our centre we have established an "expedited" list of patients with ESVA (bilateral central vein occlusion and survival, deemed by the MDT, to be <1 year on haemodialysis). It has been locally agreed that kidneys from DCD donors aged >50 years old will first be allocated to these patients if a match exists. We describe our early (4 year) experience of such a policy.
Results: 22 patients with ESVA were identified. 18 have been transplanted during the study period (9 via the "expedited" list, 6 via the national allocation policy, 3 live donors). The two patients with ESVA who remain on the "expedited transplant list" and have not yet been transplanted both have a cRF of 100% and match score of 1. Half of those who were transplanted also had cRF >95%. Mean age and waiting time for patients getting "expedited' transplantation (n=9) was comparable to the general transplant population (n=420) (46.3+/-10 vs 48.8+/-12.9 years; p=0.56 and 1305.4+/-925.5 days). 1-year patient and graft survival was 88.9%. 44.4% had DGF. Mean eGFR at 1 year was comparable to the general transplant cohort (62.0+/13.4 vs 58.4+/-20.9ml/min/1.73m2; p=0.71). In all but one case, the patient who would have been allocated the kidney according to the national algorithm, was transplanted within the subsequent year.
Conclusions: At our centre, priority allocation of DCD kidneys to patients with failing vascular access has proven effective with acceptable outcomes and minimal negative impact on the global transplant population.

423.9

Efficiency savings through reduction of red blood cell cross-match and transfusion in renal transplantation

Sarah-Jayne Edmondson, Irene Mosca, Ami Mehta, Neal Banga.
Department of Renal Transplantation, Royal Free Hosptial NHS Foundation Trust, London, United Kingdom.

Introduction: Blood products, including donated red blood cells (RBCs) are a valuable and limited resource. Transfusion of RBCs in transplant recipients is not only undesirable from an immunological perspective, but is also expensive. In the UK, each RBC unit costs £122 to produce and store, a recipient "group and save" sample costs £22 to process, while cross-matching and issuing a RBC unit costs between £22-160, depending upon the patient's antibody status. Our unit has recently switched from a policy of routine cross-match of 2 RBC units for renal transplantation to the use of "electronic issue", a process similar to virtual cross-match, where patients without red cell antibodies are allocated group-specific RBC units without a physical cross-match taking place. 90% of the general population are thought suitable for electronic issue. We aimed to quantify the use of RBC cross-match and transfusion in our unit, to assess the efficiency savings that could be achieved through electronic issue, and also identify variables that could predict the transplant recipient's transfusion requirement.
Methods: Transfusion data for all renal transplants performed in a one-year period (April 2014–March 2015) in our department were retrospectively analysed. Electronic issue was routinely performed for all recipients without red cell antibodies in the final four months of the study. Results collated included: initial number of RBC units cross-matched and transfused in the immediate peri-transplant period; total number of units cross-matched and transfused during index admission; proportion of patients receiving a transfusion; and the effect of admission haemoglobin on transfusion requirement.
Results: 140 kidney transplants were performed during the study period. 32/140 (23%) of grafts were from a living donor (LD) and 108/140 (77%) were from a cadaveric donor (CD). 62/140 (44.3%) patients required a RBC transfusion during their index admission. In the initial peri-transplant period, the mean number of units, cross-matched was 2.79 (range 0-10) and, transfused was 0.49 (range 0-4) per patient. There was no difference in transfusion requirements between LD and CD graft recipients. LD transplant recipients were more likely to require a RBC transfusion if their admission haemoglobin level was <100g/L (75.0% vs 20.8%, p=0.005). No similar association was seen in recipients of CD grafts. Following the introduction of electronic issue, 24/46 patients (56%) had electronic issue performed rather than a physical cross-match. This resulted in a £1056 cost saving, and could lead to a future 280 x 52% x £22 (£3,203) saving per annum.
Discussion: The blood transfusion requirement in patients undergoing renal transplantation during index admission is low. There is a clear benefit in optimising pre-operative haemoglobin in LD transplant recipients. Electronic issue has meant that patients no longer require multiple RBC cross-matches, which in turn will result in cost savings.

423.10

Kidney transplantation in the elderly: evaluation of effects of donor age on short- and long-term clinical outcome

Martin Ronaghi, Erik Berglund, Lars Wennberg.
Division of Transplant Surgery, Clinical Science, Intervention and Technology, Solna, Sweden.

Introduction: The population is continuously ageing, and the number of patients with end stage renal disease, and consequently, patients in need of a kidney transplant, are steadily growing. As organ shortage remains one of the main challenges in kidney transplant surgery, the use of elderly donors has become a common strategy to enlarge the donor pool. Herein, the short- and long-term effects of donor age on recipient and graft survival, and graft function, were determined at Karolinska University Hospital in Sweden.
Materials and Methods: Adult recipients of their first kidney allograft from either living or deceased donors, transplanted between the years 2001-2012, were included (n=548). The recipients were stratified and assessed separately depending on the donor's age: <60 (n=367), 60-64 (n=76), 65-69 (n=63), and ≥70 (n=42) years. Data on demographics, patient- and graft survival, as well as graft function was collected retrospectively from patient medical records. Survival was evaluated using Kaplan-Meier estimation and Cox regression analysis, adjusted for recipient age, and gender, as well as living contra deceased donor. Graft function was defined as estimated glomerular filtration rate according to CKD-EPI.
Results and Discussion: The recipients of grafts from donors aged <60 years experienced lower risk for death and graft loss (adjusted HR 0,4-0,7; p <0.05), and higher glomerular filtration rate compared to the other groups. The recipients of grafts from the eldest donors (≥70 years) experienced lower risk for graft loss and death compared to the recipients of grafts from donors aged 60-69 years. There was no significant difference in glomerular filtration rate between the three groups that received grafts from donors ≥60 years.
Conclusion: Recipients of kidneys from donors <60 years had significantly better outcomes compared to the other groups. However, progressively inferior outcomes with advancing donor age could not be shown in recipients of grafts from donors ≥60. These results indicate that utilization of older donors is likely to be a sustainable strategy to improve graft availability, and that no upper age-limit should be set for kidney donation. Our results also emphasize the necessity of further studies in Sweden, for a better understanding of how the donor source is best utilized.

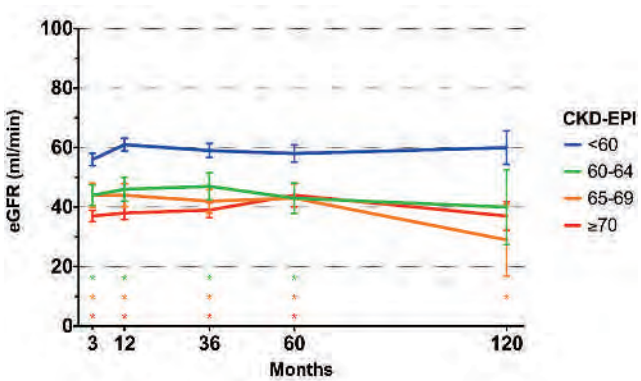


Figure 1. Estimated glomerular filtration rate during 10 years. Connected scatter plot showing estimated glomerular filtration rate over time from transplantation date, using Cockroft-Gault estimation. Error bars represent 95% CI. Subjects are grouped by donor age; <60, 60-64, 65-69, and ≥70. Asterisk denote significant (p<0.05) difference compared to the group <60.

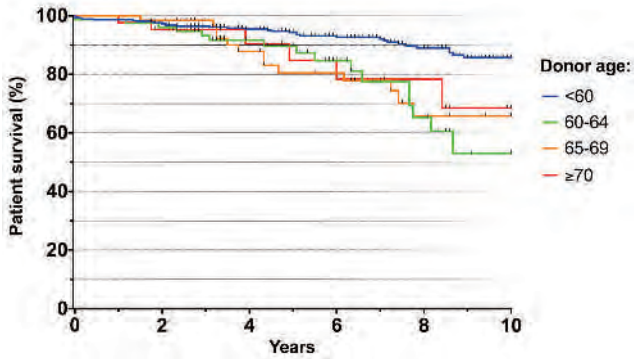


Figure 2. Patient survival. Kaplan-Meier plot showing the proportion of recipients alive expressed as function of time from transplantation. Recipients are stratified and grouped by donor age; <60, 60-64, 65-69, and ≥70.

423.11

Avoiding unnecessary healthcare utilization: The advantages of preemptive kidney transplantation

Patrick Dean[1,2], Kristi M.Swanson[3], James M.Naessens[3], Mark D.Stegall[1,2].
[1]Division of Transplantation Surgery, Mayo Clinic, Rochester, MN, United States; [2]William J. von Liebig Center for Transplantation and Regenerative Medicine, Mayo Clinic, Rochester, MN, United States; [3]Robert D. and Patricia E. Kern Center for the Science of Health Care Delivery, Mayo Clinic, Rochester, MN, United States.

Introduction: Delivering timely, definitive treatment can reduce healthcare costs. Performing a kidney transplant without ever undergoing dialysis (PreKTx) is associated with improved patient survival and quality of life and also might decrease health care utilization and costs. However, most patients—even those with living donors—do not undergo PreKTx. The goal of this study was to compare healthcare utilization in PreKTx vs. non-PreKTx.
Materials and Methods: We identified recipients of first kidney transplants from 2008-2014 utilizing the Optum Labs Data Warehouse, a database including administrative claims data on over 100 million privately insured and Medicare Advantage enrollees in the United States. Patients were identified as receiving a PreKTx vs. non-PreKTx based on claims indicating receipt of dialysis therapy. Healthcare utilization measures were compared across groups.
Results: Of 1334 KTx recipients, 595 (45%) received PreKTx [469 living donor (LD) + 126 deceased donor (DD)] and 739 (55%) received non-PreKTx (379 LD + 360 DD). In LD PreKTx, 20% (93/469) underwent 163 imaging or surgical procedures related to dialysis access, including 55 who underwent creation of an arteriovenous fistula. PreKTx was associated with fewer hospitalizations and emergency department visits before and after transplant (Table 1).

Table 1. Demographics and 90 Day Utilization Measures

	Preemptive (N=595)		Non-Preemptive (N=739)		
	Number or Mean (Median)	% or Std. Dev. (IQR)	Number or Mean (Median)	% or Std. Dev. (IQR)	P-Value
Age at transplant					0.018
≤ 18	36	6.1%	33	4.5%	
19 to 34	71	11.9%	70	9.5%	
35 to 44	103	17.3%	116	15.7%	
45 to 54	155	26.0%	192	26.0%	
55 to 64	172	28.9%	210	28.4%	
65+	58	9.8%	118	16.0%	
Race					0.015
White	291	48.9%	335	45.3%	
Black	21	3.5%	58	7.8%	
Hispanic	39	6.6%	51	6.9%	
Asian	16	2.7%	26	3.5%	
Unknown	228	38.3%	269	36.4%	
Office visits pre-transplant	25.7 (22.0)	15.1 (16)	23.6 (21.0)	14.7 (18)	0.004*
Office visits post-transplant	9.4 (9.0)	5.2 (6)	9.9 (9.0)	6.2 (8)	0.39*
ED visits pre-transplant	1.1 (0)	2.2 (2)	3.3 (2.0)	4.6 (4)	<0.0001*
ED visits post-transplant	0.4 (0)	0.9 (0)	0.6 (0)	1.2 (1)	0.03*
Hospitalizations pre-transplant	1.1 (0)	2.1 (1)	2.7 (2)	3.2 (2)	<0.0001*
Hospitalizations post-transplant	1.3 (2)	2.2 (2)	1.7 (1)	3.0 (2)	0.006*

Conclusions: PreKTx results in lower utilization of healthcare resources. These data suggest that "Transplant First" should be a priority rather than current Federal Government guidelines for "Fistula First"[1].
References:
[1] Centers for Medicare and Medicaid Services. CMS launches breakthrough initiative for major improvement in care for kidney patients. Press release, Office of Media Affairs, March 2005.

423.12

The end of the expanded criteria donor era: Lessons learned

Robert Stratta[1], Alan Farney[1], Giuseppe Orlando[1], Hany El-Hennawy[1], Muhammad Khan[1], Amber Reeves-Daniel[2], Amudha Palanisamy[2], Michael Gautreaux[3], Samy Iskandar[3], William Doares[4], Scott Kaczmorski[4], Jeffrey Rogers[1].
[1]Surgery, Wake Forest, Winston-Salem, NC, United States; [2]Internal Medicine, Wake Forest, Winston-Salem, NC, United States; [3]Pathology, Wake Forest, Winston-Salem, NC, United States; [4]Pharmacy, Wake Forest, Winston-Salem, NC, United States.

Introduction: Expanded criteria donor (ECD) kidneys increase the donor organ pool but their discard rate remains high and their value has been questioned because of expected lower long-term survival outcomes. With the development of the Kidney Donor Profile Index (KDPI) and a new allocation system in the US, the end of the ECD kidney era has occurred coincident with regional and national sharing of high KDPI (ECD) kidneys. The study purpose was to retrospectively review our single center experience with ECD kidney transplants (KT) prior to implementation of the new scoring and allocation systems.
Methods: All patients (pts) received antibody induction with tacrolimus/mycophenolate ± steroids. Standardized management algorithms were implemented to preserve nephron function (including machine preservation) and pt selection was based on low immunological risk (primary transplant, low panel reactive antibody [PRA] level) and predicted limited nephron need (older age, lower BMI).
Results: Over a 12.8 year period, we performed 497 ECD KTs including 247 local and 250 imported from other donor service areas. Mean KDPI was 82%. A total of 55 (11%) dual KTs were performed, including 31 (12.5%) from local and 24 (9.6%) from import ECDs. The import ECD group had more donors (16% vs 8.5%) and recipients (23% vs 16%) ≥ age 70, a higher recipient BMI (27.4 vs 25.4 kg/m^2), more zero HLA-mismatches (14% vs 2%), more pts with a PRA >20% (17% vs 9%), more KTs with a cold ischemia time >30 hours (46% vs 19%), fewer donation after cardio-circulatory death (DCD) ECDs (5% vs 9%), a shorter duration of pretransplant dialysis (mean 26 vs 34 months), higher pump resistance (mean 0.27 vs 0.20 mm Hg/ml/min) and fewer kidneys managed with pump preservation (78% vs 92%, all $p \leq 0.05$) compared to the local ECD group. With a mean follow-up of 5 years (minimum 1 year), actual pt and graft survival rates were 71% and 57.6% in import vs 76% and 57.9% in local ECD KTs, respectively. Death-censored graft survival rates were 70% in import vs 69% in local ECD KTs. Delayed graft function occurred in 28% import vs 23% (p=NS) local ECD KTs. There were no differences in other outcomes. One- and 2-year renal function (eGFR 42 ml/min/1.73 m^2) was similar in both groups. In 104 cases, right and left mate ECD kidney pairs were transplanted as single allografts into 208 pts at our center; outcomes were similar irrespective of kidney laterality.
Conclusions: In spite of a number of differences in donor, recipient, immunologic, and preservation characteristics, intermediate-term outcomes are remarkably similar for import vs local ECD KTs, suggesting that broader sharing of ECD kidneys may improve utilization without compromising outcomes. In addition, by appropriate donor and recipient profiling and the use of management algorithms to project and protect renal function, acceptable outcomes can be achieved with ECD kidney transplants irrespective of kidney laterality.

423.13

Financial cost of cold ischemia time in kidney transplantation

Oscar Serrano[1], David Vock[2], Ty B. Dunn[1], Srinath Chinnakotla[1], Raja Kandaswamy[1], Timothy L. Pruett[1], Arthur J. Matas[1], William D. Payne[1], Roger Feldman[3], Erik B. Finger[1].
[1]Surgery, University of Minnesota, Minneapolis, United States; [2]Biostatistics, University of Minnesota, Minneapolis, United States; [3]Health Policy & Management, University of Minnesota, Minneapolis, United States.

Purpose: A new kidney allocation system (KAS) was implemented in December 2014 using the kidney donor profile index (KDPI), which prioritizes historically-disadvantaged populations. While KAS aims to maximize the equitable sharing of kidney allografts, concerns remain that its implementation through wider geographical sharing may lead to increased cold ischemia time (CIT) and CIT-associated adverse effects, namely delayed graft function (DFG), and ultimately, transplant-related costs.
Methodology: Between 2006 and 2014, 1495 kidney transplants (KTx) were performed at our institution. Donor and recipient demographics, intraoperative parameters, short- and long-term outcomes and hospitalization cost data were collected. A linear regression model was constructed adjusting for recipient age, race, gender, pre-KTx hemodialysis (HD) status, KDPI, and donor type (DD, LD) to assess the impact of CIT on DGF, LOS (LOSTx) and cost (cTx) of transplant admission, and overall 1-year post-transplant in-hospital LOS (LOS1Y) and cost (c1Y).

Table 1. Effect of Cold Ischemia Time on Kidney Transplant Outcomes

Outcome	Expected Increase per 6 hr CIT [95% CI]	*p*-value
DGF	32% Odds of DGF [12-55%]	<0.001
LOS (Tx admission)	0.58 days [0.34-0.81]	<0.001
Cost (Tx admission)	$2078 [$1057-3098]	<0.001
LOS (1-year)	1.06 days [0.33-1.79]	<0.01
Cost (1-year)	$3135 [$651-5619]	0.013

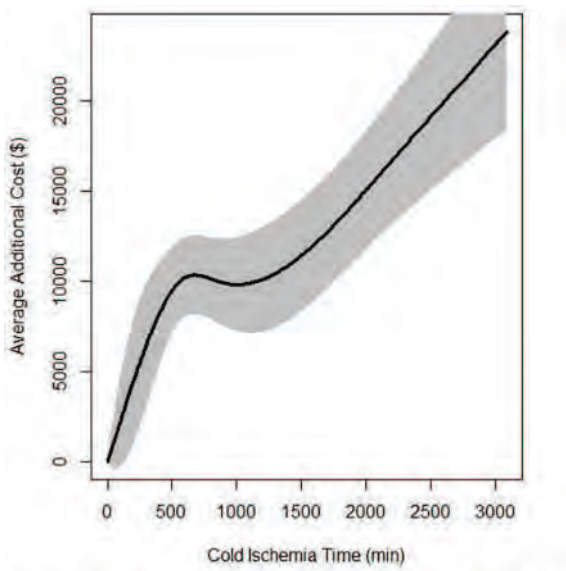


Figure 1. Average additional cost (±95% confidence interval) of transplant admission for each minute of cold ischemia time after adjusting for recipient age, gender, race, hemodialysis, donor type, year of transplant, and KDPI. A restricted cubic spline basis transformation was used to measure the effect of CIT on average cost.

Results: A total of 1200 KTx recipients had financial records available for analysis. Median age at transplant was 48.7 years (IQR 32.6, 59.7). The majority of our patients were male (62.5%), Caucasian (80.9%) and on pre-KTx HD (60%); 43% received a DD kidney. In multivariate analysis, CIT was associated with increased DGF ($p<0.001$), LOSTx ($p<0.001$), cTx ($p<0.001$), LOS1 ($p<0.01$) and c1Y ($p=0.013$). The incremental effect of CIT on KTx outcomes is listed in Table 1 and the average additional cost per minute of CIT is depicted in Figure 1. Further analysis of the cost breakdown revealed an incremental cost effect of CIT on direct, indirect, fixed and variable costs.
Conclusion: One of the unintended consequences of KAS is the prolongation of CIT, which has the potential to increase DGF rates. In turn, we have seen increased DGF rates, leading to increased LOS and overall hospital costs.

424.1

Kinetics of CMV-specific t-cells from pre-to-posttransplantation predict outcomes in CMV-seronegative kidney transplant recipients

Thomas Schachtner[1,2], Maik Stein[2], Pera Reinke[1,2].
[1]Nephrology and Internal Intensive Care, Charité Campus Virchow Clinic, Berlin, Germany; [2]Berlin-Brandenburg Center for Regenerative Therapies, Charité Campus Virchow Clinic, Berlin, Germany.

Introduction: CMV-seronegative kidney transplant recipients (KTRs) from CMV-seropositive donors are at highly increased risk of primary CMV-infection with associated inferior patient and allograft outcomes. Presence of preformed CMV-specific T-cells despite CMV-seronegativity, however, may result from absence of detectable circulating antibodies despite CMV-specific memory B-cells or cross-reactivity due to allogeneic presensitization.
Material and Methods: We studied all CMV-seronegative KTRs between 2008 and 2013 for the presence of CMV-specific T-cells pretransplantation. Among 87 CMV-seronegative KTRs, 49 KTRs (56%) received an allograft from a CMV-seropositive and 38 KTRs (44%) from a CMV-seronegative donor. Samples were collected pretransplantation, at +1, +2, and +3 months posttransplantation. CMV-specific T-cells to CMV-IE1, CMV-pp65, and alloreactive T-cells were measured using an interferon-γ Elispot assay.
Results and Discussion: Among 49 CMV-seronegative KTRs from CMV-seropositive donors, 11 KTRs (22%) showed detectable CMV-specific T-cells pretransplantation. Interestingly, no differences were observed for the incidence of CMV-replication between KTRs with preformed CMV-specific T-cells and those without ($p>0.05$). However, KTRs with preformed CMV-specific T-cells presented with shorter duration of CMV-replication, lower initial and peak CMV-loads, and less need for intravenous antiviral therapy ($p<0.05$). The loss of CMV-specific immunity from pre- to posttransplantation was highly associated with CMV-replication ($p<0.05$). KTRs with stable or increasing CMV-specific immunity from pre- to posttransplantation showed protection from CMV-replication. KTRs with no CMV-specific T-cells pretransplantation showed inferior patient survival and allograft function. We didn't observe an association between preformed CMV-specific T-cells and preformed alloreactive T-cells ($p>0.05$). However, KTRs developing CMV-replication showed a higher incidence of preformed alloreactive T-cells and acute cellular rejection episodes ($p<0.05$).
Conclusion: A relevant proportion of CMV-seronegative KTRs shows CMV-specific T-cells pretransplantation with less severe primary CMV-infection and superior allograft outcomes compared to KTRs without CMV-specific T-cells. However, not the presence of preformed CMV-specific immunity, but the kinetics of CMV-specific immunity from pre- to posttransplantation identifies KTRs at increased risk of CMV-replication. Higher frequencies of alloreactive T-cells may contribute to the higher incidence of acute cellular rejection in KTRs with CMV-replication.

424.2

Treatment of Carbapenem resistant Enterobacteriaceae with reduced susceptibility to polymyxin among kidney transplant recipients experience during an outbreak period

Maristela Freire[1], Flavio J.De Paula[2], Luiz Sergio Azevedo[2], Ana Carolina Lazaro[4], Flavia Rossi[3], Elias David-Neto[2], Willian Nahas[2], Ligia C. Pierrotti[4].
[1]Infection Control Team, Hospital das Clínicas, School of Medicine, University of São Paulo, São Paulo, Brazil; [2]Kidney Transplantation Service, Hospital das Clínicas, School of Medicine, University of São Paulo, São Paulo, Brazil; [3]Laboratory Division of Microbiology, Hospital das Clínicas, School of Medicine, University of São Paulo, São Paulo, Brazil; [4]Infectious Diseases , Hospital das Clínicas, School of Medicine, University of São Paulo, São Paulo, Brazil.

Carbapenem resistant Enterobacteriaceae (CRE) is an emergence worldwide pathogen, with few therapeutic options. Treatment of CRE with reduced susceptibility to polymyxin (MIC ≥16) has been a further challenge, with no established gold standard therapy.
The aim of this study is described the treatment of infections with CRE with reduced susceptibility to polymyxin (MIC ≥16) (CREPR) in kidney transplant recipients (KTr). We included all cases of CREPR infection from Jan 2013 to Dec 2015 that have at least 48 hours of treatment with at least one antimicrobial agent having proven in vitro activity against the isolated strain. Treatment failure was defined as persistence or relapsed of infection within the first 30 days after the end of the initial treatment. Recurrence was defined as new signs or symptoms of infection at the same site, with a positive culture for CREPR of the same phenotype and no evidence of deep infection, after 30 days to the end of the initial treatment. Criteria for infection definitions were those outlines by NHSN.
Association between variables was performed by Chi-square or Fisher test for dichotomous variables and Mann-Whitney test for continuous ones. Multivariate analysis was performed by logistic regression.
We analyzed 21 CREPR infection episodes in 17 patients. The median time between the transplant to first infection episode by CREPR was 78 days (12-4613). Median age of KTr was 57 years (33-72). Two patients had combined transplant (pancreas-kidney and liver-kidney), 16 (94.1%) had a deceased donor, and 13 (76.5%) had induction therapy with ATG. All infections episodes were due to K. pneumoniae, except two patients which infections were caused by S. marcenscens. Sites of infection were urinary tract in 9 cases (42.9%), surgical site infection (SSI) in 11 (52.4%) and pneumonia in 1 (4.8%) case. Twelve infections (57.1%) had positive blood culture. All SSI evolved with deep collection and need surgical or radiological intervention for focus control. The median time from first positive culture to initial of effective therapy was 2 days (0-63). Combined therapy was used in 14 (66.7%) treatment, among those only 6 (28.6%) had more than one drug with in vitro susceptibility. Drugs used in treatment were: polymyxin in 5 cases (23.8%), aminoglycoside in 13 (61.9%), oral fosfomycin in 6 (28.6%), carbapenem in 11 (52.4%) and tigecycline in 7 (33.3%). Among infections treated with carbapenem, the MIC for this antibiotic was under 16mg/dl only in 3 cases. Median length of therapy was 14 days (7-30). Seven (33.3%) patients evolved with treatment failure: 4 (19.0%) died and 3 (14.3%) had relapsed of infection within first 30 days. One KTr had infection recurrence. Risk factors associated to treatment failure in univariate analysis were polymyxin used (P 0.001), higher SOFA score at diagnosis (P 0.002); protective factor was aminoglycoside used (P 0.05). In multivariate analysis, only higher SOFA score was associated to treatment failure. In conclusion infections with CREPR in KTr had high rate of treatment failure and the inclusion of aminoglycoside in therapy may be a protective effect.
References:
[1] Rafailidis PI, Falagas ME. Options for treating carbapenem-resistant Enterobacteriaceae. *Curr Opin Infect Dis* . 2014;27:479-83.

424.3

VZV-specific T-cell memory responses after reactivation in transplant recipients and after vaccination in transplant candidates

Nicole M. van Besouw[1], Marcia M.L. Kho[1], Joke M. Zuijderwijk[1], Jeroen W.I. Jpelaar[1], Marieken J. Boer-Verschragen[1], Herold J. Metselaar[2], Rogier A.S. Hoek[3], Olivier C.Manintveld[4], Annemiek A. van der Eijk[5], Georges M.G.M. Verjans[5], Willem Weimar[1].
[1]Internal Medicine - Nephrology & Transplantation, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands; [2]Gastroenterology & Hepatology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands; [3]Respiratory Medicine, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands; [4]Cardiology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands; [5]Viroscience, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands.

Introduction: Primary varicella zoster virus (VZV) infection causes varicella and lifelong latent infection in neural ganglia from which it may reactivate leading to herpes zoster (HZ). Transplant patients are at high risk to develop HZ and severe clinical complications. In addition, we reported that VZV-reactive memory T-cells are significantly lower in transplant recipients compared to healthy controls (J Med Virol 2012). First, we investigated the incidence of HZ after organ transplantation. Second, we questioned whether HZ after transplantation leads to adequate VZV-reactive memory T-cell responses. Third, we studied the effect of a VZV booster vaccination on T-cell memory.
Methods: The medical records of 958 transplant recipients after first kidney (KTx: n=420), first liver (LTx: n=224), heart (HTx: n=195) and lung (LuTx: n=119) transplantation were analyzed for HZ. The VZV-specific T-cell memory responses before and after HZ (n=5) were compared to patients without HZ (n=5). The VZV-specific T-cell memory responses before and 1 year after Zostavax® vaccination were analysed in 19 patients, aged at least 50 years and older, awaiting renal transplantation. Twenty gender and aged-matched living donors served as controls.
Results: The overall incidence rate of HZ post-KTx (14.4 cases/1000 PY), LTx (24.5 cases/1000 PY), HTx (30.8 cases/1000 PY) and LuTx (38.2 cases/1000 PY) was significantly higher than in the general population of 50-70 years of age (7-8 cases/1000 PY). The percentage of both VZV-reactive CD4 and CD8 central (CM: p=0.06, p=0.02) and effector (EM: p=0.02, p=0.03) memory T-cells increased in all patients after HZ to significantly higher frequencies compared to those without HZ. One year after booster vaccination, the percentage of CD4 CM had increased in both patients (p=0.004) and donors (p=0.004) and the CD4 EM were increased in donors (p=0.009) compared to before vaccination.
Conclusion: HZ is a frequent complication after transplantation and increases VZV-specific T-cell memory responses. Boosting the VZV adaptive immune response by prophylactic VZV vaccination before transplantation may limit the incidence of HZ after transplantation.

424.4

Still an equal B-cell response to a VZV-booster in both renal transplant recipients and healthy individuals at one year post vaccination

Marcia Kho[1], Willem Weimar[1], Marieken J.Boer-Verschragen[1], Annemieke A.van der Eijk[2], Nicole M.van Besouw[1].
[1]Internal Medicine – Nephrology and Transplantation, Erasmus Medical Center, Rotterdam, Netherlands; [2]Viroscience, Erasmus Medical Center, Rotterdam, Netherlands.

Introduction: Herpes zoster incidence and complication rate is much higher in solid organ transplant recipients than in the general population of the same age. This reactivation of the varicella zoster virus could be prevented by booster vaccination. However, end stage renal disease (ESRD) patients are known to respond much poorer to vaccinations, e.g. against hepatitis B and influenza, than healthy individuals do. Therefore, we studied the effect of a VZV booster vaccine on B-cell response in patients with ESRD as well as in healthy individuals.
Methods: In a prospective study, 26 patients, aged at least 50 years, and awaiting renal transplantation were vaccinated with Zostavax®. Gender and age-matched living kidney donors were included as controls (n=27). Varicella Zoster Virus (VZV) specific IgG titres were measured before, at 1, 3 and 12 months post vaccination.
Results: All patients and donors reached month 3 after vaccination, 25 patients and 26 donors reached month 12 after vaccination. Both in patients and healthy individuals, VZV-specific IgG titers were significantly higher at all time points after vaccination compared to before (M1: $p<0.0001$, $p<0.0001$; M3: $p=0.0002$, $p<0.0001$; M12: $p=0.01$, $p=0.0005$; patients and controls, respectively). The patients' IgG titers were comparable to the donors' IgG titers at all time points (pre: $p=0.64$, M1: $p=0.94$, M3: $p=0.90$, M12: $p=0.84$). No difference was found between the patients who received a renal transplant within one year after vaccination and the patients who are still awaiting transplantation.
Discussion: VZV booster vaccination equally increases VZV-specific IgG titers for at least 1 year after vaccination in ESRD patients compared to healthy individuals. Prophylactic VZV vaccination before transplantation could reduce herpes zoster incidence and severity after transplantation.

424.5

Diagnostic potential of interferon gamma release assay to detect latent tuberculosis infection in kidney transplant recipients in endemic area

Hassan Aleid[1], Jameela Edathodu[2], Bright Varghese[3], Abdulrahman Alrajhi[2], Ahmad Nazmi[1], Mohammed Shoukri [4], Attia Ashraff[1], Hazem Elgamal[1], Ihab Mahmoud[1], Fahad Alrabiah[2], Sahal Al Hajoj[3]. [1]Department of Kidney and Pancreas Transplantation, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia; [2]Medicine, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia; [3]Department of Infection and Immunity, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia; [4]National Biotechnology Centre, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia.

Background: Latent tuberculosis infection (LTBI) is now being screened by using clinical assessment, tuberculin skin test (TST), and chest radiography. Interferon gamma release assays, which measure gamma interferon production by lymphocytes in response to exposure to Mycobacterium tuberculosis specific antigens, are now licensed to diagnose latent tuberculosis. The objective of this study was to evaluate the utility of QuantiFERON®-TB Gold in Tube (QFT) test for diagnosing LTBI in patients planned for kidney transplantation.
Methods: All adult patients with end-stage renal disease, evaluated for kidney transplantation in a referral center from August 2008 till May 2013, were enrolled, after consenting in a prospective, observational, non-interventional study. LTBI diagnosis was conducted by TST, chest X-ray and clinical assessment. Interferon Gamma Release Assay (IGRA) was carried out on all candidates by QuantiFERON®-TB Gold In-tube Test.
Results: Overall, 278 patients were enrolled and kidney transplantation was performed in 173 patients. Contributed follow-up was 836.5 patient-years, and tuberculosis-free transplant duration was 478.5 patient-years. By standard methods, latent tuberculosis was diagnosed in 14 patients. Peri-transplant chemoprophylaxis was given to 53 patients, including recipients of organs from all deceased donors and living donors with LTBI. QFT was positive in 70 patients, negative in 200 patients and indeterminate in 8. The agreement between latent tuberculosis diagnosis using standard methods and IGRA by QFT was poor (Kappa: 0.089 + 0.046, p-value = 0.017). 27 of the patients, who had positive QFT, were transplanted and none except one was given isoniazid preventive therapy. None of the transplant recipients developed tuberculosis after a median follow-up of 25 months (range 2-58 months, mean 27 months).
Conclusions: The agreement of the QuantiFERON®-TB Gold In-tube test with standard diagnosis of LTBI in kidney transplant recipients was poor.

References:

[1] 1. Organization World Health. Global Tuberculosis Report 2013. Geneva: WHO, 2013.
[2] 2. Dye C, Watt CJ, Bleed DM, Hosseini SM, Raviglione MC. Evolution of tuberculosis control and prospects for reducing tuberculosis incidence, prevalence, and deaths globally. Jama. 2005 Jun 8;293(22):2767-75.
[3] 3. Selwyn PA, Hartel D, Lewis VA, Schoenbaum EE, Vermund SH, Klein RS, et al. A prospective study of the risk of tuberculosis among intravenous drug users with human immunodeficiency virus infection. The New England journal of medicine. 1989 Mar 2;320(9):545-50.
[4] 4. Qunibi WY, al-Sibai MB, Taher S, Harder EJ, de Vol E, al-Furayh O, et al. Mycobacterial infection after renal transplantation—report of 14 cases and review of the literature. The Quarterly journal of medicine. 1990 Oct;77(282):1039-60.
[5] 5. al-Homrany M. Successful therapy of tuberculosis in hemodialysis patients. American journal of nephrology. 1997;17(1):32-5.
[6] 6. Hussein M, Mooij J, Roujouleh H. Tuberculosis in hemodialysis patients. Saudi journal of kidney diseases and transplantation : an official publication of the Saudi Center for Organ Transplantation, Saudi Arabia. 1996 Jan-Mar;7(1):6-9.
[7] 6. Hussein M, Mooij J, Roujouleh H. Tuberculosis in hemodialysis patients. Saudi journal of kidney diseases and transplantation : an official publication of the Saudi Center for Organ Transplantation, Saudi Arabia. 1996 Jan-Mar;7(1):6-9.
[8] 7. Shohaib SA, Scrimgeour EM, Shaerya F. Tuberculosis in active dialysis patients in Jeddah. American journal of nephrology. 1999;19(1):34-7.
[9] 8. Targeted tuberculin testing and treatment of latent tuberculosis infection. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. This is a Joint Statement of the American Thoracic Society (ATS) and the Centers for Disease Control and Prevention (CDC). This statement was endorsed by the Council of the Infectious Diseases Society of America. (IDSA), September 1999, and the sections of this statement. American journal of respiratory and critical care medicine. 2000 Apr;161(4 Pt 2):S221-47.

424.6

Early bloodstream infections among solid organ transplant recipients

Aysegul Yesilkaya[1], Ebru Soy[2], Mehtap Akcil Ok[3], Ozlem Kurt Azap[1], Hande Arslan[1], Gokhan Moray[2], Atilla Sezgin[4], Fatos Berdan[1], Mehmet Haberal[2].
[1]Infectious Diseases and Clinical Microbiology, Baskent University, Ankara, Turkey; [2]General Surgery, Baskent University, Ankara, Turkey; [3]Nutrition and Dietetics, Baskent University, Ankara, Turkey; [4]Cardiovascular Surgery, Baskent University, Ankara, Turkey.

Introduction: To evaluate the incidence and spectrum of etiologic agents of early bloodstream infections (BSI) among solid organ transplantation (SOT) recipients
Materials and Methods: This was the preliminary report of an ongoing prospective observational study (project no: KA14/120). The data of this report were collected from medical records for each SOT recipient from April 15th 2014 to December 31th 2015. BSI within the first month after SOT was defined as early BSI. Polymicrobial BSI was defined as the growth of more than one organism in blood culture, excluding coagulase-negative staphylococci, *Bacillus* spp. and *Corynebacterium* spp. The etiologic agents and antimicrobial resistance profiles of pathogens of bacteremia episodes were evaluated. Methicillin resistance in *Staphylococcus aureus* strains; extended-spectrum β-lactamase (ESBL) production both in *Escherichia coli* and in *Klebsiella pneumoniae* strains; and multidrug-resistance (MDR) and extensively drug-resistance (XDR) in *Acinetobacter baumannii*, *Pseudomonas aeruginosa* were evaluated. The interim standard definitions for MDR and XDR were used as appropriate[1]. SPSS version 11.0 was used and $p < 0.05$ was considered to be statistically significant. Pearson chi-square test was used as appropriate.

Table 1. Characteristics of the recipients of a SOT with early BSI

	Kidney	Liver	Heart	Total
Transplants performed	110	51	23	184
Living donor	80	37	0	117
Number of BSI episodes	13	20	6	39
Number of BSI episodes >1	1	3	0	4
Number of patients with BSI	12 (10%) (Cl_{95} 4.4-15.6)	17 (33%) (Cl_{95} 20.1-45.9)	6 (26%) (Cl_{95} 8.1-43.9)	35 (19%) (Cl_{95} 13.4-24.6)
Ratio BSI episodes/patients	1.1	1.2	1.0	1.1
Incidence by episodes	11.8%	39.2%	26.1%	21.2%
Incidence by patients	10.9%	33.3%	26.1%	19%
Microbiology of BSI				
Gram negative	11	14	4	29
Gram positive	2	9	0	11
Candidemia*	0	1	2	3
Polymicrobial	0	4	0	4
Leucopenia	5 (39%)	5 (25%)	0	10 (26%)
Crude mortality¶	1 (8%)	2 (10%)	1 (17%)	4 (10%)

Candida dubliniensis (1), Candida glabrata (1), Candida tropicalis (1)

¶Mortality within 30 days after the diagnosis of early BSI, number

Results: One hundred eighty five patients had 187 SOT (one patient had both liver and kidney transplantation, one patient had retransplantation) during the study period. As a total, 113 kidney, 51 liver and 23 heart transplantations were performed. Three SOT recipients living outside Turkey were exluded, as they were unlikely to return to Turkey for the treatment of BSI. The study population comprised of 182 patients with 184 SOT.
We diagnosed 39 early BSI episodes in 35 patients (19.2%). Four recipients had two episodes each: three liver recipients, one kidney recipient. The incidences of early BSI by type of transplantation were demonstrated in Table 1.
Early BSI was seen more in liver than kidney or heart transplantation (p= 0.001). Nine of 55 pediatric recipients had 11 early BSI episodes (28%, 11/39episodes). The median age of SOT recipients at the time of early BSI was 40 years (interquartile range: 11-49 years). The male gender (26/35, 74%) was prominent in the recipients with early BSI.
The source of early BSI episodes by type of transplantation was demonstrated in Table-2.

Table 2. The source of early BSI by type of transplantation

Source of early BSI	Kidney (N=13) N (%)	Liver (N=20) N (%)	Heart (N=6) N (%)	Total (N=39) N (%)
Catheter-related	0	3 (15%)	1 (17%)	4 (10%)
Urinary	7 (54%)	1	1 (17%)	9 (23%)
Biliary	0	3 (15%)	0	3 (8%)
Abdominal nonbiliary	4 (31%)	4 (20%)	0	8 (21%)
Surgical incisional wound	1 (8%)	0	0	1 (3%)
Mediastinitis	0	0	1 (17%)	1 (3%)
Unknown	1 (8%)	9 (45%)	3 (50%)	13 (33%)

Fourty three microorganisms (29 gram-negative, 11 gram-positive and three *Candida*) were isolated. The distribution of 29 gram-negative isolates were: 21 enteric (10 *K. pneumoniae*, 10 *E. coli*, 1 *Serratia* spp) and 8 nonfermentative (3 *A. baumannii*, 2 *Pseudomonas putida*, 1 *Pseudomonas aeruginosa*, 1 *Stenotrophomonas* and 1 *Burkholderia* spp.). ESBL production was 60% (6/10) in *K. pneumoniae* and 50% (5/10) in *E. coli*. One *P. aeruginosa* isolate was XDR. All three (100%) *A. baumannii* isolates were XDR. Among 11 gram-positive isolates; five methicillin resistant coagulase-negative staphylococci, two methicillin-sensitive *S. aureus*, four enterococci isolated.
Discussion: Liver transplant recipients are at the highest risk for developing early BSI after transplantation in accordance with previous studies[2],[3].
Conclusion: Prompt diagnosis and appropriate antimicrobial therapy are important in the management of BSI. Local antimicrobial resistance profiles are helpful in deciding empirical antibacterial therapy.
References:
[1] 1. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*. 2012; 18: 268-281.
[2] 2. Linares L, Garcìa-Goez JF, Cervera C, Almela M, Sanclemente G, Cofàn F, et al. Early bacteremia after solid organ transplantation. *Transplant Proc*. 2009; 41: 2262-2264.
[3] 3. Yesilkaya A, Azap OK, Demirkaya MH, Ok MA, Arslan H, Akdur A. Bloodstream infections among solid organ transplant recipients: eight years' experience from a Turkish university hospital. *Balkan Med J*. 2013; 30: 282-286.

424.7

Efficacy of INH prophylaxis in renal transplant recipients: a follow up of 5 years

Anwar Naqvi[1], Rubina Naqvi[2], Sohail Akhtar[3], Tahir Aziz[2], Asma Naseem[4], Sunil Kumar[4].
[1]Urology and Transplantation, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [2]Nephrology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [3]Pulmonology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [4]Infectious Diseases, Sindh Institute of Urology and Transplantation, Karachi, Pakistan.

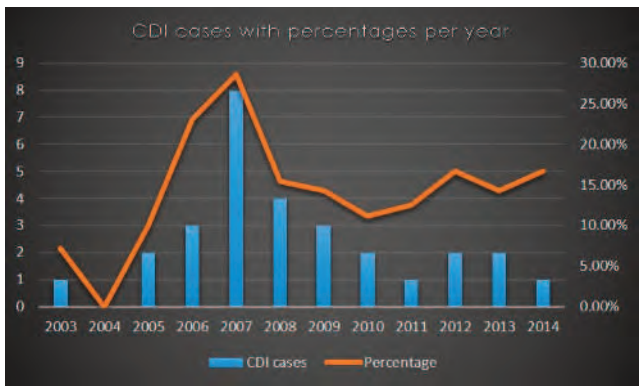
Introduction: Renal transplant recipients are at high risk of tuberculosis due to immunosuppressives they receive. The incidence of tuberculosis in transplant recipients is found to be 20 to 70 time more than in the general population. A prospective randomized study on Isoniazid (INH) prophylaxis at our center showed good efficacy. We have started INH prophylaxis in all renal transplant recipients from April 2009.
Objectives: We seek to follow up all patients on INH prophylaxis and will study the incidence of TB, tolerability of INH and INH resistance. Methods: This is a retrospective observational study. The files of renal transplant recipients from April 2009 to December 2011 were reviewed till June 2015. We noted the incidence of TB, INH resistance, and the tolerability of INH prophylaxis.
Results: A total of 910 patients were followed till June 2015. Eight hundred and twenty six (91%) completed INH prophylaxis successfully. Overall 1.42% had hepatotoxicity due to INH. Fortysix patients (5%) developed tuberculosis. Nearly half of the patients (43%) developed TB >2yrs post prophylaxis. Twenty two (48%) had Pulmonary tuberculosis and 12 (26%) had extrapulmonary TB. Out of 14 cultures only one isolate was INH resistant (7%). There was no significant effect of immunosuppression on the onset of tuberculosis. Thirty seven (84%) got cured and tolerated Rifampicin based regimen very well with no rejection episode.Six patients (13%) died, 3 (6.5%) died due to fulminant TB.
Conclusion: NH prophylaxis is well l tolerated with minimal side effects. The incident TB has significantly decreased during the first 2 years post prophylaxis. INH seems to be effective in curtailing TB in the first two years post transplant. However we should consider prolonging the duration of INH prophylaxis in countries where TB is endemic.

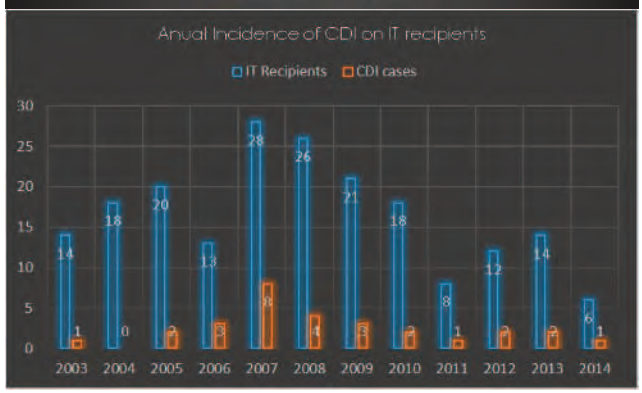
424.8

Epidemiology of Clostridium difficile infection (CDI) in Intestinal Transplant (IT) recipients

Luis F. Guzman Vinasco[1], Fang Qiu[3], Andre C. Kalil[1], David Mercer[2], Alan Langnas[2], Diana F. Florescu[1].
[1]Infectious Diseases, University of Nebraska Medical Center, Omaha, NE, United States; [2]Transplantation Surgery, University of Nebraska Medical Center, Omaha, NE, United States; [3]Department of Biostatistics, University of Nebraska Medical Center, Omaha, NE, United States.

Introduction: IT has become an alternative treatment option for patients with intestinal failure. C. difficile is the most common cause of healthcare-associated infectious diarrhea. One previous report estimated the prevalence of C difficile infection (CDI) in IT recipients at 8%[2]. The aim of our study was to describe the epidemiology of CDI in IT recipients during the first year post-transplantation.
Methods: We performed a retrospective cohort study in pediatric IT recipients who were transplanted at University of Nebraska Medical Center from January 2003 to June 2014. Recipients who developed CDI during the first year post-transplant were analyzed, and only the first episode was taken in consideration. Descriptive statistics are presented for categorical variables, and medians (range) for continuous variables.
Results: 198 patients were transplanted during the study period, with a median age at transplantation of 19 months (range 3 – 443 months); 92(46%) recipients were female, 104 (53%) were Caucasian. CMV sero-status: 27(14%) D+/R+, 54 (27%) D+/R-, 31(16%) D-/R+, 86(43%) D-/R-. Allograft type: 37(19%) patients underwent isolated IT, 155(78%) liver-IT and 6(3%) liver-kidney-IT. 197 recipients were induced with basiliximab and 1 with basiliximab-thymoglobulin. Maintenance immunosuppression: 198(100%) oral steroids, 188(95%) tacrolimus, 22(11%) mycophenolate, 7(3.5 %) sirolimus and 1(0.5 %) everolimus.
29(15%) recipients developed CDI. The graph describes the annual incidence of CDI.





Of the recipients with CDI: 13(44%) were exposed to antibiotics, 10(34%) had surgery, 23(79%) were on proton pump inhibitors, 2(6.9%) had jejunostomy, 23(79%) gastrostomy, 20(68%) ileostomy, 3(10%) received IVIG, 25(86%) were on tube feedings; median serum levels of tacrolimus during the month prior to diagnosis of CDI was 13.6 ng/mL (range 3.3 – 28.7). 25(86%) patients were treated with oral metronidazole; in 26(89%) patients the length of treatment was 14 days. There was only 1(3.3%) episode of recurrent CDI. Of the 29 patients with CDI, 7(24%) had an episode of rejection: 4 before and 3 after CDI. 6(20.6%) died during the first year post-transplant.
Conclusion: CDI is a frequent (15%) infectious etiology of diarrhea in IT recipients during the first year post-transplant.
References:
[1] David Ziring, Robert Tran, Susan Edelstein, Sue V. McDiarmid, Nupoor Gajjar, Galen Cortina. Infectious Enteritis After Intestinal Transplantation:Incidence, Timing, and Outcome. Transplantation • Volume 79, Number 6, March 27, 2005.
[2] Suresh Paudel, Ioannis M. Zacharioudakis, Fainareti N. Zervou, Panayiotis D. Ziakas. Prevalence of Clostridium difficile Infection.PLOS ONE, April 17, 2015.
[3] David J. Riddle and Erik R. Dubberke. Clostridium difficile infection in solid organ transplant recipients. Current Opinion in Organ Transplantation 2008.
[4] J. P. Donnelly, H. E. Wang, J. E. Locke. Hospital-Onset Clostridium difficile Infection Among Solid Organ Transplant Recipients. American Journal of Transplantation 2015; 15: 2970–2977.
[5] Pant C, Deshpande A, Desai M, Jani BS, Sferra TJ, Gilroy R, Olyaee M. Outcomes of Clostridium difficile infection in pediatric solid organ transplant recipients. Transpl Infect Dis. 2015 Nov 5.

424.9

Incidence and outcome of parasitic infection after kidney transplantation in Thailand

Siriorn Watcharananan[1], Kaenchai Pipatpannawong[2], Viroon Mavichak[2], Nattakan Naitook[2].
[1]Medicine, Ramathibodi hospital, Mahidol University, Bangkok, Thailand; [2]Kidney transplant institute, Praram 9 hospital, Bangkok, Thailand.

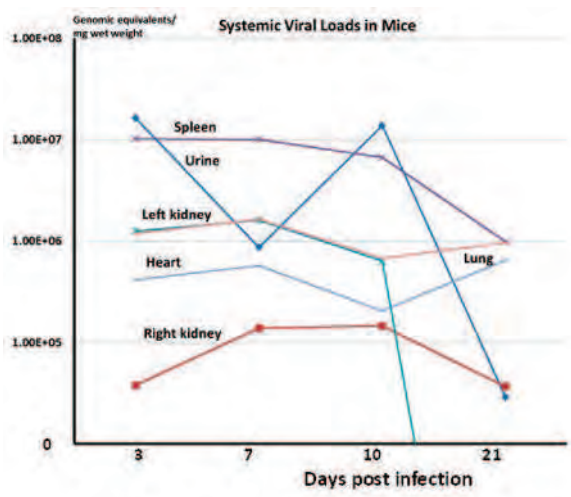
Background: Little is known about the parasitic infection among kidney transplant (KT) recipient.
Methods: We prospectively screened 374 adult (age >18 years old) KT candidates for pretransplant latent infections during 2006 and 2016. Majority of the patients were from Thailand (66.3%), Myanmar (26.2%), and Cambodian (4.3%). Parasitic screenings including blood serology for strongyloides and rapid screen for malaria for patients from endemic area. Endemic parasites (Gnathosomiasis spp., Angiostrongylus spp., Strongyloides spp.) were tested by serology and stool examination among the case with peripheral eosinophilia. Medical records of patients with parasitic infection before or after KT were reviewed for demographic characteristics, clinical presentation, laboratory findings, treatment and outcome.
Result: A total of 357 (95.45%) and 39 (10.43%) patients were tested for strongyloides antibody and rapid screen for malaria before KT, respectively. Only one (0.28%) was screened positive for strongyloides without notable peripheral eosinophilia, nor clinical symptoms. None were screened positive for malaria. Two (0.53%) had peripheral eosinophilia and were tested positive for gnathosomiasis. Patient with seropositive strongyloides received 2 courses of oral ivermectin (before and soon after transplantation). Long term (> 3 years) success was noted without repeated course of treatment. Of the 2 gnathosomiasis cases, one completed oral albendazole before transplantation and the long term follow up showed no relapse of eosinophila. Another received oral ivermectin plus albendazole within 2 weeks before transplantation, followed by the completion of albendazole course after KT. There was no relapse of eosinophila at 6 weeks follow up. After KT, there were 2 (0.53%) cases with clinically evident parasitic infections. Both were previously tested negative for strongyloides antibody before KT but they were diagnosed with strongyloides hyperinfection after transplantation. One had disseminated strongyloidiasis 5 months after KT. Despite the treatment with oral and parenteral ivermectin plus albendazole, he subsequently died from ARDS, sepsis with multiorgan failure. Another was diagnosed with eosinophilic appendicitis 4 years after KT. This patient initially presented with acute fever, abdominal pain and underwent appendectomy. A history of intermittent abdominal pain and chronic diarrhea was also noted. Stool examination showed viable larva of Strongyloises stercolaris which persisted despite 2 days of oral ivermectin. Extending the treatment to 1 more week resulted in resolution of the clinical symptoms. There was no relapse of parasites in the stool, nor eosinophilia at 3 months follow up.
Conclusion: Parasitic infection is uncommon among KT recipients in endemic area but the infection result in a significant complication after transplantation. Serology for strongyloides antibody might not be helpful to prevent infection after transplantation.

424.10

Pathogenesis of polyomavirus-associated nephropathy: insights from a mouse model

Gang Huang[1], Gang Zeng[2], Yuchen Huang[2], Parmjeet Randhawa[2].
[1]Department of Organ Transplantation, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, People's Republic of China; [2]Department of Pathology, University of Pittsburgh, School of Medicine, Pittsburgh, United States.

Background: Polyomavirus-associated nephropathy is an important cause of dysfunction and failure of renal transplants. Because it typically occurs in kidney transplant recipients, immunosuppression and renal ischemia-reperfusion injury are considered important factor in its pathogenesis. We sought to test this notion experimentally and develop an animal model that would facilitate development of novel therapies for this distressing disease.
Methods: 4 week old, 15-20 gram, male, C57BL/6J mice were used as kidney ischemia- reperfusion model. The left kidney pedicle was clamped for 3 hours and then released. Tacrolimus (0.2 mg/Kg) and Mycophenolate mofetil (200 mg/Kg) were given by the intragastric route daily. Mouse PyV (MPyV) LID -1 strain was propagated in NIH 3T3 cells and 3×109 genomic equivalents were used to infect mice by the intraperitoneal route 2 days after clamping of the renal artery. DNA was extracted using the Qiagen DNeasy Blood & Tissue Kit, QIAamp Fast DNA Stool Mini Kit, or QIAamp DNA Mini Kit (for urine samples). Viral load kinetics was studied in groups of 4-6 mice, sacrificed 3, 7, 10, 14, 21 and 28 days after infection. For quantitation of viral load, MPyV A2 large T antigen gene derived primers were used in a SyBrGreen based PCR run on the ABI Prism 7500 System using a MPyV-A3 plasmid based standard curve.
Results: Following infection, MPyV DNA was readily detected at virtually all time points with a peak observed on day 3, 7 or 10. MPV can replicate in many organs including not widely appreciated sites, namely the lung, aorta, heart, and liver. Viral loads were in the range of 105-107 genomic equivalents /mg wet weight. The two organs with the highest load were the spleen and aorta. Complete viral clearance occurred in the left kidney by 21 dpi, while clearance in other organs was partial with a 10-1000 fold decrease in viral load. Although some of the left kidneys with ischemic damage developed very high loads, there was considerable variability, and some organs showed no demonstrable infection, possibly because the tissue saved for viral DNA was not completely viable. Histopathologic examination did not produce the characteristic pathology of human polyomavirus nephropathy. There was also no viral cytopathic effect in any other organ.



Conclusion: A combination of ischemia and immunosuppression in the non-transplanted kidney failed to recapitulate human polyomavirus nephropathy. It appears that alloimmune mononuclear infiltrates provide a critically needed cytokine milieu that engenders viral replication and triggers the biochemical pathways that cause acute tubular injury and interstitial nephritis, which are the hallmark lesions of polyomavirus infection in the allograft kidney.

References:

[1] 1. Han Lee ED, Kemball CC, Wang J, et al. A mouse model for polyomavirus-associated nephropathy of kidney transplants. Am J Transplant 2006; 6:913-22.
[2] 2. Kemball CC, Pack CD, Guay HM, et al. The antiviral CD8+ T cell response is differentially dependent on CD4+ T cell help over the course of persistent infection. J Immunol 2007; 179:1113-21.
[3] 3. Albrecht JA, Dong Y, Wang J, et al. Adaptive immunity rather than viral cytopathology mediates polyomavirus-associated nephropathy in mice.Am J Transplant. 2012;12:1419-28.
[4] 4. Ribeiro A, Wörnle M, Motamedi N, et al. Activation of innate immune defense mechanisms contributes to polyomavirus BK-associated nephropathy. Kidney Int 2012;81:100-11.
[5] 5. Ribeiro A, Merkle M, et al.BK virus infection activates the TNFα/TNF receptor system in Polyomavirus-associated nephropathy.Mol Cell Biochem. 2015 Oct 7.

424.11

Pneumocystis pneumonia cluster outbreak at a Scottish Renal Transplant Centre

Andrew McClarey, Paul J.Phelan, Ian Laurenson, Dáire T.O'Shea, Lorna K.Henderson.
Royal Infirmary of Edinburgh, Edinburgh, United Kingdom.

Introduction: Pneumocystis Pneumonia (PCP) caused by Pneumocystis jirovecii is a potentially fatal opportunistic infection occurring primarily in immunocompromised hosts. Cluster outbreaks of PCP amongst renal transplant recipients have been documented worldwide. We describe a cluster of PCP diagnosed in nine renal transplant recipients from a single centre between November 2014 and January 2016.
Methods: A restrospective analysis of cases was carried out within our transplant centre, collating demographic and clinical data from the hospital database.
Results: During November 2014 to January 2016, nine renal transplant recipients (including one combined kidney pancreas recipient) were diagnosed with PCP, a notable rise in our centre. All suspected cases were confirmed by real time polymerase chain reaction testing of induced sputum (n=7) and bronchoalveolar lavage (n=2). All patients had radiological features consistent with PCP but two had no clinical symptoms initially other than pyrexia.
The median age at presentation was 65 years (range 24-77 years). All patients had a functioning graft (mean eGFR 30ml/min/1.73m2 (range 15-47ml/min/1.73m2). Median time to disease onset was 5.8 years post transplant (range 0.5-10.4 years). Eight patients were receiving tacrolimus or sirolimus based immunosuppression at diagnosis together with an anti-metabolite (n=7) and/or steroids (n=6). Mean MMF dose was 1.5 g (range 1-2 g). Mean tacrolimus trough was 6.6 ug/l (range 4-8 ug/l). Two patients received ATG therapy for vascular rejection within 12 months of presentation. All patients received pneumocystis prophylaxis for three months post transplantation and following ATG therapy but none were taking prophylaxis when diagnosed. Three patients had received treatment for CMV disease prior to index presentation, CMV viremia was simultaneously detected in a further three patients at presentation. All patients were lymphopenic at diagnosis (mean $0.2x10^9$/L) with six of nine demonstrating lymphopenia in the 12 months prior to diagnosis (mean $0.9 x10^9$/L).
Four patients required mechanical ventilation and two required dialysis. Four patients died within three months of presentation. Graft failure occurred in one further survivor.
Conclusion: This study characterises a heterogenous cluster of renal transplant patients developing PCP. Notable clinical features included a universal lymphopenia, low eGFR, high rates of co-morbidities (rejection episodes, CMV viremia) and several patients being a considerable time out from transplant. A number of the cases overlapped in time and place such that cross-transmission amongst susceptible hosts is suspected and further characterisation of this cluster by molecular typing of clinical specimens has been undertaken. This cluster illustrates the persistent risk of opportunistic infection, many years from transplantation, and represents a precedent in Scotland, and an emerging challenge to the long-term success of renal transplantation.

424.12

Neutrophil function assay in renal transplant recipients with bacterial and fungal infections

Sabiha Anis[1], Hafsa Tanwari[1], Mahjabeen Imam[1], Rana Muzaffar[1], Tahir Aziz[2], Anwar Naqvi[3], Adib Rizvi[3].
[1]Molecular Biology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [2]Nephrology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [3]Urology and Transplantation, Sindh Institute of Urology and Transplantation, Karachi, Pakistan.

Background: Neutrophils are the cells of the innate immune system that kill microorganisms by generating oxidative burst. This may be impaired in renal transplant recipients (RTx) due to immunosuppressive drugs and may predispose to develop bacterial and fungal infections. In this study we have evaluated the neutrophil functions in renal transplant recipients with bacterial and fungal infections by Nitro Blue Tetrazolium (NBT) slide test.
Methods: Neutrophil function was assessed in 41 RTx and 12 healthy controls. The neutrophils were stimulated with E. coli endotoxin and Candida extracts. The NBT scoring was done based on percentage of NBT positive cells from total neutrophils counted with or without formazan. Reference cut-off was taken from NBT scores of the control group. The data was analyzed using spss version 20. $P \leq 0.05$ was taken as significant difference between groups.
Results: The mean NBT score was significantly higher in unstimulated (US) and stimulated neutrophils RTx compared to control group (14.2 ± 8 vs. 7.3 ± 3.2; $P = 0.006$, 63.1 ± 17 vs. 54 ± 9.2; $P = 0.007$ and 42 ± 14.4 vs. 32 ± 6.2; $P = 0.020$ respectively)
There was a significant association of mean NBT scores of US neutrophils with the renal dysfunction compared to normal renal functions (16.4 ± 8.3 vs. 10 ± 6.0; $P = 0.013$). Rejection episodes were found in 11 recipients (42%). A high mean scores of NBT results was seen with Candida stimulation in patients with rejection compared to non-rejection (46 ± 12 vs. 25 ± 15.3; $P = 0.007$).
Conclusions: The neutrophil function assay has a major role in assessing the status of innate immune system in transplant recipients. A significant association of high NBT score in US neutrophils with active infections and renal dysfunction may indicate a need of aggressive treatment strategies in these patients. Low NBT positive results may signify considerable degree of immunosuppression, requiring drug modification. A prospective study with a larger sample size may help to elucidate these important findings in RTx.

424.13

Factors associated with clostridium difficile infection after heart and heart-lung transplantation

Jackrapong Bruminhent[1,2], Kelly A.Cawcutt[2], Charat Thongprayoon[5], Tanya M.Petterson[3], Walter K.Kremers[3], Raymund R.Razonable[2,4].
[1]Department of Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; [2]Division of Infectious Diseases, Mayo Clinic, Rochester, MN, United States; [3]Division of Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN, United States; [4]William J. von Liebig Center for Transplantation and Clinical Regeneration, Mayo Clinic, Rochester, MN, United States; [5]Division of Critical Care, Mayo Clinic, Rochester, MN, United States.

Background: Clostridium difficile is a major cause of diarrhea in thoracic organ transplant recipients. We investigated risk factors of Clostridium difficile infection (CDI) in heart and heart-lung transplant (HT) recipients.
Materials and Methods: This is a retrospective study of HT recipients at a single US transplant center from 2004-2014. CDI was defined by diarrhea and a positive toxigenic Clostridium difficile in stool measured by toxin enzyme immunoassay (EIA) (2004-2006) or polymerase chain reaction (2007-2014). Cox proportional hazards regression was used to model the association of baseline risk factors and the time dependent variable, acute cellular rejection (ACR), with time to CDI following transplant.
Results: There were 254 (246 heart and 8 heart-lung) HT recipients, with a median age was 53 years (IQR, 45-60); 34% were female. During the median follow-up of 96 months (IQR, 40- 185), 22 (8.7%) patients developed CDI. In univariate analysis, CDI in HT recipients was associated with female sex, having combined heart-lung transplant, redo-transplantation, acute cellular rejection (ACR), and maintenance with tacrolimus and prednisone (Table 1). In multivariate analysis, ACR was associated with lower risk [HR=0.24; 95% CI, 0.09-0.69 (p=0.008)] while maintenance with prednisone was associated with higher risk [HR 10.60; 95% CI, 1.43-78.83 (p = 0.02)] of CDI after transplantation.
Conclusion: CDI after HT is less common among patients who have ACR and more common among those maintained on prednisone.

Table 1
Univariate analysis of risk factors for CDI after heart and heart-lung transplantation

Risk factors	HR	95%confidence interval	P-value
Recipient age (years)	0.98	0.95-1.01	0.16
Recipient female sex	3.07	1.31-7.18	0.01
Recipient BMI (kg/m^2)	0.93	0.84-1.03	0.15
Heart-lung transplant	6.26	1.85-21.21	0.003
CMV mismatch	0.75	0.31-1.85	0.54
Redo-transplant	5.92	1.34-26.12	0.02
Prior episode of CDI	2.43	0.56-10.58	0.24
Donor age (years)	1.00	0.97-1.04	0.87
Donor female sex	1.79	0.77-4.15	0.17
Ischemic time	1.00	0.99-1.01	0.91
Perioperative prophylaxis with cephalosporins	1.17	0.35-3.96	0.80
Induction with Anti-thymocyte globulin	1.61	0.60-4.32	0.35
Maintenance therapy			
-Cyclosporine	0.81	0.32-2.02	0.65
-Tacrolimus	3.63	1.54-8.53	0.003
-Azathioprine	0.85	0.32-2.22	0.73
-Mycophenolate mofetil	0.48	0.21-1.13	0.09
-Prednisone	10.13	1.36-75.33	0.02
Acute cellular rejection	0.26	0.09-0.74	0.01

References:
[1] Johnson S, Gerding DN. Clostridium difficile—associated diarrhea. Clin Infect Dis 1998 May;26(5):1027-34; quiz 35-6. 2.
[2] Boutros M, Al-Shaibi M, Chan G, et al. Clostridium difficile colitis: increasing incidence, risk factors, and outcomes in solid organ transplant recipients. Transplantation 2012 May 27;93(10):1051-7. 3.
[3] Riddle DJ, Dubberke ER. Clostridium difficile infection in solid organ transplant recipients. Curr Opin Organ Tran 2008 Dec;13(6):592-600.
[4] Lee JT, Kelly RF, Hertz MI, Dunitz JM, Shumway SJ. Clostridium difficile infection increases mortality risk in lung transplant recipients. J Heart Lung Transplant 2013 Oct;32(10):1020-6.

425.1

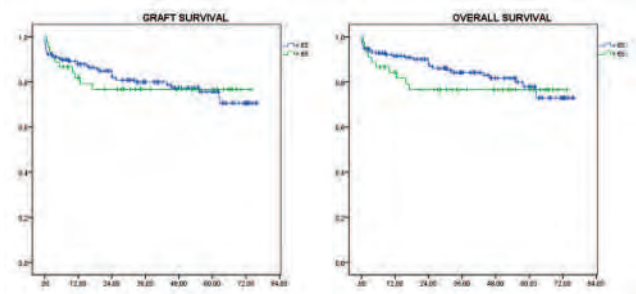
Liver transplantation in the elderly: An increasing and appropriate indication

Luis Moulin, Andres Fraile, Diego Ramisch, Silvina Yantorno, Gabriel Gondolesi, Valeria Descalzi.
Multiorgan Transplant Institute, Favaloro's Foundation University Hospital, Ciudad Autonoma de Buenos Aires, Argentina.

Continues increase in general life expectancy and improvements achieved in medical therapies for cirrhotic patients (pts), have rise the age of potential candidates for Liver Transplantation (LT). Age is no longer an absolute contraindication for LT or donation. Previous presentations from our center have shown a progressive increase from 5% to 15% of elderly patients transplanted from 1995-2000 to the 2006-2009 periods.
Aim: To evaluate number and outcomes of LT performed on patients older (OP) than 65 years of age at a single center.
Methods: A retrospective study, based on a prospective database including 216 LT from 9/2009 to 12/2015. OP patients were compared to the younger population (YP). Demographics and transplant variables, ethiologies and outcomes were analyzed using SPSS v 17.0.
Results: Over the last 6 years, 45 OP (20.8%) and 171 YP were transplanted. Analyzed variables are presented in table 1. Actuarial patient survivals at 1 and 3 years were: OP group 84% and 77%, vs. YP group: 91 and 84% respectively (p: NS). Graft survivals were: 82 and 77% for the OP vs. 88 and 80% for the YP (Figure 1).

		OP 45 (20.8%)		YP 171 (79.2%)		p
Mean Age (years±SD)		68 ± 2.25		50 ± 11.4		<0.05
Sex	*Males (n; %)*	25	55%	101	59%	NS
MELD score (±SD)		21 ± 8.3		18 ± 7.1		<0.05
DRI (±SD)		1.50 ± 0.33		1.52 ± 0.29		NS
Ethiology (*)	*Alcohol(n; %)*	15	33%	57	33%	NS
	HCV(n; %)	12	27%	36	21%	NS
	Autoimmune (n; %)	1	2%	12	7%	<0.05
	Cryptog./NASH (n; %)	11	24%	21	12%	<0.05
	PBC (n; %)	2	4%	16	9%	NS
	ALF (n; %)	2	4%	21	12%	NS
	HCC (n; %)	19	43%	37	22%	<0.05

Table 1: Variables analyzed. Categorical variables were analyzed using: χ^2-test, Fischer exact test and t-test. (*) We included most frequent etiologies for both groups. *PBC*: primary biliary cirrhosis. *ALF*: acute liver failure. *HCC*: hepatocarcinoma.



References:
[1] Tapia Mantilla et al. - ¿Debe Ofrecerse El Trasplante Hepatico A Mayores de 65 Anos? - Congreso Argentino de Hepatologia (AAEEH); june 2011 - Oral Presentation.

425.2

Long term outcome results using predictive scores in liver re-transplantation

Andres Fraile, Nestor Pedraza Alonso, Maria Eugenia Serio, Victorio Cervera, Valeria Descalzi, Pablo Barros Schelotto, Gabriel E. Gondolesi.
Instituto de Trasplante Multiorgánico, Hospital Universitario Fundación Favaloro, Ciudad Autónoma de Buenos Aires, Argentina.

Liver re-transplantation (re-LT) is an infrequent indication for LT, with a reported prevalence close to 6% in different reported series. It is the unique therapeutic option when graft failure occurs. Although is a high risk population with poor prognosis, so a strict selection of candidates is mandatory.
Aims: 1) to evaluate the prevalence and outcome of re-LT at our centre, 2) To identify predictors of survival after re-LT. Patients and Methods: From 6/95 to 12/15, 876 consecutive adult liver transplantations were performed. Clinical, biochemical, and demographic variables, and survival prediction scores (Rosen, Markmann and UCLA) were retrospectively applied and analyzed from donors and receptors of re-LT. Statistical Analysis: Chi-square, T-Test, Actuarial Survival Rate by Kaplan Meier.
Results: 55 out of 876 were re-LT (6 %) and 4 received a 2nd re-LT (0.4 %). Median age of re-LT was 43±14 years, 57 % were males with median time between LT and re-LT of 2.2 years. Main causes for re-LT were recurrence disease (29%), biliary (22%),ductopenia (20%), vascular (18%) and primary non-function (8%). Causes for 2nd re-LT were: biliary 2 (50%), recurrence disease 1 (25%) and ductopenia 1 (25%). According to priority for re-LT, in 37 patients (72%) re-LT was elective (>60 days) and urgent in 14 (27%). From the 51 re-LT patients, 23 died, with an overall, elective and urgent survival rate at 3 months, 1, 5 and 10 years of 84-83-64%, 77-78-64%, 72-76-48%, 58-63-48% (p=ns). Among studied variables (donor and recipients age, gender, underling liver disease, time between LT and re-LT, bilirrubin, creatinine, DRI and survival prediction scores) only cold ischemia time (CIT) >495 minutes was associated with poor outcome (p=0.002). Markmann score was statistically significantly associated with 1 year mortality (p=0.015). Actuarial survival rate of 2nd re-LT at 3 months, 1 and 5 years were 50, 50 and 25% respectively. The re- LT scores did not predict early mortality in the 2nd re-LT.
Conclusions: 1) Re-LT represents a safety option for patients with graft failure, with survival rate over 70% at 5 year post re-LT 2) CIT > 495 minutes for re-LT was the only useful predictor of outcome in our series 3) Although Markmann score was associated with 1 year mortality, re-LT predictive scores when were applied individually, didn't show enough sensibility and specificity to predict poor outcome.

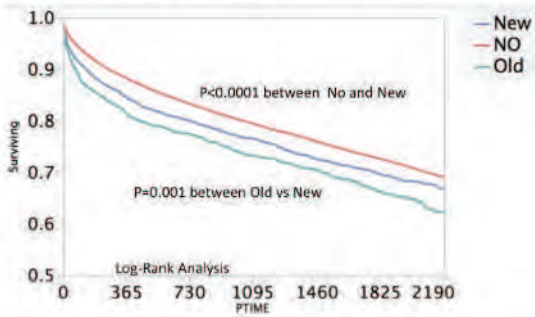
425.3

Patients on the liver transplant waiting list at risk for developing new onset portal vein thrombosis

Martin Montenovo, Jorge Reyes, James Perkins.
Surgery, University of Washington, Seattle, United States.

Introduction: Portal vein thrombosis (PVT) is quite common in patients with cirrhosis. The risks factors of developing PVT while on the waitlist and the impact on patient survival after liver transplantation are not well defined. The objective of this study was to determine risk factors for developing PTV while in the waitlist and the impact in patient survival after liver transplantation..
Methods: We conducted a retrospective cohort analysis using the United Network for Organ Sharing database from January 2002 through June 2014, including adults (³18 years old) who were listed and received a primary OLT. We looked at rate of PVT during the study period at the time of listing and at the time of transplant, and the effect of PVT on patient survival after the liver transplantation. Cox Proportional Hazards models were constructed to adjust for recipient characteristics in order to estimate the risk factors associated with development of PVT while in the waitlist.
Results: A total of 63.265 patients listed and transplanted for primary OLT were identified. 3,612 patients were listed without PVT but developed PVT by the time of transplant; 1,708 patients were listed with PVT and had PVT at the time of transplant; 57,945 patients never had PVT at listing or time of transplant. PVT at listing and at the time of transplant has a negative impact in patient survival after liver transplantation (fig 1).

Fig 1. Survival among patient with new, old and no PVT.



On the multivariate analysis several risk factors were independently associated with the development of PVT while in the waitlist (table 1).

Table 1. Logistic Regression Analysis of Factors Associated with the Development of PVT while in the waitlist.

Days Waiting	1.0008	1.0007	1.0009	<0.0001
Age	1.01	1.009	1.018	<0.0001
Prior Abd Surg	1.27	1.01	1.38	<0.0001
Exception for HCC	1.21	1.08	1.37	0.0009
Albumin at Listing	0.90	0.84	0.97	0.004
Slight Ascites	1.30	1.16	1.46	<0.0001
Moderate Ascites	1.5	1.31	1.71	<0.0001
Previous Malignancy	1.32	1.17	1.49	<0.0001
Variceal Bleeding Yes	1.4	1.14	1.7	0.001
SBP	1.23	1.04	1.44	0.02
INR at listing	0.90	0.84	0.96	0.001
Serum Creatine at Listing	0.95	0.91	0.98	0.05
Diabetes	1.28	1.16	1.4	<0.001

Conclusions: PVT has a significant negative impact in patient survival after liver transplantation. Patients at increased risk for PVT should undergo a more aggressive screening for early identification and potential treatment.

425.4

Determination of alcohol abstinence in transplant candidates by ethyl glucuronide hair analysis

Brigitta Globke[1], Christina Papachristou[2], Martin Hastedt[3], Franziska Krumbiegel[3], Johann Pratschke[1], Andreas Pascher[1].
[1]Department of General, Visceral and Transplantation Surgery, Charité - Universitaetsmedizin Berlin, Berlin, Germany; [2]Department of Psychosomatic Medicine, Charité - Universitaetsmedizin Berlin, Berlin, Germany; [3]Institute of Legal Medicine and Forensic Sciences, Charité - Universitaetsmedizin Berlin, Berlin, Germany.

Introduction: In the Eurotransplant region listing for liver transplantation requires abstinence of at least 6 months in patients with alcoholic liver disease. The assessment of alcohol abstinence is a complex process and so far comprised a thorough taking of the history of alcohol consumption as well as the presentation to the department of psychosomatic medicine. The use of biomarkers to objectify alcohol abstinence is an important addendum. Ethylglucuronide (EtG) is a direct alcohol metabolite, with a high sensitivity and specificity for detection of alcohol consumption for a period of a few days in urine samples and three months in hair samples, respectively. As of mid 2015 the german authorities implemented a testing for EtG in urine (uEtG) samples to the mandatory evaluation process, hair testing (hEtG) is an optional test.
Materials and Methods: In our patient collective EtG was determined using liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS) in hair samples of transplant candidates referred to our hospital for alcoholic-, cryptogenic- or suspected alcohol abuse between 2012 and 2015. We analysed samples from 221 patients (m:f 149:72) and compared patients statements concerning their alcohol consumption habits, with EtG results and psychosomatic assessment.
Results: When the hair sample was taken 90% of patients claimed abstinence of at least three months time, but only in 76% of the samples the patients statement was confirmed by the test results. In a multivariate analysis "favourable psychosomatic assessment," a "long duration of abstinence" and "male gender" proofed to be predictors for negative hEtG results. In 147 cases the EtG test results matched the psychosomatic assessment, whereas in 35 cases we received contradictory results. Those cases were re-assessed individually.
Conclusion: EtG hair analysis is the only generally available method that allows detection of alcohol consumption over a period of time that comes close to the demanded period of abstinence for placement on the Eurotransplant list. Therefore, we are enabled to offer support to people who still need to overcome alcoholism before they become the transplant candidates. We also have a tool at hand that might enable patients with cryptogenic cirrhosis to fend off allegations of alcohol abuse. In our hospital we established an algorithm combining psychosomatic evaluation, mandatory uEtG analysis and optional hEtG analysis in the liver transplant evaluation process.

425.5

Influencing factors of fatigue in liver transplant recipients

Hongxia Liu, Xiaohong Lin, Sha Teng, Yabin Shang.
School of Nursing, Beijing University of Chinese Medicine, Beijing, People's Republic of China.

Aims and Objectives: The purpose of this study was to examine the intensity, interference, duration, and prevalence of fatigue in liver transplant recipients and to explore the influencing factors of post-transplantation fatigue.
Background: Fatigue is a highly prevalent symptom experienced by patients who underwent the liver transplantation. However, the influencing factors of fatigue are poorly understood. More studies are needed to document fatigue and its influencing factors in patients after liver transplantation. Design A cross-sectional design was used in this study.
Methods: A convenience sample of liver transplant recipients was recruited at an outpatient liver transplant clinic in Beijing, China. Self-report survey data were provided by liver transplant recipients using the Fatigue Symptom Inventory (FSI), the Hospital Anxiety and Depression Scale (HADS), the Perceived Social Support Scale (PSSS), and the Athens Insomnia Scale (AIS). Demographic, clinical, psychosocial parameters were evaluated as influencing factors of fatigue.
Results: Participants (n=285) included 69 women and 216 men. Fatigue was found in 87.02% of liver transplant recipients. Mean scores of fatigue intensity items were 4.47±2.85, 1.93±1.97, 3.15±2.13, 2.73±2.42 (most fatigue, least fatigue, average fatigue, fatigue now). Mean score of fatigue interference were 2.27±2.09. Number of days having fatigue in the previous week was 2.26±2.02 and the mean percentage of fatigue time each day was 2.75±2.44. Spearman Correlation Analysis showed that fatigue intensity was associated with anxiety, depression, and insomnia ($P<0.001$ for all), while fatigue interference was associated with gender, anxiety, depression, and insomnia ($P<0.05$ for all). In the multiple linear regression analysis, anxiety and insomnia were associated with fatigue intensity ($P<0.001$), and insomnia, depression and anxiety were associated with fatigue interference ($P<0.001$).
Conclusions: Fatigue is common and pervasive in liver transplant recipients, and it is strongly associated with insomnia, anxiety, and depression.
Relevance to Clinical Practice: Health professionals need to pay attention to fatigue in liver transplant recipients and take appropriate measures to reduce patients' fatigue.
This study was supported by Beijing Natural Science Foundation, grant number:7142098
References:
[1] Rodrigue J, Nelson D, Reed A, et al. Fatigue and sleep quality before and after liver transplantation. *Progress in Transplantation*. 2010; 20(3): 221-233.
[2] van den Berg-Emons R J G, van Ginneken B T J, Nooijen C F J, et al. Fatigue after liver transplantation: effects of a rehabilitation program including exercise training and physical activity counseling. *Physical therapy*, 2014; 94(6): 857-865.
[3] van den Berg-Emons R, van Ginneken B, Wijffels M, et al. Fatigue is a major problem after liver transplantation. *Liver transplantation*. 2006; 12(6): 928-933.
[4] van Ginneken B T J, Den Berg-Emons V, Rita J G, et al. Persistent fatigue in liver transplant recipients: a two-year follow-up study. *Clinical transplantation*. 2010; 24(1): E10-E16.
[5] van Ginneken B T J, van den Berg-Emons R J G, Kazemier G, et al. Physical fitness, fatigue, and quality of life after liver transplantation. *European journal of applied physiology*. 2007; 100(3): 345-353.
[6] van Ginneken B T J, van den Berg-Emons R J G, Kazemier G, et al. Physical fitness, fatigue, and quality of life after liver transplantation. *European journal of applied physiology*. 2007; 100(3): 345-353.

425.6

Predictors of mortality in living donor liver transplantation

Shaimaa Elkholy, Sherif Mogawer, Mostafa Elshazli, Ula M.Al-Jarhi, Samah Abdel-Hamed, Adel Hosny, Ayman Salah, Noman El-Garem, Amany Abdel-Maksoud, Mona El-Amir, Mohamed S.Abdel-Aziz, Ahmed Nabil.
Liver Transplantation Unit, Faculty of Medicine, Cairo University, Cairo, Egypt.

Background: Liver transplantation had become now the definite treatment of patients with end stage liver disease (ESLD). Due to ethical and cultural background, living donor liver transplantation (LDLT) is the only available option for liver transplantation in some countries. Being the only source of donors it's of extreme importance to perform a detailed analysis for the whole process aiming to improve the overall outcome of LDLT. Minimizing the Mortality had been the target of this study.
Post-operative infection is a leading cause of morbidity and mortality in patients undergoing Living Donor Liver Transplantation (LDLT). This study aims to develop a practical and efficient prognostic index for possible predictors of post-transplant mortality using risk factors identified by multivariate analysis.
Materials and Methods: This study was done to analyze data from patients with post hepatitic cirrhosis, HCV positive, genotype 4, Child B, C or MELD score (13-25) undergoing LDLT. It included data from 100 Egyptian patients who had LDLT conducted at Kasr AlAiny Hospital, Liver Transplantation Department, Faculty of Medicine, Cairo University. The Study included 90 males and 10 females with ages ranging between 20 and 63 years.Patient management and decisions were independent from the study and totally subject to our centre protocol. More than 160 fields per patient were documented including pre-operative, operative and post-operative data. Data included patient history, clinical, laboratory, and sonographic information as well as complications and outcome. All potential predictors of mortality were analyzed by backward logistic regression. Cut-off values were obtained from ROC curve analysis.
Results: Sepsis was the main cause of death in the studied group. Early infectious complications were associated with a significantly higher mortality rate 34(50.7%) in patients who encountered post-operative infection versus 11(33.3%) in patients who did not (p-value < 0.005). TLC, total bilirubin, early biliary complications, fever and CRP were highly correlated to morbidity and mortality. By performing multivariate analysis (logistic regression) it was found that the presence of infection, fungal infection and HCC recurrence were the most prominent predictors of mortality.
Conclusion: Post LDLT infections remains on the top of the list as a cause of mortalitiy. Prompt initiation of risk-stratified management plans may improve the outcome of LDLT.

425.7

Liver transplantation in elderly recipients: when is old too old?

Nicholas Gilbo, Ina Jochmans, Mauricio Sainz, Jacques Pirenne, Diethard Monbaliu.
Abdominal Transplantation Surgery and Coordination, University Hospitals of Leuven, Leuven, Belgium.

Background: Recipient age is regarded a risk factor of inferior outcome after Liver Transplantation (LT) because of increased co-morbidities, including cardiovascular disease. Nevertheless, the average age of LT recipients is increasing. We therefore compared post-LT outcome between recipients younger and older than 70y.
Methods: We retrospectively compared donor and recipient demographics, transplant and outcome data between LT recipients >70y (R>70) and <70y (R<70) in a cohort of 643 consecutive LT in (01/2003-12/2014). The influence of recipient age on patient and graft survival was evaluated by means of multivariable regression. Data are presented as median and IQR.
Results: 30 LT and 3 ReLT out of 643 transplants (5%) were performed in R>70. Compared to R<70 [IQR 49-64y], all R>70 [IQR 72-74y] received grafts from brain dead donors (100% vs 87.2%, p:0.1), more frequently extended criteria donors (66.7% vs 48.2%, p=0.048) of older age [69y (53.5-79) vs 53y (43-63), p<0.0001] although with no difference in DRI [2.39 (1.99-2.61) vs 2.13 (1.8-2.51), p=0.08]. Recipients >70y had lower lab-MELD [12.5 (8.45-18.42) vs 15.6 (10.8-24), p=0.01] but higher BAR score [4 (4-4) vs 2 (2-4), p<0.0001] and were less frequently admitted in intensive care at the time of LT (6% vs 14%, p=0.1). R>70 were more frequently transplanted for HCC (54.5% vs 29.5%, p=0.004). Cold ischemia was shorter in R>70 [6.17h (5.3-7.55) vs 7.36h (5.65-8.9), p=0.02]. Peak AST post-LT [R>70 626IU/L (299-1058) vs R<70 693IU/L (365-1323), p=0.27], incidence of early allograft dysfunction (R>70 15% vs R<70 29.3%, p=0.1) or acute kidney injury (R>70 21.2% vs R<70 22.8%, p=1), permanence in intensive care and length of hospitalization did not differ amongst groups. The incidence of non-anastomotic biliary strictures within 1y post-LT was similar in both groups (R>70 15.2% vs R<70 12.1%, p=0.5). One and five year graft survival were similar between R>70 and R<70 (93.9% vs 87.6%, 75.4% vs 70.8% respectively, p=ns); likewise 1 and 5 y patient survival did not differ (100% vs 90.9%, 78.7% vs 75.8% respectively, p=ns). Multivariable Cox regression did not reveal an impact of recipient age when considered either as continuous or dichotomous variable on patient and graft survival (R>70 HR:0.84, 95%CI:0.42-1.7 and HR:1.01, 95%CI:0.99-1.04) in contrast to the presence of HCC which was independent risk factor of impaired recipient survival (HR:1.53, 95%CI:1.08-2.2, p=0.017).
Conclusion: In our experience, transplanting stable elderly recipients did not increase post-operative morbidity and achieved survival comparable to that one of younger patients. Patient selection for LT should therefore not be limited by age.

425.8

Extracorporeal membrane oxygenation support for critically Ill liver transplantation recipient

Kyo Won Lee[1], Min Jung Kim [2], Nuri Lee[1], Seounghyun Kim[1], Ji Soo Lee[1], Gyuseong Choi[1], Kyeong Sik Kim[1], Jae-Won Joh[1].
[1]Department of Surgery , Samsung Medical Center , Seoul, Korea; [2]Department of Surgery, Jeju National University Hospital , Jeju, Korea.

Introduction: After liver transplantation (LT), recipients occasionally come into cardiopulomonary dysfunction refractory to optimal medical management and ventilator care. Extracorporeal membrane oxygenation (ECMO) can provide life support in those cases. This study was designed to assess the outcome of the ECMO and predict the prognosis of those cases.
Methods: From February 2008 to October 2012, ECMO was used in 25 cases (24 patients) due to cardiogenic shock (n=5), septic shock (n=9), respiratory failure (n=10), hypovolemic shock (n=1). Laboratory values including lactate level just before starting ECMO were obtained and Sequential Organ Failure Assessment (SOFA) score, Acute Physiology and Chronic Health Evaluation (APATCH) II score and Simplified Acute Physiology Score (SAPS) II were calculated. Subsequent SOFA, APATCH II, SAPS II and lactate clearance (LC) after 24 hours of starting ECMO were also calculated. Comparisons were made between cases who did and did not succeed in weaning ECMO and between who did and did not survive.
Results: Successful weaning occurred in 8 cases (7 patients) and 4 cases (4 patients) were survived. APATCH II and SAPS II were significantly low($p = 0.011$, 0.016, respectively) and LC and Δ-SOFA(0-24) were significantly high in weaning success group ($p = 0.009$, 0.020, respectively). In survival group, SAPS II and APATCH II were low ($p = 0.047$, 0.038, respectively).
Conclusions: ECMO can be used for critically ill LT recipients who do not respond to conventional management. SAPS II and APATCH II could be good indicators of prognosis in terms of both weaning and survival.
References:
[1] Choi, N. K, Hepato-Gastroenterology 59(116): 1189-1193.
[2] Park, Y. H., et al. Transplant Proc 44(3): 757-761.
[3] Yoo, C. S., et al. Korean J Anesthesiol 65(2): 151-157.
[4] Park, T. K., et al. Eur J Cardiothorac Surg 47(2): e68-74.
[5] Auzinger, G., et al. Liver Transplantation 20(9): 1141-1144.
[6] Tejani, M., et al. Liver Transplantation 21(3): 410-414.

425.9

Pre transplant recipient frailty combined with disease severity predicts post liver transplant (OLT) mortality

Dympna Kelly[1], Lisa Louwers[1], Kareem Eid[1], Shetal Shah[3], Pilar Bayona[3], Fouzeyah Ali[3], Kevin Chagin[2], Bijan Eghtesad[1], Nafatali Presser[1], Julia Muntean[1], Michael Kattan[2], Charles Miller[1], John Fung[1].
[1]General Surgery, Cleveland Clinic, Cleveland, United States; [2]Quantitative Health Sciences, Cleveland Clinic, Cleveland, United States; [3]Imaging, Cleveland Clinic, Cleveland, United States.

As we transplant sicker patients we are challenged in identifying patients with poor post OLT outcomes. This study aimed to identify pre-OLT parameters which can predict early post OLT mortality.
Methods: Data for all adult primary OLT recipients (01/10-08/14) was retrospectively reviewed (IRB 15-1443). Pre OLT parameters were identified in the frailty domains of: 1) Age 2) Functional status: Karnofsky score (KS), location at transplant 3) Cognition: encephalopathy, history of depression 4) Nutrition: BMI, albumin, Hct, refractory ascites, sarcopenia 5) Comorbidities: heart disease, diabetes, renal and thyroid disease, PVD, PUD, tobacco use) 6) Extrinsic frailty: support at home 7) Measures of disease severity: chemical MELD, PT, INR, creatinine, bilirubin. Sarcopenia was assessed using psoas and paraspinal muscle vol. (mean) and density (HU) (mean) on CT scans within 90 days prior to OLT on a Leonardo Workstation using Oncocare® software (Seimens Healthcare, Erlangen, Germany). Data was analyzed using multivariate logistic regression to identify predictors of in-hospital mortality (death during the OLT admission) and 1yr mortality. Two patients with graft failure were excluded from mortality analysis.
Results: 523 adult, liver only, recipients were transplanted. CT scans within 90 days of OLT were available in 278/523 (53%) patients. Data was analyzed for the entire population (n=523) without sarcopenia parameters, and all data, including sarcopenia, was analyzed for 278 patients with CT scans.
In-Hospital Mortality: 20/522 (4%) patients died in-hospital. Excluding sarcopenia the model predicting in-hospital mortality had a c-statistic of 0.757 (95% CI 0.66-0.85). 15/277 (5%) patients with all data including sarcopenia died in-hospital. The final model predicting in-hospital mortality with the inclusion of sarcopenia (n=277) had c-statistic of 0.875 (95% CI 0.81-0.93).

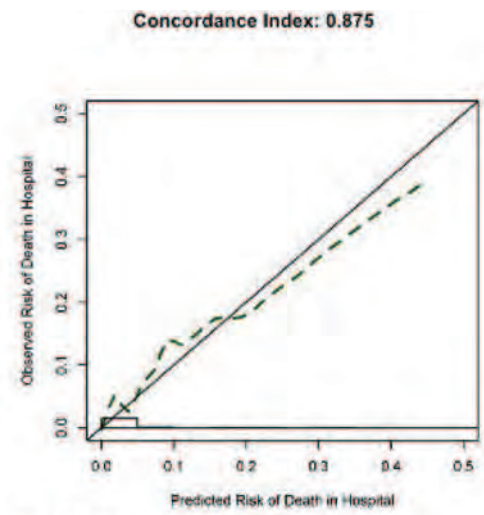


Fig. 1: In Hospital Mortality.

Variables with the highest predicting value were MELD (Odds Ratio 1.12), KS (OR 0.94), Bilirubin (OR 0.92), Psoas vol. (0.44), Paraspinal vol. (OR 1.3)

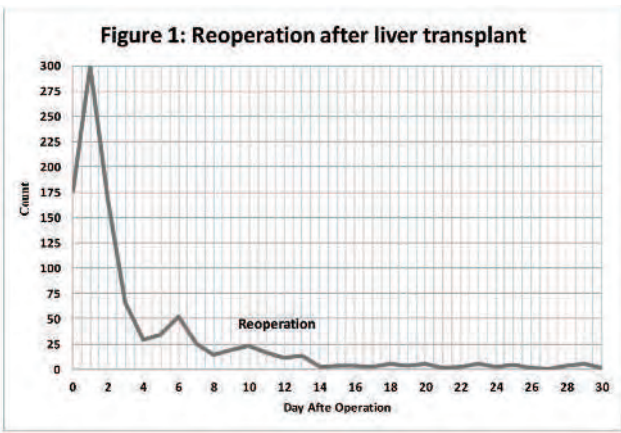
One Year Mortality: 52/521 (10%) patients 52 died within 1yr. The c-statistic was 0.688 (95% CI 0.62-0.77). 31/276 (11%) patients with all data including sarcopenia died within 1yr. The final model predicting 1yr mortality with the inclusion of sarcopenia (n=276) had c-statistic of 0.823 (95% CI 0.76 and 0.89). Variables predicting 1yr mortality were male gender (OR 2.8), KS (OR 1.1), depression (OR 2.9), BMI (OR 0.9), Living alone (OR 0.1), Psoas density (OR 1.1), Psoas vol. (OR 0.4).
Conclusion: The sub group of patients with sarcopenia data (n=278) is representative of the entire cohort including mortality rates. Frailty parameters, including sarcopenia, combined with disease severity significantly improves our ability to predict post OLT mortality, with a C Index of 0.877 for in-hospital mortality and 0.823 for 1yr mortality. This model has the potential to identify poor risk patients prior to proceeding to OLT.

425.10

A nationwide analysis of reoperation after liver transplant

Zhobin Moghadamyeghaneh[1], Gennaro Selvaggi [1], Seigo Nishida [1], Linda Chen[1], Rodrigo Vianna [1], Giselle Guerra[2].
[1]Miami Transplant Institute, University of Miami/ Jackson Memorial Hospital, Miami, United States; [2]Transplant Surgery, Miami Transplant Institute, Miami, FL, United States.

Objectives: This study aimed to report the rate and short-term outcomes of patients underwent reoperation following liver transplant in the United States.
Methods: The NIS database was used to examine the clinical data of patients underwent liver transplant and reoperation during same hospitalization during 2002-2012. Multivariate regression analysis was performed to compare outcomes of patients with and without reoperation.
Results: We sampled a total of 59,166 patients who underwent liver transplant. Of these, 7373 (12.5%) had reoperation during the same hospitalization. The most common day of reoperation was the first day after transplant (Figure 1). Hemorrhagic complication with 68% was the most common reason of reoperation followed by biliary tract anastomosis complications (14.8%) and vascular complications (10%). Also, 3.6% of patient underwent reoperation for wound disruption. Patients with reoperation had a significantly higher mortality (11.6% vs. 3.8%, AOR: 3.01, P<0.01). Preoperative coagulopathy (AOR: 1.71, P<0.01) and renal failure (AOR: 1.57, P<0.01) were associated with hemorrhagic complications. Also, preoperative peripheral vascular disorders (AOR: 2.15, P<0.01) and coagulopathy (AOR: 1.32, P<0.01) were significantly associated with vascular complications. Risk of wound disruption was significantly higher in patients with chronic pulmonary disease (AOR: 1.50, P<0.01).
Conclusion: Reoperation after liver transplant occurs in 12.5% of cases. However, it is associated with a significantly increased mortality. The first day after transplant and hemorrhagic complication are the most common day and reason of reoperation respectively. Controlling coagulation disorders preoperatively may decrease hemorrhagic and vascular complication. Patients with chronic pulmonary disease are at risk of wound disruption.



425.11

Effect of graft weight to recipient body weight ratio on hemodynamic and metabolic parameters in pediatric liver transplantation: A retrospective analysis

Zeynep Ersoy[1], Şerife Kaplan[1], Aycan Özdemirkıran[1], Adnan Torgay[1], Gülnaz Arslan[1], Arash Pirat[1], Mehmet Haberal[2].
[1]Anesthesiology and Reanimation, Baskent University Hospital, Ankara, Turkey; [2]General Surgery, Baskent University Hospital, Ankara, Turkey.

Background: The ratio of graft weight to the recipient body weight (G/R ratio) in liver transplantation (LT) is one of the major risk factors affecting patient outcome. We hypothesize that a high G/R ratio (>4%) in pediatric LT adversely affects the intra and early postoperative hemodynamic and metabolic parameters.
Methods: In this retrospective study, we screened data of children who underwent LT between 2005 and 2015 at Ankara Baskent University Hospital. LT recipients were divided into two groups based on their G/R ratio: liver transplant recipients with a G/R > 4% (large for size) and those with a G/R ≤ 4% (normal for size). Collected parameters included demographic data, preoperative laboratory findings, intraoperative metabolic and hemodynamic parameters, and intensive care unit follow-up parameters.
Results: A total of 130 pediatric liver transplant recipients' data were included in the analyses. The median Child and PELD scores were 9.0 and 17.0, respectively. Child and PELD scores were significantly different between two groups (9.6±1.3 and 8.5±2.1, p=0.013; 27.8±11.3 and 14.5±15.2, p<0.001).
When we compared the two groups the patients in group G/R > 4% received higher amounts of colloid solutions (57.7±20.1 ml/kg and 45.1±21.9 ml/kg, p=0.08) and higher doses of furosemide (0.7±0.6 mg/kg and 0.4±0.7 mg/kg, p= 0.018).
Patients in group G/R > 4% had lower mean pH and pO2 values during newhepatic phase than those in group G/R ≤ 4% (7.1±0.1 and 7.2±0.1, p=0.004; 115.4±44.6 mmHg and 147.6±49.3 mmHg, p=0.004). Compared to group G/R ≤ 4%, recipients in group G/R > 4% had higher values of blood glucose (352.8±96.9 mg/dl and 262.8±88.2 mg/dl, p<0.001) and lower mean body temperature during newhepatic phase (34.8±0.7oC and 35.2±0.6oC, p=0.016). Compared to group G/R ≤ 4%, group G/R > 4% received higher amounts of packed red blood cell transfusion during both anhepatic and newhepatic phases (30.3±24.3 ml/kg and 18.8±21.8 ml/kg, p=0.013; 17.7±20.4 ml/kg and 10.3±15.5 ml/kg, p=0.031, respectively) and received higher amounts of fresh frozen plasma during newhepatic phase (13.6±17.6ml/kg and 6.2±10.2ml/kg, p=0.012).
Recipients in group G/R > 4% were more likely to be hypotensive in terms of systolic and diastolic blood pressures intraoperatively and also more hypothermic during all phases (p<0.05).
Recipients in group G/R > 4% more commonly received norepinefrine infusion intraoperatively (44% and 22%, p <0.05) . More patients in group G/R > 4% compared to group G/R ≤ 4 were mechanically ventilated in ICU (56% vs 31%, p=0.035). There were no significant differences between two groups in terms of postoperative acute renal dysfunction, graft rejection or lost, infections, length of ICU stay, and mortality (p>0.05).
Conclusion: The high G/R are associated with adverse metabolic and hemodynamic changes during LT and early postoperative period. These results emphasize the importance of using an appropriate size of the graft in liver transplantation.

References:

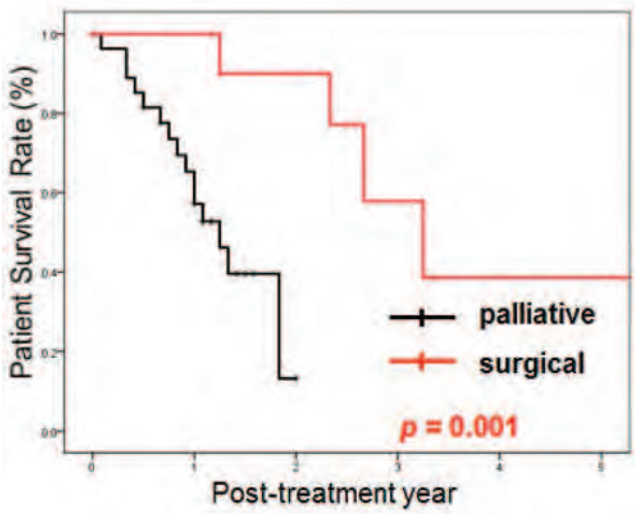
[1] Hill M J, Hughes M, Jie T, Cohen M, Lake J, Payne W D, Humar A. Graft weight/recipient weight ratio: how well does it predictoutcome after partialliver transplants? *Liver Transpl*. 2009, 15:1056-1062.
[2] Desai CS,Sharma S, Gruessner A, Fishbein T, Kaufman S, Khan KM. Effect of small donor weight and donor-recipient weight ratio on the outcome of liver transplantation in children. *Pediatr Transplant*. 2015; 19: 366-370.
[3] Levesque E, Duclos J, Ciacio O, Adam R, Castaing D, Vibert E. Influence of larger graft weight to recipient weight on the post-liver transplantation course. *Clin Transplant*. 2013; 27: 239-247.

425.12

The role of curative intent surgical resection for the recurrent hepatocellular carcinoma after liver transplantation

Seung Hwan Song[1], Jee Youn Lee[1], Su-Kyung Kwon[1], Jae Geun Lee[1], Juhan Lee[1], Dai Hoon Han[1], Kyu Ha Huh[1], Gi Hong Choi[1], Myoung Soo Kim[1], Yu Seun Kim[1], Jin Sub Choi[1], Soon Il Kim[1], Dong Jin Joo[1].
[1]Department of Surgery and The Research Institute for Transplantation, 1Department of Surgery and 2 The Research Institute for Transplantation, Seoul, Korea.

Introduction: Liver transplantation (LT) is one of the best treatment for hepatocellular carcinoma (HCC). However, there could be HCC recurrence in around 10-20% of the transplant patients. The Recurrent HCC after LT remains one of the major causes to graft failure and patient death. Because HCC recurrence is known for systemic disease, systemic therapy may be considered. However the optimal treatment of recurrent HCC is not established. The aim of this study is to evaluate the difference of graft survival rate between palliative therapy and curative intent surgical therapy after LT.
Methods: A total 292 recipients with HCC who underwent LT between January 2007 and April 2015 in Severance hospital were retrospectively reviewed. Among 292 patients, 41 patients developed hepatic or extra-hepatic recurrent HCC. We compared the outcomes of the recurred patients according to the therapeutic approaches.
The Results: The mean age of the HCC recurrence group was younger than non-recurrence group (51.4 ± 6.3 vs 54.8 ± 6.9, p<0.003). There was no significant difference of the etiology of HCC between the groups. The patients above Milan criteria showed a higher tumor recurrence rate than those within Milan criteria(Odd ratio 4.717, p<0.001). The curative intent surgical therapy was performed in 13 patients. Among them, resection only in 2, adjuvant chemotherapy after resection in 4, adjuvant radiation therapy (RT) in 4, adjuvant transarterial chemoembolization (TACE) and chemotherapy in 1, adjuvant TACE and RT in 1, and adjuvant TACE and RFA in 1 case. The palliative therapy was consisted of TACE, chemotherapy, or RT. Among the patients received curative intent surgical therapy, 3 patients had intrahepatic recurrence and 10 patients had extrahepatic recurrence. The 5 year graft survival was higher in curative intent surgical therapy group than in palliative therapy group (51.1% vs 306%, P=0. 026)
Conclusion: The curative intent surgical therapy showed the superior graft survival than palliative therapy. The curative intent surgical therapy is not applicable in every recurrent case. However the patient received curative intent therapy if possible, it is increased with the graft survival significantly.



	HCC recur (n=41)	No HCC recur (n=251)	p-value
Age(mean ± SD)	51.4 ± 6.3	54.8 ± 6.9	0.003
Sex (M) %	36(80.9%)	203(80.9%)	0.286
Etiology (%)			0.325
HBV	35(85.4%)	209(83.2%)	
HCV	4(9.8%)	20(8.0%)	
HBV + HCV	1(2.4%)	1(0.4%)	
Alcoholic	0(0%)	12(4.8%)	
Other	1(2.4%)	9(3.8%)	
AFP	284.7 ± 594.6	54.5 ± 207.2	0.001
Criteria			0.001
Within Milan	21(51.2%)	209(83.3%)	
Above Milan	20(48.8%)	42(16.7%)	
Pre-Treatment	41 (100%)	169(67.3%)	0.001
Pathology			
Total necrosis	1(2.4%)	42(16.7%)	0.001
Partial / No response	34(82.9%)	103(41.2%)	
Microvascular invasion	6(14.6%)	13(5.2%)	0.035

425.13

Intraoperative blood loss is an independent promoter of tumor recurrence in liver transplant patients with advanced hepatocellular carcinoma

Arno Kornberg[1], Ulrike Witt[1], Jennifer Kornberg[2], Hemut Friess[1], Katharina Thrum[3].
[1]Surgery, Technical University, Munich, Germany; [2]Anesthesiology, LMU, Munich, Germany; [3]Pathology, Helios Klinikum , Berlin, Germany.

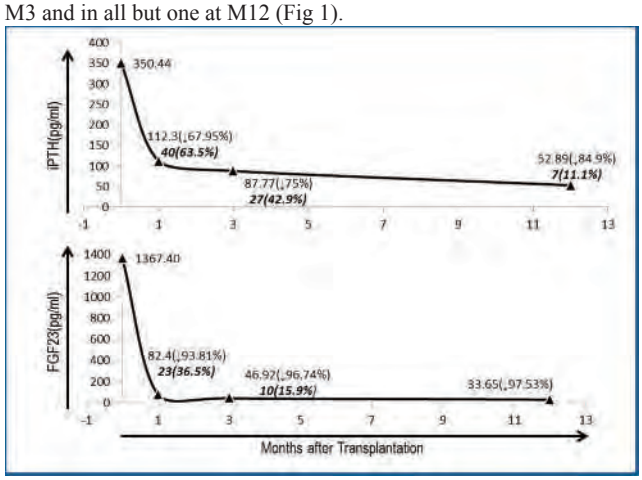
Introduction: Mechanisms of ischemia-reperfusion (I/R) injury were shown to promote the risk of hepatocellular carcinoma (HCC) recurrence after liver transplantation (LT). Along with ischemia times, intraoperative blood loss (IOBL) is a major trigger of I/R damage to the liver graft. The aim of this trial was to analyze the impact of IOBL on risk of tumor recurrence in liver transplant patients with HCC.
Material and Methods: A total of 108 liver transplant patients with HCC were included. Prior to LT, 18F-fluoro-D-glucose (18F-FDG) positron emission tomography (PET) was performed in all of them to identify PET-positive (18F-FDG-avid) and PET-negative (18F-FDG-non-avid) HCC. According to final pretransplant radiographic staging, tumors were classified as Milan In (meeting the Milan criteria) and Milan Out (exceeding the Milan criteria). The impact of IOBL on tumor-specific outcome was analyzed by uni- and multivariate analysis, along with other established prognostic variables.
Results: Mean IOBL was 1500 ml (median: 1493 ml). Twenty-six patients (24.1%) developed HCC recurrence. IOBL was significantly higher in patients with HCC relapse (2196 ml) than in patients without tumor recurrence (1270 ml; $P < 0.001$). Increased IOBL was associated with a significantly higher post-LT C-reactive protein level (4.7 versus 3.5 mg/dl; $P < 0.05$). In multivariate analysis, IOBL $\leq$ 1500 proved to be an independent promoter of recurrence-free long-term survival (Hazard ratio [HR] 3.3; $P = 0.013$), along with negative PET-status (HR 6.15; $P < 0.001$), alpha-fetoprotein level $\leq$ 400 ng/ml (HR 4.13; $P = 0.001$) and well/moderate tumor grading (HR 2.91; $P = 0.014$). Five-year recurrence-free survival was 91.9% in patients with low, but only 37.1% in those with increased IOBL (log rank < 0.001). Blood loss had no independent prognostic impact in patients with HCC meeting the Milan criteria. In contrast, IOBL $\leq$ 1500 ml was identified as an independent promoter of recurrence-free survival in Milan In patients (HR 9.08; $P = 0.003$), along with tumor grading (HR 36.12; $P < 0.001$), PET-status (HR 15.58; $P = 0.002$) and red blood transfusion (HR 4.83; $P = 0.022$). Five-year recurrence-free survival rates were comparable between Milan In recipients and Milan Out patients with low IOBL (86.2% versus 79.2%; log rank = 0.433) and, thus, significantly better than in Milan Out patients with IOBL exceeding 1500 ml (26.9%, log rank = 0.001).
Discussion: IOBL is an independent promoter of HCC recurrence following LT, possibly by inducing pro-inflammatory and pro-cancerogenic proccesses. Particularly in patients with advanced HCC, IOBL should be significantly restricted in order to improve oncological outcome post-LT. Our results suggest that monitoring and considering IOBL might be useful to improve posttransplant follow-up surveillance.

426.1

Biointact FGF-23 normalizes faster and correlates better with inorganic phosphorus as compared to intact PTH in living donor renal allograft recipients

Narayan Prasad[1], Akhilesh Jaiswal[1], Vikas Agarwal [2], Shashi Kumar[1], R. K. Sharma[1], Shaurabh Chaturbedi[2], Dharmendra S.Bhadauria[1].
[1]Nephrology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India; [2]Clinical Immunology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India.

Introduction: Fibroblast growth factor-23 (FGF23) and Parathyroid hormone (PTH), are phosphaturic hormones and both are usually elevated in end stage renal failure (ESRF) patients to maintain homeostasis of serum phosphorus and calcium level. Hypophosphatemia is not uncommon in post transplant period. There is paucity of data on serial changes in these parameters particularly FGF-23 level in living donor post renal transplant period.
Aims: We aimed this study to analyze the serial changes in iFGF-23, iPTH, serum calcium and inorganic phosphate level in renal transplant patients.
Materials and Methods: Biointact PTH(iPTH), calcidiol, biointact FGF-23, albumin corrected calcium, and inorganic phosphorus (iP) were analyzed in 63 ESRD (Male 58, mean age 35.65±11.79 years) patients who underwent living donor renal transplantation with average eGFR 9.58±4.01 ml/min before transplantation (preTx) and month 1 (M1), 3 (M3) and 12 (M12) of transplantation. The native kidney diseases of these patients were chronic glomerulonephritis (n=36), interstitial nephropathy (n=20), diabetic nephropathy (n=6) and polycystic kidney disease (n=1). The upper normal cutoff value of iPTH was considered as >65 pg/ml, and iFGF23 >50 pg/mL, hypo and hyperphosphatemia was defined with iP <2.5 and >5.5mg/dl respectively, hypo and hypercalcemia with corrected for serum albumin <8.5 and >10.8 mg/dL, respectively.
Results: Pre-tx eGFR was inversely correlated with logFGF23 (r=-0.668, p=0.001) and log PTH (r=-0.516, p=0.001). Compared to pre-tx level FGF23 decreased at M1 by 94%, 16.6 fold and PTH by 68%, 3.1 fold; at M3 FGF23 decreased by 97%, 29.1 fold and PTH by 75%, 4 fold; and at M12 FGF deceased by 98%, 40.6 fold, and PTH by 85%, 6.6 fold respectively. FGF23 level normalized in 64% at M1, 84% at M3 and in all but one at M12 (Fig 1).



However, prevalence of hyperparathyroidism was 63%, 43%, 11% at M1, M3 and M12 respectively. At M1, 17(27%) hypophosphatemic patients had higher FGF23 (pre-tx, p<0.01, M1, p<0.001) and iPTH (pre-tx, p<0.048, M1, p=0.002) compared to normophosphatemic. At pre-tx, 26(41%) hypocalcemic patients had higher values of iPTH(p=0.016), and FGF23(p=0.008) as compared to values in normocalcemics. Serum calcium normalized in all except one patient at M1 and in all at M12. On linear regression using a backward stepwise

model, serum iP was significantly correlated with FGF23 levels and eGFR in pre-tx and M1, not with iPTH (Fig 2).

variable	Pre-Tx	Month 1	Month 3	Month 12	Friedman Test (p)
iPTH (pg/ml)	350.44±235.16	112.3±84.38	87.77±65.92	52.89±22.17	0.001
Hyperparathyroidism (>65 pg/ml)	63(100%)	40 (63.5%)	27 (42.9%)	7(11.1%)	
FGF23 (pg/ml)	1367.4±807.24	82.40±79.78	46.92±33.63	33.65±15.32	0.001
FGF23 (>50 pg/ml)	58(92%)	23(36.5%)	10(15.9%)	0	
25(OH) Vit D (ng/ml)	27.29±12.35	23.54±9.56	27.22±7.60+	28.17±6.29	0.002
eGFR (ml/min)	9.58±4.01	81.02±48.64	84.86±64.90	95.71±49.3	0.001
Inorganic phosphate (mg/dl)	6.42±2.12	2.92±0.85	3.19±0.71	3.18±0.51	0.001
Hypophosphatemia (<2.5mg/dl)		17(27%)	5(8%)	0	
creatinine (mg/dl)	7.49±1.93	1.19±0.25	1.20±0.25	1.21±0.26	0.001
BUN (mg/dl)	53.54±22.51	23.26±9.14	19.64±7.91	18.53±7.05	0.001
uric acid ()	6.76±1.99	4.33±1.32	4.21±1.20	4.24±1.37	0.001
calcium (mg/dl)	8.62±0.82	9.24±0.50	9.45±0.50	9.42±0.49	0.001
Hypocalcemia (<8.5mg/dl)	26(41.3%)				
alkaline phosphatase (U/l)	182.02±97.75	99.86±50.27	83.22±48.34	82.87±33.42	0.001

Conclusions: In conclusion, our data indicate that FGF23 normalizes faster than iPTH after successful renal transplantation.iPTH remains high in 11% of patients at end of one year while FGF23 normalizes in all. FGF23 is better correlated with hypophophatemia as compared to iPTH at one month post-transplantation in living donor renal allograft recipients.

We acknowledge Sanofi Synthelabo (India) Ltd. to support FGF23 kit for this study

426.2

Are there risks to fathering pregnancies after solid organ transplantation?

Serban Constantinescu[1,2], Lisa Coscia[2], Dawn P.Armenti[2], Michael J.Moritz[2,3].
[1]Medicine, Temple University, Philadelphia, PA, United States; [2]National Transplantation Pregnancy Registry (NTPR), Gift of Life Institute, Philadelphia, PA, United States; [3]Surgery, Lehigh Valley Health Network, Allentown, PA, United States.

The purpose of this study was to analyze 1343 pregnancy outcomes fathered by 870 male solid organ transplant recipients with emphasis on exposure to mycophenolic acid products (MPA) and sirolimus. Data were collected by the National Transplantation Pregnancy Registry (NTPR) via telephone interviews and review of medical records. Overall pregnancy outcomes are similar to the general population (Table 1).

Table 1. NTPR Fathered Pregnancies

Organ(s)	Recipients	Pregnancies/ Outcomes[1]	Fetal loss[2] (%)	Live births (%)	Mean Gestational Age (wks)	Mean Birthweight (g)	Birth defects (%)
Kidney	619	968/987	6.5	93	39±2.4	3320±659	2.7
Kidney-Pancreas	33	43/45	9	91	38.7±2.4	3321±589	2.4
Liver/Multi-organ	91	147/154	11	88	39±1.9	3326±611	4.4
Thoracic organs	127	185/190	8.4	89	38.8±2.4	3380±614	1.8
General Population			16-21		39	3389	4-6

[1]includes multiple births; [2]miscarriages and stillbirths

Females are advised to avoid MPA during pregnancy due to higher fetal loss rate and the increased incidence and pattern of malformations in maternal MPA exposures. Between 11/1996-6/2015, 268 pregnancies (278 outcomes) fathered by male recipients taking MPA (exposed) were compared to 251 pregnancies (263 outcomes) fathered by male recipients not taking MPA (unexposed) during the same time period. In the exposed group, the fetal loss rate was 10%, and the birth defect rate was 2.8% with no observed pattern of malformations. These rates are similar to the general population. There was no significant difference in live births, fetal loss and birth defect rates between the exposed and unexposed groups (Table 2).

Table 2. NTPR Fathered Pregnancies: MPA Exposed vs. Unexposed

	Exposed	%	Unexposed	%	p-value (Fisher's exact test)
Pregnancies	268		251		
Outcomes	278		263		
Live births	250	89.9	244	92.8	0.29
Fetal loss rate	28	10	18	6.8	0.22
Birth defect rate	7	2.8	6	2.5	1

There were 16 (18 outcomes) fathered pregnancies with exposure to sirolimus; all resulted in live births with no reported birth defects.

Conclusions: The outcomes of pregnancies fathered by male transplant recipients are comparable to the general population. The results of this study do not support the avoidance of MPA for male recipients considering parenthood. Data regarding sirolimus remains limited, however, to date there does not appear to be increased risks for pregnancies fathered while taking sirolimus.

The NTPR is supported by grants from Astellas Pharma, US Inc., Bristol-Myers Squibb Co. and Pfizer Inc.

426.3

Impact of induction therapy on incident cardiovascular events in kidney transplant recipients

Shaifali Sandal[1], Sunjae Bae[2], Allan Massie[2], Marcelo Cantarovich[1], Dorry Segev[2].
[1]Medicine, McGill University Health Center, Montreal, Canada; [2]Surgery, Johns Hopkins University, Baltimore, United States.

Cardiovascular events (CVE) are one of leading causes of mortality in kidney transplant (KT) recipients and ATG use is hypothesized as a risk factor. To better understand the relationship between induction and post-KT CVE, we compared CVE incidence among patients who received no induction, alemtuzumab (ALM), anti-thymocyte globulin (ATG) and IL-2 receptor antagonist (IL-2RA).

Methods: Medicare-primary KT recipients from 1999 -2010 were studied using USRDS data. The outcome of interest was time to first post-KT CVE hospitalization, ascertained using validated ICD-9 codes for myocardial infarction, congestive heart failure, stroke and transient ischemic attack. We used Cox regression to model the association between induction therapy and CVE, adjusting for recipient, donor and transplant characteristics.

Results: A total of 86,541 first-time KT recipients were eligible for analysis. Compared with the no induction group, ATG and IL2 were at a similar risk for CVE [table1]. However, KT recipients who received ALM as induction therapy had 18% higher CVE. When stratified by donor type, this association was only found among recipients of kidneys from deceased donors (22% higher CVE).

Conclusion: Deceased donor recipients receiving alemtuzumab, particularly those with high baseline cardiovascular risk profile, should be closely monitored for CVE and/or should receive other induction agents. No other induction agent appears to be associated with post-KT CVE.

Adjusted hazard of CVD-related readmission (Ref: No induction)

	aHR	95% CI	p value
Overall			
IL-2 RA	0.97	0.92-1.02	0.2
ATG	0.96	0.91-1.02	0.2
ALM	**1.18**	**1.06-1.30**	**0.001**
LDKT only			
IL-2 RA	1.03	0.93-1.14	0.6
ATG	1.01	0.89-1.14	0.9
ALM	1.00	0.80-1.26	1.0
DDKT only			
IL-2 RA	0.96	0.90-1.01	0.1
ATG	0.96	0.90-1.01	0.1
ALM	**1.22**	**1.10-1.37**	**<0.001**

426.4

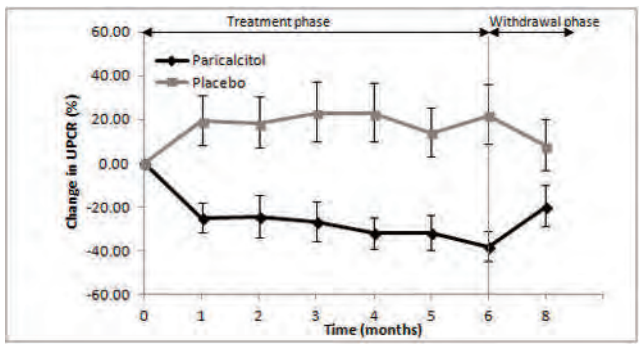
Paricalcitol versus placebo for reduction of proteinuria in kidney transplant recipients: A double-blind, randomized controlled trial

Miha Arnol[1,2], Manca Oblak[1], Gregor Mlinšek[1], Jadranka Buturović-Ponikvar[1,2], Aljoša Kandus[1].
[1]Department of Nephrology, University Medical Centre Ljubljana, Ljubljana, Slovenia; [2]Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia.

Introduction: Kidney transplant recipients with proteinuria have an increased risk of allograft failure[1]. We aimed to assess whether paricalcitol could be used to reduce proteinuria in patients with increased urinary protein excretion despite treatment with renin-angiotensin-aldosteron system (RAAS) inhibitors.

Materials and Methods: In this single-center, placebo-controlled, double-blind trial, we enrolled a national cohort of kidney transplant recipients at least 3 months' post-transplant with urinary protein-to-creatinine ratio (UPCR) ≥20 mg/mmol despite optimization of the RAAS blockade during the run-in phase. Patients were randomly assigned to receive 24 weeks' treatment with 2 µg/day paricalcitol or placebo. The primary endpoint was the percent change in geometric mean UPCR, and secondary endpoints were the percent change in geometric mean urinary albumin-to-creatinine ratio (UACR) and mean 24-hour proteinuria from baseline to last measurement during treatment. Analysis was by intention to treat. This trial is registered with ClinicalTrials.gov, number NCT01436747.

Results: After completion of the run-in phase in 2012, 190 of 572 prevalent kidney transplant recipients (33%) had UPCR ≥20 mg/mmol. Of 168 patients who consented to undergo randomization, 83 were allocated to paricalcitol, and 85 were allocated to placebo; all patients on paricalcitol and placebo received at least one dose of study drug and had UPCR data at baseline and at least one timepoint during treatment. Change in UPCR was -38% (from 74 to 46 mg/mmol; 95% CI -45% to -31%) in the paricalcitol group and 21% (from 52 to 63 mg/mmol; 95% CI 9% to 36%) in the placebo group, with a between-group difference *vs.* placebo of -28% (95% CI -45% to -4%; $P<0.001$). Patients on 2 µg paricalcitol showed a sustained reduction in UPCR, ranging from -25% to -39% ($P<0.001$ *vs.* placebo, Figure).



Change in UACR and 24-hour proteinuria was -47% (95% CI -54% to -38%) and -35% (95% CI -42% to -28%) in the paricalcitol group, and 11% (95% CI -5% to 29%) and 19% (95% CI 8% to 30%) in the placebo group, respectively. Significant reduction in IL-6 and TGF-beta plasma concentrations were recorded in the paricalcitol group. Incidences of hypercalcemia, adverse events, and serious adverse events were similar between both patient groups.

Discussion: We have shown that 24 weeks' treatment with 2 µg paricalcitol daily reduced proteinuria in kidney transplant recipients who were on stable doses of RAAS inhibitors. The effect occurred within 4 weeks and then progressively increased during treatment, and returned towards baseline after drug withdrawal, indicating that the effect was real. Biomarker analysis suggested that renoprotection may be caused by antiproliferative and antifibrotic effects.

Conclusion: In kidney transplant recipients, addition of 2 µg/day paricalcitol to RAAS inhibition safely lowers proteinuria, and could be used as an effective approach to lower allograft failure risk.

The study has been supported by University Medical Centre Ljubljana (research grant 20110090), Slovenian Research Agency (research grant P3-0323), and AbbVie.

References:

[1] Knoll G. Proteinuria in kidney transplant recipients: prevalence, prognosis and evidence-based management. Am J Kidney Dis 2009; 54: 1131-1144.

426.5

Effect of the RANKL inhibitor denosumab on early loss of bone mineral density and skeletal microstructure in first-year kidney transplant recipients

Rudolf Wuthrich[1], Diana Frey[2], Thomas Mueller[1], Nicole Graf[3], Marco Bonani[1].
[1]Division of Nephrology, University Hospital, Zurich, Switzerland; [2]Division of Rheumatology, University Hospital, Zurich, Switzerland; [3]Graf, Biostatistics, Winterthur, Switzerland.

We performed a randomized controlled clinical trial in kidney transplant recipients (NCT01377467) to examine whether RANKL inhibition with denosumab improved areal and volumetric bone mineral density (aBMD, vBMD) and skeletal microstructure when given during the first year after renal transplantation.

We randomly assigned 90 kidney transplant recipients (age 49 ± 13 yrs, 63% male, all on CNI, mycophenolate, steroids, and calcium/vitamin D supplementation) 16 ± 6 days after surgery in a 1:1 ratio to receive 60 mg denosumab s.c. at baseline and after 6 months, or no treatment. At baseline and after 6 and 12 months, aBMD was measured at the lumbar spine and the hip by DXA (n=90). Cortical and trabecular vBMD (Ct.vBMD, Tb.vBMD), and cortical thickness and porosity (Ct.Th, Ct.Po) were assessed at the distal tibia and radius by HR-pQCT (XtremeCT®) at baseline and after 12 months (n=24).

The denosumab group (n=46) had significantly increased bone mineral density at the lumbar spine (+5.1%; 95% CI 3.1-7.0%; P<0.001) and at the hip (+1.9%; 95% CI 0.1-3.7%; P=0.035) over that in the control group (n=44) at 12 months. Biomarkers of bone resorption (beta-CTX) and bone formation (P1NP) markedly decreased with denosumab (P<0.001). Treatment with denosumab led to a significant increase of the vBMD and the Ct.Th at the distal tibia and radius, but did not change Tb.vBMD and Ct.Po. The Ct.vBMD, bone stiffness and failure load showed significant improvements at the tibia. Denosumab was well tolerated, except for more frequent episodes of cystitis and asymptomatic hypocalcemia, whereas graft function, rate of rejections and incidence of opportunistic infections were similar in the denosumab and control groups. Three-year safety follow-up showed excellent graft function in both groups.

In summary, denosumab improved bone density and bone quality in first-year kidney transplant recipients at risk to develop osteoporosis.

426.6

Arteriovenous fistula (AVF) maturation among prevalent hemodialysis patients with failed kidney transplants: Results from the united states renal data system (USRDS)

Kenneth Woodside[1], Sarah Bell[1], Brett W.Plattner[1], Douglas E.Schaubel[1], Sudipta Dasmunshi[1], Purna Mukhopadhyay[2], Jeffrey Pearson[2], Ronald L.Pisoni[2], Rajiv Saran[1].
[1]Kidney Epidemiology and Cost Center, University of Michigan, Ann Arbor, MI, United States; [2]Arbor Research Collaborative for Health, Ann Arbor, MI, United States.

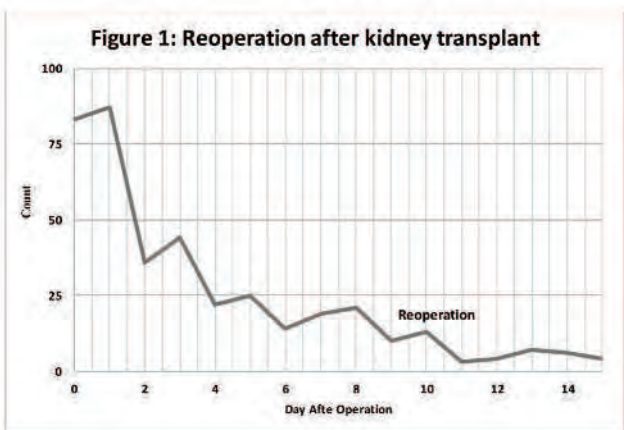
Kidney transplant patients with failed allografts who have returned to dialysis have more difficulty establishing access and are more likely to suffer from central venous catheter (CVC)-related complications. The recent addition of Consolidated Renal Operations in a Web-Enabled Network (CROWNWeb) data to the USRDS allows more granular analysis of dialysis care, including vascular access use. We sought to describe AVF maturation from placement until time to first use for prevalent hemodialysis patients with failed kidney transplants in the US.
Methods: We examined prevalent hemodialysis patients with previous failed kidney transplants who had new AVF placements during 2013 using Medicare claims. Failure of maturation was identified by non-use following placement using CROWNWeb, where patient access use is reported monthly by the facility. Patients were followed until end 2014. We assessed the relationship between patient characteristics and time-to-first-use of AVF with a Cox model (HR < 1.0 indicating longer time to first use).
Results: In 2013, a total of 1353 AVF were placed among 1186 failed kidney transplant patients followed through 2014. No subsequent evidence of use occurred in 34.4% of the placements. Of successfully used AVF, median time to first use was 108 days (IQR 70 – 209). AVF in females were associated with longer time to AVF use (HR 0.68, 95%CI 0.59-0.79), as were AVF in black patients (HR 0.84, 95%CI 0.71-0.98). Comorbidities including cardiovascular disease (HR 0.83, 95%CI 0.70-0.98), diabetes (HR 0.85, 95%CI 0.72-0.99), tobacco use (HR 0.69, 95%CI 0.51-0.94), and needing assistance (HR 0.57, 95%CI 0.40-0.82) had similar association with increased time to AVF use. Patient age, dialysis vintage, hypertensive history, and ESRD Network Region associations with AVF maturation were mostly non-significant, as was previous dialysis access history.
Conclusions: We have characterized AVF maturation in the subset of failed kidney transplant patients in a national sample and identified important associations with multiple patient-level factors. Patients who were female, black, frail, smokers, or had cardiovascular or diabetic disease required additional time for maturation, suggesting that such patients may require earlier referral as part of their access placement plan. Research is urgently required into the importance of patient, region, and practice factors that could improve AVF placement and maturation, decrease CVC use, and improve outcomes in patients with failed kidney transplants.

426.7

A nationwide analysis of reoperation after kidney transplant

Zhobin Moghadamyeghaneh, Mahmoud Alameddine, George Burke, Gaetano Ciancio, Linda Chen.
Miami Transplant Institute, University of Miami/ Jackson Memorial Hospital, Miami, United States.

Objectives: We aim to report the rate and short-term outcomes of patients undergoing reoperation following kidney transplant in the United States.
Methods: The NIS database was used to examine the clinical data of patients undergoing kidney transplant and reoperation during same the hospitalization from 2002-2012. Multivariate regression analysis was performed to compare outcomes of patients with and without reoperation.
Results: We sampled a total of 172,586 patients who underwent kidney transplant. Of these, 3802 (2.2%) had reoperation during the same hospitalization. Reoperation was associated with a significant increase in mortality (3.1% vs. 0.4%, AOR: 5.40, P<0.01), mean total hospital charges ($249425 vs. $145403, P<0.01), and mean hospitalization length of patients (18 days vs. 7 days, P<0.01). The most common day of reoperation was POD 1 (Figure 1). Hemorrhagic complication (64.1%) was the most common reason for reoperation followed by urinary tract complications (8.9%). Renal vein complications needing reoperation was more than seven times higher than renal artery complications (2.9% vs. 0.4%, P<0.01). Preoperative coagulopathy (AOR: 3.65, P<0.01) was the strongest predictor of need for reoperation and renal artery (AOR: 1.50, P=0.04) and renal vein complications (AOR: 3.62, P<0.01). Also, diabetes (AOR: 1.26, P<0.01) and hypertension (AOR: 1.88, P<0.01) had associations with need for reoperation.
Conclusion: Reoperation after kidney transplant occurs in 2.2% of cases. However, it is associated with a significantly increased mortality, hospitalization length, and total hospital charges. The first day after transplant and hemorrhagic complication are the most common day and reason of reoperation respectively. Preoperative coagulopathy was the strongest factor in prediction of need for reoperation, renal artery complications, and renal vein complications. Controlling coagulation disorders preoperatively may decrease need for reoperation, hospitalization length, and total hospital charges.



426.8

Efficacy of using FloSeal to prevent post renal transplantation lymphocele formation

Yiu Chung Lam, Chun Ki Chan, Chi Man Ng, Yi Chiu, Kwok Leung Ho, Tin Yu Chu, Fu Keung Cheung.
Surgery, Princess Margaret Hospital, Hong Kong, Hong Kong.

Introduction: Preliminary data suggests the use of FloSeal® hemostatic matrix can reduce lymphocele formation after pelvic lymphadenopathy for prostate cancer. We investigate the efficacy of using FloSeal® to reduce the incidence of post renal transplantation lymphocele.
Materials and Methods: We retrospectively reviewed the data of renal transplantation performed from June 2002 to January 2016 in our center. From May 2013 onwards, FloSeal® was applied over graft kidney hilum and segment of iliac artery dissection. Patients' demographics and peri-operative parameters were analysed. Symptomatic lymphocele was defined as those causing either mass effect, impaired renal function or infection requiring intervention. Chi-square and t-test were used for statistical analysis of categorical and continuous variables respectively.
Results: Total 238 patients (203 cadaveric and 35 living-related renal transplantation) were included in this 15 years review. The mean age was 36.6 years old (range 4-69 years old) and FloSeal® was applied in 62 (26.1%) patients. There was no significant difference in patients' demographics such as age, gender and duration of renal replacement therapy between the FloSeal® and non-FloSeal® groups. The incidence of lymphocele formation was significantly reduced in FloSeal® group (9.7% vs 22.2%, p=0.031). Also, the incidence of symptomatic lymphocele formation was lower in FloSeal® group as well (4.8% vs 14.2%, p=0.049). Total drain output was significant less in FloSeal® group (464ml vs 890ml, p=0.004) and drains could be removed earlier (4.3 days vs 6.2 days, p=0.000). No adverse effect associated with the usage of FloSeal® occurred.
Discussion: FloSeal® is a hemostatic matrix using in both open and laparoscopic procedures. It consists of bovine-derived gelatin matrix mixed with human-derived thrombin before usage. The effectiveness of FloSeal® in lymphocele prevention may come from several hypotheses. Firstly, the swelling of gelatin granules by up to 20% after local application may offer a local tamponade effect resulting in occlusion of leaky lymphatic channel. Moreover, as lymph contains a limited amount of fibrinogen, it may form a fibrin clot with the thrombin solution leading to further blockage of the disrupted lymphatic chain. Lastly, the cross-linked gelatin granules facilitates conformation to irregular wound geometries. Thereby it maintains contiguous contact of tissue with the active site of lymphorrhea so that leakage site of lymphatic channel is minimized and tissue healing is promoted. The promising result of FloSeal® in preventing post renal transplantation lymphocele formation is strongly back up these hypotheses.
Conclusion: The results of this study suggest FloSeal® can reduce lymphocele formation after renal transplantation and associated intervention. It also helps decrease drain output and earlier drain removal. This findings warrant a randomized controlled study to further confirm the use of FloSeal® in such aspect.

426.9

External ureteral stenting in kidney transplantation; does the type of stent matter?

Liselotte Ooms[1], Laura G.Spaans[1], Michiel G.H.Betjes[2], Jan N.M.IJzermans[1], Turkan Terkivatan[1], Robert Minnee[1].
[1]Transplant Surgery, Erasmus University Medical Center, Rotterdam, Netherlands; [2]Nephrology, Erasmus University Medical Center, Rotterdam, Netherlands.

Introduction: In our center, we prefer the use of external stents over double J stents to prevent urological complications after kidney transplantation. we think external stents have several advantages like the possibility of monitoring the grafts urine production and the simplicity of stent removal without a cystoscopy. The aim of this study was to evaluate the effect of two types of external ureteral stents on the number of urological complications after kidney transplantation.
Materials and Methods: Data were retrospectively collected from 366 consecutive transplantations performed between January 2013 and January 2015 in our hospital, in which an external ureteral stent was placed during surgery. A urological complication was defined as urinary leakage or ureteral stricture requiring percutaneous nephrostomy (PCN) placement.. PCN placement was indicated when the post-operative ultrasound revealed hydronephrosis in combination with a rising serum creatinine level or a peri-renal urinoma indicating urine leakage, confirmed by ultrasonography or MAG-3 scanning.
Results and Discussion: A total of 197 patients received a straight stent with two larger side holes (type A; 8 Fr 'Covidien' tube) and 169 patients received a single J stent with 7 smaller side holes (type B; 7 Fr 'Teleflex' single J stent). In total 50/366 patients (14%) required PCN placement, of whom 37 patients (10%) underwent a PCN placement due to hydronephrosis and 13 patients (4%) due to urinary leakage. We found a significant higher number PCN placements in type A stenting: 34 (17%) PCN interventions versus 16 (9%) in type B stenting (p= 0.030). Reason for PCN placement, stent dysfunction and early removal (< 8 days) was equally distributed in both groups (p= 0.397) while incidence of rejection and urinary tract infection were higher in type B stenting. The 3 months, 6 months and 1 year graft survival rate in the type A stenting group was respectively 97%, 96% and 95% and in the type B stenting group 98%, 97% and 95% (p= 0.781). The 3 months, 6 months and 1 year patient survival rate in the type A stenting group was respectively 99%, 98% and 98% and in the type B stenting group 99%, 98% and 95% (p= 0.093).
Conclusion: 'Stent type B' is associated with less urological complications compared to 'stent type A'. The type of stent does not affect patient and graft survival.

426.10

Standardized intraoperative application of HaemoCer(trademark) may reduce the incidence of lymphocele after kidney transplantation

Christopher Burghuber[1], Daniela Kandioler[1], Stephanie Strobl[1], Martina Mittlböck[3], Georg Böhmig[2], Thomas Soliman[1], Gabriela Berlakovich[1].
[1]Surgery, Division of Transplantation, Medical University Vienna, Vienna, Austria; [2]Nephrology, Medical University Vienna, Vienna, Austria; [3]Medical Statistics, Medical University Vienna, Vienna, Austria.

Introduction: Lymphoceles can occur after kidney transplantation with a reported frequency of around 20% in the literature. Aim of this prospective, non-randomized academic study was to assess whether the standardized application of HaemoCer™ absorbable polysaccharide hemostatic powder (BioCer Entwicklungs-GmbH CE1275), available at our center for facilitating hemostasis, has an effect on the lymphocele rate.
Materials and Methods: The center specific lymphocele rate after kidney transplantation was retrospectively assessed at Vienna University Hospital, Division of Transplantation, over a defined period of one year (April 2011- April 2012). Lymphoceles were diagnosed via routine ultrasound assessment. During this time frame the lymphocele rate in 183 consecutively transplanted patients was 20.3%. We proposed a reduction of 50% in lymphocele rate to be a significant result. In order to detect this difference using a 1-group $\chi 2$ test with a two-sided significance level of 5% and a power of 80% we calculated the number of patients to treat to be at least 108. For one year HaemoCer™ was prospectively used intraoperatively in all patients undergoing kidney transplantation. The study was approved by the ethics committee of the Medical University of Vienna; EK 1125/2013).
Results and Discussion: In the study period 156 patients were transplanted and included into the prospective evaluation. Two patients did not receive HaemoCer™. Of the remaining 154 patients, 19 developed a lymphocele (12.3 %, 95 % CI: 8.0-18.5 %). Compared to the earlier assessed center specific lymphocele rate after kidney transplantation of 20.3%, this represents a significant reduction (p= 0.0174).
Conclusion: Lymphoceles after kidney transplantation seemed to be significantly reduced in a period of time when Haemocer™ was applied intraoperatively compared to a period without standardized use. The magnitude of the effect warrants randomized evaluation.

426.11

Pregnancy after renal transplantation: A single-center experience

Toyofumi Abe[1], Yukito Kokado[2], Nariaki Nakazawa[1], Kazuaki Yamanaka[1], Yoichi Kakuta[1], Ryoichi Imamura[1], Naotsugu Ichimaru[3], Shiro Takahara[3], Norio Nonomura[1].
[1]Department of Urology, Osaka University Hospital, Suita, Japan; [2]Takahashi Clinic, Toyonaka, Japan; [3]Department of Advanced Technology for Transplantation, Osaka University Hospital, Suita, Japan.

Background: Pregnant women with renal allografts often face several complications such as chronic deterioration of graft function and increased risk of preterm delivery. We examined women with renal transplants who became pregnant and delivered at our hospital.
Methods: Of 36 women who underwent renal transplantation and became pregnant and delivered at Osaka University Hospital between 1977 and 2014, 28 women with complete data served as subjects.
Results: Mean recipient age at the time of transplantation was 27.4 (19-38) years. Mean duration from transplantation to delivery was 5.9 years. The 28 recipients became pregnant a total of 40 times after renal transplantation, and neonates were delivered in 32 of the 40 pregnancies. Four recipients delivered twice and 2 recipients delivered twins. As a result, 34 neonates were delivered. Mean gestational period was 36.3 (27-41) weeks, and mean birth weight was 2423 (724-3544) g. Intrauterine growth retardation was observed in 3. One child was born with a double-outlet right ventricle and another child had congenital unilateral hydronephrosis. In 7 of the 32 deliveries (22%), renal function worsened after delivery. Rates of graft survival for the 28 recipients at 1, 5 and 10 years after delivery were 100%, 86% and 74%, respectively. Prognosis for renal transplantation was significantly poorer for recipients with hypertension prior to pregnancy than for recipients without hypertension (log-rank test, p=0.0008).
Conclusions: Rates of graft survival after delivery were mostly favorable. In patients with drug-treated hypertension prior to pregnancy, subsequent renal function may be adversely affected.

426.12

A multidisciplinary approach to reviewing patient care in abdominal transplantation

Susan Lerner, Vinay Nair, Morgan Resta-Flarer, Ron Shapiro, Sander Florman, Leona Kim-Schluger.
Recanati Miller Transplantation Institute, Mount Sinai, New York, NY, United States.

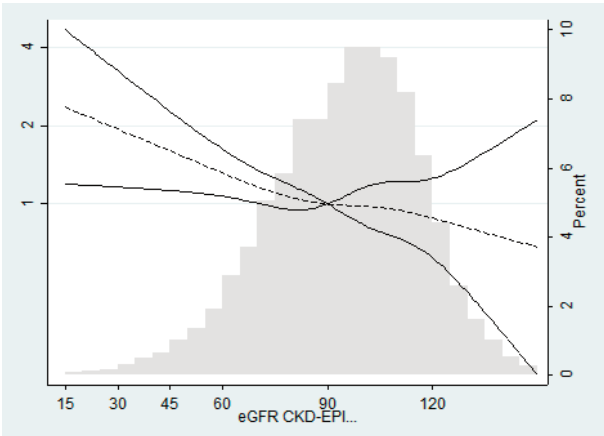
Purpose: Unbiased review of patient care in solid organ transplantation is essential for improving patient safety. In October 2013 we created a process by which select events were reviewed by a multidisciplinary team which then determined if standard of care (SOC) was met, and if a corrective action plan (CAP) needed to be implemented. We present results of 694 reviewed cases from our abdominal transplant program.
Methods: All cases presented for review between 10/2013 and 12/2015 were included. Cases came from adult and pediatric kidney, pancreas, liver, small bowel transplantation and hepatobiliary surgery. Cases were selected based on the following criteria: unexpected death, 1 year graft failure, operative complication, unplanned reoperation, medical complication, adverse event, near miss, reported medical event, 30 day readmission, donor disease transmission, and living organ donor adverse event. Members of the review committee include program leadership, director of quality, social worker, and physicians from the transplant department. After review cases are graded as SOC met, SOC not met, or SOC met with room for improvement.
Results: We reviewed 694 cases since 10/2013. Adult liver service (liver transplant, pre and post, and hepatobiliary surgery) accounted for 426 cases and kidney/pancreas, small bowel and pediatric liver accounted for 198, 58 and 12 cases respectively. Death within 1 year of transplant, other death (on the waiting list, while on service, or more than 1 year post-transplant), 30 day readmissions, unplanned reoperations, graft failure within one year, operative/procedural complications, medical complications, adverse events, near misses, reported medical events, donor disease transmissions, and living donor adverse events represent 5.2, 39.9, 28.1, 13.83, 1.59, 6.63, 3.03, 0.14, 0.29, 0.29, 0.72, and 0.29 percent of the cases, respectively. SOC was met in 95.1% of adult liver cases; 91.9% of kidney cases; 94.8% of small bowel cases; 83.3% of pediatric liver cases. Cases where SOC was not met led to the creation and implementation of 43 CAPs.
Conclusions: We have created a process for multidisciplinary review of problematic clinical cases in abdominal transplantation. This has led to the discovery of patient safety problems and the creation of CAPs to prevent recurrence. We believe our approach can help guide other centers in quality and process improvement with benefits in both patient safety and long term clinical outcomes.

426.13

The impact of renal function on post-operative respiratory complications in bariatric surgery

Akihiro Shimomura[1], Yoshitsugu Obi[2,3], Reza Fazl Alizadeh[1], Shiri Li[1], Clarence EFoster[1], Hirohito Ichii[1].
[1]Department of Surgery, Division of Transplantation, University of California, Irvine, Orange, CA, United States; [2]Harold Simmons Center for Kidney Disease Research and Epidemiology, University of California, Irvine, Orange, CA, United States; [3]Division of Nephrology & Hypertension, University of California, Irvine, Orange, CA, United States.

Introduction: As the obesity epidemic in the United States has spread to encompass almost a third of adults older than 20 years, the prevalence of obesity is also increasing among patients with chronic kidney disease. Kidney transplant candidates, most of whom have severely impaired kidney function, often undergo a bariatric surgery in order to lose their weight before transplantation because morbidly obese patients are at greater risk for postoperative complications. However, the prevalence of restrictive spirometric abnormalities is common among dialysis patients, and little is known about the risk of respiratory complications after bariatric surgery among patients with decreased kidney function.
Subjects and Methods: From the American College of Surgeons National Surgical Quality Improvement Program (ACS-NSQIP) database, we identified patients who underwent laparoscopic bariatric surgery between 2005 and 2013. We calculated estimated glomerular filtration rate (eGFR) by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula using preoperative serum creatinine concentrations. We then conducted multivariate logistic regression analyses with restricted cubic spline functions to examine the association of eGFR with the development of post-operative respiratory complications including pneumonia, being on ventilator >48 hours, or unplanned re-intubation after extubation.
Results: Patients were 45±12 years old, 79% female, 15% non-Hispanic Black, and 27% diabetic. Median eGFR and body mass index were 97 (IQR, 81 to 110) mL/min/1.73m^2 and 44.5 (IQR, 40.5 to 49.7) kg/m^2, respectively. Among 112,844 patients who underwent laparoscopic bariatric surgery, 744 (0.7%) experienced respiratory complications during the post-operative period. The incidence of respiratory complications was 0.5%, 0.7%, 1.7%, and 2.5% in patients with eGFR ≥90, 60 to <90, 30 to <60, and 15 to <30 mL/min/1.73m^2, respectively (P for trend = 0.000). Lower eGFR showed higher likelihood of respiratory complication rates after adjustment for demographics, comorbidities, and laboratory variables; 60, 30, and 15 mL/min/1.73m^2 in eGFR showed adjusted odds ratios (95%CIs) of 1.32 (1.07-1.62), 1.95 (1.16-3.25), and 2.36 (1.20-4.66), respectively (reference: 90 mL/min/1.73m^2).



Conclusion: These data demonstrated higher risks of respiratory complications after bariatric surgery among patients with decreased kidney function. The involved mechanisms have yet to be examined, careful perioperative evaluation and management of cardiovascular and respiratory functions may be necessary when obese kidney transplant candidates are undergoing bariatric surgery.

427.1

Imbalance of t helper 17 cells and T regulatory cells is associated with chronic active antibody mediated rejection in renal allograft recipients

Brijesh Yadav[1], Narayan Prasad[1], Vikas Agarwal [2], Vinita Agarwal [3], R. K. Sharma[1].
[1]Nephrology and Renal Transplantation, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India; [2]Clinical Immunology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India; [3]Pathology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India.

Introduction: Chronic antibody mediated rejection (CABMR) poses challenges for the clinicians and researchers as well. The link between Th17 and antibody formation has been explored. IL-17 deficient mice exhibit impaired T-dependent antibody production.Th17 cells can function as B-cell helpers and IL-17 alone or in combination with B cell-activating factor control the survival and proliferation of human B cells and their differentiation into immunoglobulin-secreting cells.Tregs and Th17 cells share a differentiation pathway involving TGFβ with IL-6 serving as a switch favoring Th17 differentiation.
Aim: To study the frequency of Th1, Th2, Th17 and Tregs; soluble and biointact cytokines in peripheral blood and PBMCs culture supernatant, their mRNA transcript (T-bet, GATA-3, RORC and FoxP3) expression in allograft tissue of patient with CABMR and stable graft function (SGF).
Methods: Total 42 recipients, 10 SGF patients (age 40.90±12.67) with SGF for 6 months without proteinuria and <10% cortical surface area with IF/TA on biopsy; and 32 (age 37.93±12.78 year) in CABMR group as per defined Banff 2013 criteria. Frequency of Th1, Th2, Th17 and Treg was analyzed by flow cytometry and IFN-γ, IL-4, IL-17A, IL-6 and IL-10 level was analyzed from Serum and PBMC by ELISA and mRNA transcript expression by Taqman Real time PCR from graft specimen. Fold change was calculated by 2^^-ct method relative to SGF.
Result: Mean post-transplant interval at biopsy in CABMR Versus SGF was (70.18±34.4 and 49.70±22.71) months. Serum creatinine (2.40±0.8 and 1.23±0.18; P=0.001) mg/dl; daily urine protein (2.94±1.53 and 0.13±0.10; P<0.001) g/day respectively.
Circulating frequency in CABMR Versus SGF of Th1 cell was 23.65±5.93 and 22.2±8.79; P=0.55; Th2 2.28±1.0 and 2.51±0.80; P=0.52;, CD4+Th17 7.84±1.99 and 4.73±1.22; P<0.001; and CD4+CD25+FoxP3+ Treg was 2.25±1.02 and 3.70±1.55; P<0.001,respectively. Ratio of circulating Th17/Treg was higher in CABMR (4.30±2.30) than SGF patients (1.80±1.65; p<0.001).
Soluble cytokines level (pg/ml) in CABMR Versus SGF of IFN-γ 109.8±23.7 and 82.36±24.97; P=0.003; IL-17 ,66.97±18.27 and 42.09±15.9; P<0.001; and IL-6, 57.11±12.92 and 42.94±12.20; P=0.004 ; and IL-10 , 98.91±17.0 and 149.85±22.51, P<0.001respectively. However, IL-4 level was similar in both groups. Similarly, Bio-intact soluble IFN-γ level in patients of CABMR Versus SGF was 161.1±37.34 and 110.68±13.21; P=0.003; IL-17A 66.97±18.27 and 45.65±14.5; P<0.001; IL-6, 63.91±18.97 and 44.41±11.20; P=0.004), IL-10, 163.53±32.56 and 197.62±20.18; P=0.003 repectively. Intragraft mRNA transcript expression in CABMR Versus SGF of T-bet was 2.79±1.62 and 1.03±0.05 a.u; P<0.002; RORc 2.53±0.98 and 1.02±0.076 a.u; P<0.001; FoxP3 1.69±0.83 and 0.98±0.069 a.u; P=0.011 respectively. However GATA-3 expression was similar in both groups. Intragraft RORc/FoxP3 ratio in CABMR 1.83±1.10 was higher than that of SGF patients 1.04±0.11; P=0.032.
Discussion: Th17 is inflammatory cell secrets IL-17, IL-21,which is associated with B cell maturation and antibody class switching. Recently,study has shown role of IL-17 in acute antibody mediated rejection[1]. Increased Th17/ Treg cell in Intragraft tissue suggest their role in CABMR. Anti Th17 cell therapy may be future of CABMR.

Conclusion: Greater mRNA tissue transcript of Th17, circulating Th17 frequency, and IL-17in serum and culture supernatant; and lower Tregs in CABMR as compared to SGF suggests that Th17 is associated with CABMR.

Brijesh Yadav is a DST Inspire fellow getting fellowship from Department of Science and Technology, Govt. of India

References:

[1] A. Loverre, T. Tataranni, G. Castellano, C. Divella, M. Battaglia, P. Ditonno, M. Corcelli, M. Mangino, L. Gesualdo, F.P. Schena, and G. Grandaliano, IL-17 expression by tubular epithelial cells in renal transplant recipients with acute antibody-mediated rejection. Am J Transplant, 2011. 11 1248-59.

427.2

DEPTOR modulates alloimmunity by increasing regulatory T cell function and stabilizing Foxp3 expression

Johannes Wedel[1,2], Sarah Bruneau[1,2], Kaifeng Liu[1,2], Mathieu Laplante[3], David M.Briscoe[1,2].
[1]Division of Nephrology, Boston Children's Hospital, Boston, MA, United States; [2]Transplant Research Program, Harvard Medical School, Boston, MA, United States; [3]Centre de Recherche de l'Institut Universitaire de Cardiologie et Pneumologie de Québec (CRIUCPQ), Université Laval, Québec, QC, Canada.

DEPTOR is a recently described first-in-kind cell intrinsic mTOR-binding partner that modulates Akt signaling. While its expression and function in T cells is unknown, negative regulation of PI-3K/Akt signaling is described to be critical for $CD4^+$ Treg homeostasis and stability. By qPCR and Western blot analysis, we find high levels of DEPTOR expression in pooled populations of $CD4^+$ cells. We also observed reduced levels of expression following mitogen-dependent activation, suggesting that DEPTOR degradation may function to augment T cell activation. To investigate this possibility, we used a rtTA-inducible DEPTOR transgenic mouse (iDEP) in which DEPTOR overexpression is forced by doxycycline (dox) treatment. We initially transplanted fully MHC mismatched BALB/c ($H\text{-}2^d$) hearts into C57BL/6 wildtype (WT) or iDEP recipients (both $H\text{-}2^b$) ± dox (via chow). We found that forced overexpression of DEPTOR in iDEP recipients was associated with a significant ($p<0.001$) prolongation of graft survival (MST 35 days, n=5) *vs*. WT recipients (MST 7 days, n=14). By FACS analysis on day 6 post-transplant, iDEP recipients had a lower frequency of $CD4^+CD44^{high}CD62L^{low}$ T_{EM} cells, and a higher frequency of $CD4^+CD44^{low}CD62L^{high}$ naive T cells and $CD4^+Foxp3^+$ Treg cells *vs.* WT recipients. Administration of anti-CD25 (PC61, 250 µg on days -5, -2 and +2 peri-transplant to deplete Tregs) reversed the graft survival advantage following DEPTOR overexpression (MST 9 days, n=5). Thus, persistent expression of DEPTOR in $CD25^+$ Tregs functions to prolong graft survival. To further confirm the immunoregulatory effect of DEPTOR in $CD4^+$ T cells, we adoptively transferred $CD4^+$ iDEP T cells (± dox) into $Rag2^{-/-}\gamma c^{-/-}$ mice bearing fully MHC mismatched BALB/c allografts. Notably, dox-treated and DEPTOR overexpressing $CD4^+$ T cells were potent to prolong allograft survival (MST 37 days, n=5) *vs.* untreated cells (MST 16 days, n=6; $p<0.01$). We also cultured naive $CD4^+CD25^-$ iDEP T cells in standard iTreg-inducing media (anti-CD3/anti-CD28, TGF-β, IL-2 and retinoic acid) ± dox (3 µg/ml) and evaluated iTreg differentiation. While the addition of dox had no effect on Foxp3 expression (± dox ~80%), we found that dox-treated iTregs are more potent to inhibit T_{eff} proliferation in *in vitro* suppression assays (no dox: 58.5% suppression *vs*. dox: 91.2% suppression; $p<0.01$). In addition, DEPTOR-overexpressing iTregs were more stable upon mitogen reactivation for 7 days (no dox: ~10% $Foxp3^+$ *vs.* dox: 46% $Foxp3^+$; $p<0.001$). Collectively, these findings identify DEPTOR as a novel cell intrinsic protein in $CD4^+$ T cells that enhances Foxp3 stability and Treg function following transplantation. Our findings provide for the intriguing possibility that pharmacological agents that target DEPTOR degradation will be immunoregulatory and have promise as future therapeutics in transplant medicine.

427.3

Role of bone marrow maturity, insulin-like growth factor 1 receptor (IGF1R) and forkhead box protein N1 (FOXN1) in thymic involution and rejuvenation in a preclinical large animal model

Masayuki Tasaki[1,2,3], Vincenzo Villani[2], Mitsuhiro Sekijima[2,3], Akihiro Kawai[2,3], Akira Shimizu Shimizu[2,3], David Sachs[1,2], Kazuhiko Yamada[1,2].
[1]Columbia Center for Translational Research, Columbia University Medical Center, New York, United States; [2]Transplantation Biology Research Center, Massachusetts General Hospital, Boston, United States; [3]Center for Advanced Biomedical Science and Swine Research, Kagoshima University , Kagoshima, Japan.

Background and Aim of the Study: Thymic involution is associated with age-related changes of the immune system as well as the ability to induce tolerance. We have previously demonstrated that aged thymi were rejuvenated after transplantation into juvenile swine. Here we have studied the roles of the age of bone marrow (BM) elements and of the soluble factors forkhead-box protein N1 (FOXN1) and insulin-like growth factor (IGF)-1 in the mechanism of thymic rejuvenation and involution.
Materials and Methods: Study 1: We first examined serum testosterone and IGF1 levels in 60 naïve MHC-inbred miniature swine (age range: 1-32 months).
Study 2: We next examined the roles of the age of BM elements and of the soluble factors, FOXN1 and IGF1 in the mechanism of thymic rejuvenation and involution. 10 MHC inbred miniature swine recipients of MHC-matched, age-mismatched, vascularized thymic lobes were divided into four groups: aged donors and juvenile recipients in Group 1, juvenile donors and aged recipients in Group 2, aged donors and aged recipients in Group 3. Group 4 consisted of aged recipients who were transplanted with juvenile BM in addition to juvenile thymi. We examined thymic rejuvenation and involution by means of histology and flow cytometry. Thymic function was assessed by ability of induction of tolerance.
Results: Study 1: Serum testosterone levels in male pigs increased with age while conversely serum IGF-1 levels decreased. Consistent with this observation, IGF1 receptor (IGF1R) expression was elevated in young naïve thymic medulla but decreased in aged thymus.
Study 2: Aged thymi that were rejuvenated in a juvenile environment restored functional tolerance (Group 1), while juvenile thymi in aged recipients involuted and had a limited ability to induce tolerance (Group 2). However, juvenile BM inhibited the involution process of juvenile thymi in aged recipients, and promoted induction of kidney allograft tolerance even in an aged recipient (Group 4). Group 3 animals showed no changes in the involuted status after transplantation, showing that transplantation *per se* does not enhance rejuvenation of the thymic gland. An increase in the expression of both FOXN1 and IGF-1R was observed in juvenile thymi and rejuvenated thymi in all groups.
Conclusion: The expression of FOXN1 and IGF1R was noted to increase under conditions that stimulated rejuvenation, suggesting that these factors are involved in recover of thymic function. Juvenile BM plays a role in promoting the local thymic milieu as indicated by its ability to inhibit thymic involution in an aged animal. These data provide valuable information for the development of a novel strategy to rejuvenate aged thymi in order to induce transplant tolerance.

427.4

Rapamycin promotes allograft survival in elderly transplant recipients linked to an accumulation of IL-10+ CD4+ T cells and CD4+CD25+Foxp3+ T-regs

Jeroen Schuitenmaker, Karoline Edtinger , Midas Seyda , Hirofumi Uehara , Marcus Quante, Abdallah ElKhal , Stefan G. Tullius .
Transplant Surgery Research Laboratory, Brigham and Women's Hospital, Boston, MA, United States.

Immunosenescence is broadly affecting alloimmunity with major implications on immunosuppression. Older recipients have thus far been mostly excluded from clinical trials. Here, we dissected the effect of immunosenescence on the efficacy of Rapamycin in a fully MHC-mismatched skin transplantation model.
Skin grafts of young DBA/2 mice were transplanted onto young (2 months) and old (18 months) C57BL/6 mice that were treated with Rapamycin (1 mg per kg per day, i.p.) or PBS. Old, untreated recipients contained their grafts significantly longer (9 vs. 7 days in young-untreated recipients; p=0,006), suggesting that immunosenescence promotes allograft survival. Of particular relevance, Rapamycin prolonged graft survival in an age-specific fashion: while the median graft survival was only modestly extended in young recipients (12 days), graft survival was significantly prolonged in old recipients (19 days; p=0,008). Next, we dissected age-specific effects of Rapamycin on alloimmunity. $CD4^+$ T cells significantly declined in both young and old recipients when treated with Rapamycin (p=0.0009 and p= 0.0408 resp.) Interestingly, we observed an increase of IL-10^+ producing $CD4^+$ T cells, that are known for their immunosuppressive properties, only in old Rapamycin treated recipients (p= 0.0480). Moreover, we observed a significant decline of $CD11c^+$MHC class II^+ T cells and an increase of $CD4^+CD25^+Foxp3^+$ T-regs in old Rapamycin treated recipients (p=0.01 and p=0.0481 resp.).
Rapamycin prolonged graft survival in old recipients linked to an accumulation of IL-10^+ producing $CD4^+$ T cells and $CD4^+CD25^+Foxp3^+$ T-regs. Those age-specific capacities are of clinical relevance with increasing numbers of elderly transplant recipients.

427.5

NAD+ alters CD4+ T cell differentiation and promotes allograft survival independently of CD4+CD25+Foxp3+ regulatory T cells

A. Elkhal[1], H. Rodriguez Cetina Biefer[1,2], H. Uehara[1], T. Heinbokel[1,3], M. Quante[1], F. Krenzien[1], V. Camacho[4], M. Seyda[1], J. Schuitenmaker[1], M. A.de la Fuente[5], I. Ghiran[6], S.G. Tullius[1].
[1]Division of Transplant Surgery and Transplantation Surgery Research Laboratory, Brigham and Women's Hospital/Harvard Medical School, Boston, MA, United States; [2]Clinic for Cardiovascular Surgery, University Hospital Zurich, Zurich, Switzerland; [3]Institute of Medical Immunology, Charité, Universitätsmedizin Berlin, Berlin, Germany; [4]Flow Cytometry Core Facility, Beth Israel Deaconess Medical Center, Harvard Stem Cell Institute, Boston, United States; [5]Instituto de Biología y Genética Molecular, University of Valladolid, Valladolid, Spain; [6]Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, United States.

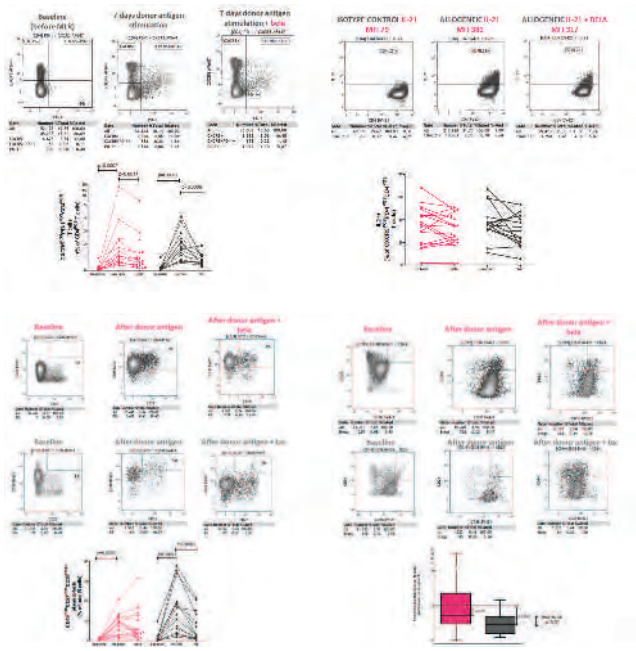
CD4+CD25+Foxp3+ Tregs have been shown to play a central role in homeostasis and to prevent from fatal inflammatory response while Th17cells have been found implicated in a myriad of diseases. We have recently shown that NAD+ protects against autoimmune diseases by regulating CD4+ T cell differentiation via a novel-signaling pathway that promotes CD4+IL-17A+TGF-b+ differentiation and induces CD4+CD25+Foxp3+ Treg-depletion. In addition, we have reported that NAD+ was able to convert naïve CD4+ T cells into IL-17A producing cells under regulatory T cell polarizing conditions. Here, we investigated in-depth the impact of NAD+ on thymus derived CD4+CD25+Foxp3+ regulatory T cells. Using a fully mismatched skin transplant mouse model, we confirmed the unique NAD+ homeostatic properties that are CD4+CD25+Foxp3+ T cell independent. More importantly, in presence of NAD+, CD4+CD25LowFoxp3+ were able to differentiate into Th17 cells. NAD+ induced a decreased expression of CD25 and IL-10 production by CD4+CD25+Foxp3+ Tregs and in parallel increased IL-17A and RORγt expression levels and promoted Th17 cell proliferation in absence of IL-23 through purinergic signaling machinery and the transcription factor STAT3. Collectively, our study unravels a novel immunosuppressive mechanism that regulates regulatory T cell development and unravels a new homeostatic pathway that is independent of CD4+CD25+Foxp3+ Tregs.

427.6

Belatacept fails to inhibit donor-reactive Tfh-B cell interaction, but favors a regulatory transitional B cell profile over tacrolimus

Gretchen de Graav, Dennis A.Hesselink, Marjolein Dieterich, Rens Kraaijeveld, Willem Weimar, Carla C.Baan.
Internal Medicine, Section Transplantation & Nephrology, Erasmus University Medical Center, Rotterdam, Netherlands.

The specialized follicular T-helper (Tfh) cells play a pivotal role in the differentiation of activated B-cells into alloreactive plasmablasts through help via co-stimulation and IL-21[1]. The co-stimulatory inhibitor belatacept effectively blocks this interaction in animal transplant models[2], while conserving regulatory transitional B-cells in stable patients ≥1 year after renal transplantation[3],[4]. This study aims to investigate whether belatacept has the same effect in patients within the first three months after renal transplantation.
Peripheral blood mononuclear cells (PBMCs) were obtained from belatacept-treated patients (n=20) or tacrolimus-treated patients (n=20). All patients had received basiliximab induction therapy, and mycophenolate mofetil and prednisone maintenance therapy. The induction and function of IL-21+ Tfh cells (CXCR5+PD-1+CD4+) and TNFα+ plasmablasts (CD19+CD27+CD38++), and the presence of transitional B-cells (CD19+CD24++CD38++) was assessed after 7-day culture of PBMCs with donor antigen. Some PBMC cultures were spiked with therapeutic concentrations of belatacept (10 µg/mL) or tacrolimus (10 ng/ml). The binding to CD80/86 and the blockade of the CD28-CD80/86 interaction by belatacept in vitro was confirmed by staining for accessible CD86 on donor-antigen activated B-cells.
Following stimulation with donor antigen, an 11-fold and 18-fold increase of Tfh cells was measured for belatacept- and tacrolimus-treated patients, respectively. In vitro addition of belatacept or tacrolimus partially inhibited this increase by a median of 30% and 43%, respectively (p=0.001). However, intracellular IL-21 production by these Tfh cells was not suppressed by belatacept or tacrolimus.



The differentiation of antigen-activated B-cells into TNFα-producing plasmablasts was also induced by donor antigen. Even though belatacept reduced free CD86-expression on donor-antigen activated B-cells more effectively than tacrolimus, median 51% vs 34%, respectively, p=0.03, the formation of plasmablasts was 63% inhibited by tacrolimus, but not by belatacept, p<0.0006. Finally, we observed

that co-culture with tacrolimus reduced the proportion of transitional B-cells by 60%, whereas belatacept preserved this population, p=0.02. In vitro belatacept favors a regulatory profile of renal transplant patients' B-cells but does not inhibit the donor-reactive B cell differentiation into plasmablasts.

Acknowledgements: A.S. Chong

References:

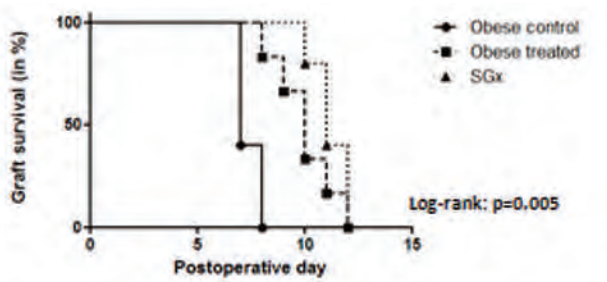
[1] de Graav et al. Clin Exp Imm 2014
[2] Kim et al. AJT 2014
[3] Leibler et al. AJT 2014
[4] Furuza-Carballeda et al. Trans Imm 2014

427.7

Metabolites associated with bariatric surgery reverse accelerated rejection and augmented alloimmunity in obese allograft recipients

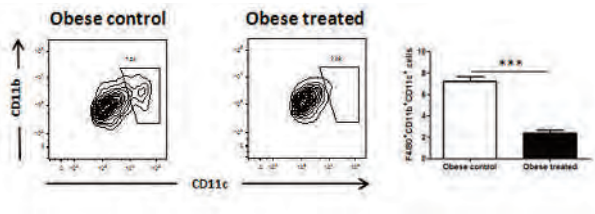
Markus Quante, Abdallah ElKhal, Midas Seyda, Jeroen Schuitenmaker, Hirofumi Uehara, Stefan G.Tullius.
Division of Transplant Surgery & Transplant Surgery Research Laboratory, Brigham and Women's Hospital/Harvard Medical School, Boston, MA, United States.

Background: The obesity epidemic has far-reaching health implications. In transplantation, obesity has become a common co-morbidity linked to impaired transplant outcomes. Here, we delineated mechanisms and consequences of obesity and surgically induced weight loss on alloimmunity and graft survival.
Materials and Methods: Allogenic skin transplantations were performed in diet-induced obese (DIO) C57/Bl6 mice and lean littermates. Additional groups underwent sleeve gastrectomies (SGx) prior to transplantation. The impact of surgery and dietary weight loss was asessed in control animals. Quantitative metabolomic profiling was performed to reveal changes in endogenous metabolites in obesity and during weight loss that might impact the immune response.
Results: Obesity resulted in accelerated allograft rejection. Bariatric surgery prolonged allograft survival beyond that observed in both, obese and lean animals (median graft survival: 7 d obese vs. 9 d lean vs. 11 d SGx; Log-rank test: p=0.005; n=5/group). On a molecular level, obese animals exhibited increased IFN-γ expression in splenic CD4+ T cells; bariatric surgery, in contrast, promoted protective, IL-10-dominated conditions (n=4-6/group by POD 6; p<0.001). Of note, a sham procedure group and a dietary intervention group that was switched to a normal chow diet showed both inferior results in allograft survival and weight loss when compared to animals that underwent SGx. Moreover, bariatric surgery resulted in a significantly compromised alloreactivity confirmed by ELISPOT (n=6/group by POD 6; p<0.05). Metabolomic profiling in obese, lean and bariatric animals identified a secondary bile acid and proteinogenic amino acid as central mediators of alloimmunity. Strikingly, combined application of both compounds reduced obesity and resulted in significant prolongation of graft survival (median allograft survival: 7 d obese controls vs. 10 d obese treated vs. 11 d SGx; n=5-6/group; Log-rank test: p=0.005, Fig.1).



Prolonged graft survival was linked to a less pro-inflammatory alloimmune response in splenic CD4+ T cells obtained from obese animals that underwent metabolic treatment. On a mechanistic level, metabolic treatment resulted in significant reduction of splenic M1-like antigen-presenting cells known to mount and promote pro-inflammatory conditions linked to obesity (n=5-6/group by POD 7; p<0.001,Fig.2).
Conclusions: Obesity augments IFNγ-driven alloimmune responses while bariatric surgery is linked to metabolic changes that induce a switch towards protective, IL-10-dominated conditions. Two metabolic compounds were identified as key mediators skewing the integrated network of metabolism and inflammation towards protective anti-inflammatory conditions. Thus, our results provide a unique

mechanistic link between obesity, metabolism and immunity with high relevance for the steadily growing number of obese transplant recipients and candidates.

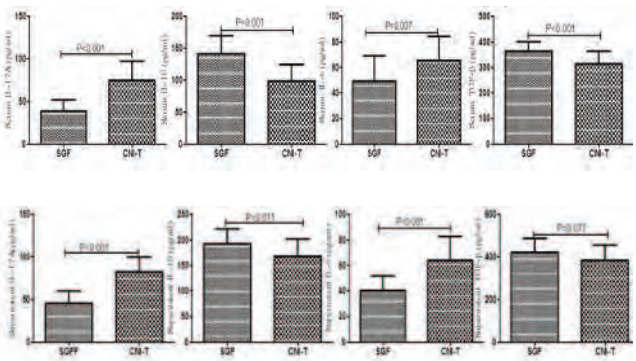


427.8

Is TH17 associated with chronic calcineurin inhibitor toxicity in renal allograft recipients?

Narayan Prasad[1], Brijesh Yadav[1], Vikas Agarwal [2], Vinita Agarwal [3], R. K. Sharma[1].
[1]Nephrology and Renal Transplantation, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India; [2]Clinical Immunology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India; [3]Pathology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India.

Introduction: Th17 a proinflamatory helper T cell, secrets IL-17A cytokines leading to activation and recruitment of other alloreactive immune cells while T regulatory cells(Tregs) suppress alloreactive immune cell and promote stable graft function (SGF). The association of IL-17 with pulmonary and myocardium fibrosis has been reported recently.Calcineurin inhibitors inhibit IL-2 synthesis which is required for T effectors,and Tregs cell differentiation, proliferation and survival as well. Both Th17 and Tregs are plastic and converts into each other also.The immune dysregulation in Chronic calcineurin inhibitor toxicity (CNIT) which is associated with striped fibrosis, arteriolar hyalinosis, and microvascular injury in allograft, is not studied in depth
Aim: To analyze the Th17 and Treg cell frequency; their soluble and biointact cytokines in serum and PBMCs culture supernatant; and their mRNA transcript (RORC and FoxP3) in allograft tissue of patient with CNIT and SGF.
Methods: We recruited total 40 patients; 10 patient with SGF who have no proteinuria and <10% cortical surface area with tubular atrophy and Interstial fibrosis on biopsy; and 30 CNIT following histopathology report of allograft biopsy (hyaline arteriolopathy, nonspecific glomerular sclerosis, striped interstitial fibrosis and tubular atrophy). To further substantiate the diagnosis of CNIT, pateints with BKV, CMV, chronic pyelonephritic changes,donor specific antibody on luminex and C4d + on IF were excluded. we have also collected peripheral blood for flow cytometry cell quantification,and serum and biointact cytokines analysis and allograft tissue specimen for mRNA transcript expression study of RORC and FoxP3 by Taq man Real time PCR.
Results: The mean age of patients in SGF versus CNIT was (40.90±12.67 Vs 38.43±8.87 years; P=0.5) month; post-transplant biopsy interval (49.70±22.71 Vs 56.13±28.37; P=0.52) months; serum creatinine (1.23±0.18 Vs 2.34±0.78; P<0.001) mg/dl and daily urine protein (0.13±0.10 Vs 0.80±0.60 g; P<0.001), respectively.
The frequency of Th17 cell in patient with SGF and CNIT was 4.73±1.22 and 10.11±2.24,P<0.001; Tregs 3.70±1.55 and 2.34±1.22,P=0.007); Th17/Treg cell ration 1.80±1.65 and 5.60±3.18; P=0.001, respectively.
Soluble serum cytokines level (pg/ml) of IL-17A and IL-6 was signicantly high in patient with CNIT and IL-10, TGF-β was signiifcantly low as compared to SGF(Fig.1) Biointact cytokines level (pg/ml) of IL-17A and IL-6 in was significantly high and IL-10, TGF-β level was low in patient of CNIT as compared to SGF (Fig 2). However,TGF-β level remain insignificant. Intragraft mRNA transcript expression of Th17 (RORC) in CNIT(2.08±0.79) was high as compared to SGF(1.02±0.076, P<0.001);and intragraft FoxP3 was also high in CNIT 2.73±1.07; P<0.001) as compared to SGF(0.98±0.069).
Discussion: We oberved increased Th17 cell frequency and IL-17 cytokines level and decreased Treg and IL-10 level in blood of CNI-T patients. However, both Intragraft Th17 and Treg expression were high in CNI-T, suggesting increased Treg cell sequestration to countercheck Th17 inflammatory activity producing CNIT. IL-17 may contribute to interstitial, stripped fibrosis of CNI-T in renal transplant as suggested by role of IL-17 in myocardial and lung graft fibrosis [1],[2].
Conclusion: CNIT is associated with Th17 cell dysregulation contributing to injury and fibrosis in CNIT.It is possible that Intragraft T-regs is not functionally active to suppress pro-inflammatory Th17 activity.



Brijesh Yadav is a DST Inspire fellow getting fellowship from Department of Science and Technology, Govt. of India

References:
[1] Faust SM, Lu G, Marini BL, Zou W, Gordon D, Iwakura Y et al. Role of T cell TGFbeta signaling and IL-17 in allograft acceptance and fibrosis associated with chronic rejection. J Immunol 2009;183(11):7297-7306. 23.
[2] Liu Y, Zhu H, Su Z, Sun C, Yin J, Yuan H et al. IL-17 contributes to cardiac fibrosis following experimental autoimmune myocarditis by a PKCbeta/Erk1/2/NF-kappaB-dependent signaling pathway. Int Immunol 2012;24(10):605-612.

427.9

Lymphotoxin on human and mouse Treg promotes migration across lymphatic endothelium

C. Colin Brinkman[1], Keli L.Hippen[2], Bruce R.Blazar[2], Jonathan Bromberg[1].
[1]Surgery, Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD, United States; [2]Division of Blood and Marrow Transplantation, Department of Pediatrics, University of Minnesota Cancer Center, Minneapolis, MN, United States.

Introduction: Lymphotoxin (LT) is involved in lymphangiogenesis, helps maintain the structure of secondary lymphoid organs, and LTβ receptor (LTβR) signaling helps promote leukocyte migration into lymph nodes (LN) via high endothelial venules (HEV) and thymocyte migration. We previously showed a requirement for LT for murine Treg to prolong islet allograft survival, and for Treg migration from tissues into afferent lymphatics in the ear and footpad via interactions with LTβR on lymphatic endothelial cells (LEC). We hypothesized that human Treg would also use LT to regulate their interactions with and migration through human LEC.
Methods: Murine Treg were generated from Foxp3GFP mice, and flow-sorted for CD25 and Foxp3GFP. Naïve human T cells were flow-sorted from peripheral blood, and effectors and induced Treg (iTreg) stimulated with anti-CD3 mAb, IL-2 and antigen presenting cells (APCs). iTreg also received TGFβ1 and rapamycin. Human thymus-derived tTreg were isolated from umbilical cord blood and expanded with anti-CD3, IL-2 and APCs. T cells were used in transmigration assays across primary mouse and human skin LEC and the murine LEC line SVEC4-10. For footpad assays murine tTreg were labeled with CFSE, injected into footpads, and analyzed in the downstream popliteal LN after 12 hours. LTβR fusion protein (LTβRIg) was used to block LTαβ interactions with LTβR.
Results: Consistent with our previous results using mouse T cells and endothelial cells, human Treg but not non-Treg CD4 T cell migration to a CCL19 gradient across human skin LEC layers was inhibited by blocking LTαβ interactions with LTβR using LTβRIg. We extended our mechanistic understanding of the expression and action of LT. Both human and murine Treg expressed more LT than did non Treg CD4+ T cells. Murine Treg and non Treg subsets also displayed different knietcis of up- and down-regulation of LT. Murine Treg migration across SVEC4-10 and primary mouse LEC layers was inhibited by blocking LTαβ-LTβR interactions, blocking VCAM-1, or inhibiting non-canonical NFκB signaling, a known downstream mediator of LTβR signaling. Migration of non-Treg T cells in vitro was not inhibited by blocking LT. Inhibitory effects of these treatments were not additive, suggesting they were part of the same pathway. Inhibitory effects were typically enhanced by simulating lymphatic fluid flow across the LEC to better model in vivo conditions. Finally, LT and VCAM-1 blockade also inhibited Treg but not non-Treg CD4+ T cell migration in vivo from footpad to draining LN. .
Conclusions: Engagement of LTβR on lymphatic endothelium by both human and murine Treg but not non-Treg promotes lymphatic migration. This mechanism involves VCAM-1 and the non-canonical NFκB pathway and operates under static conditions and is enhanced by fluid flow. As lymphatic flow is modulated during inflammation this may also result in different sensitivity to this pathway depending upon the phase of the inflammatory response.

427.10

T-bet regulates nTreg homing into draining lymph nodes to suppress alloimmune responses

Yanbao Xiong, Sarwat B.Ahmad, Daiki Iwami, C. Colin Brinkman, Jonathan Bromberg.
Surgery, Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD, United States.

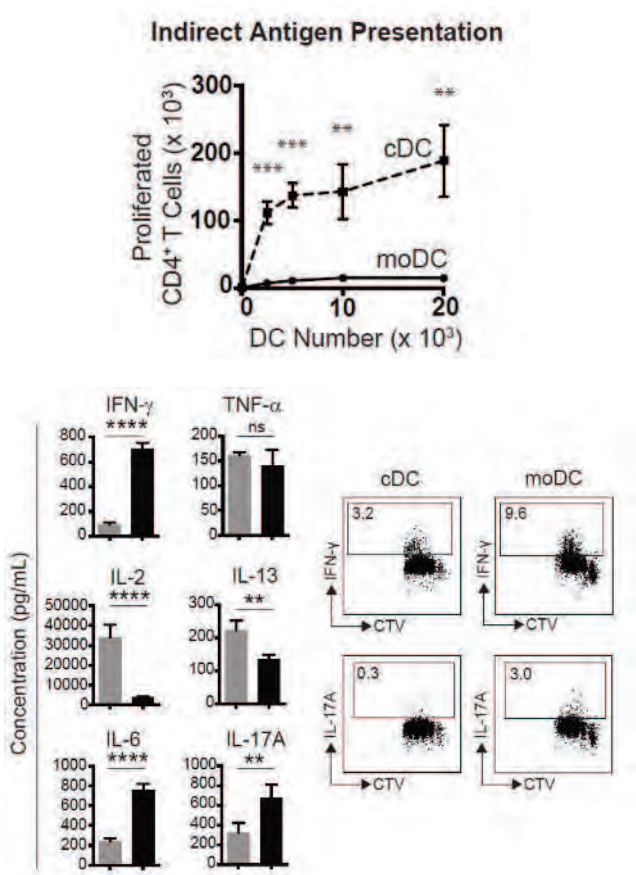
Introduction: The transcription factor T-bet is required by Treg to suppress Th1 inflammation. However, the mechanisms by which T-bet controls alloimmune responses are not fully understood. We hypothesized that T-bet regulates Treg distribution and suppressive functions, and uncovered a novel molecular mechanism for Treg migration and stability.
Methods: BALB/c (H-2d) donor islets were isolated and transplanted to the renal capsule of streptozotocin-induced diabetic C57BL/6(H-2b) wild type (WT) recipients. Blood glucose was monitored. CD4+CD25+Foxp3-GFP natural Treg (nTreg) from WT or T-bet KO C57BL/6 were isolated and adoptively transferred to allograft recipients, or used for in vitro assays. Flow cytometry and qRT-PCR were performed to analyze expression of effector, adhesion and migration molecules.
Results: T-bet deficient nTreg failed to prolong islet allograft survival as well as WT nTreg. There was no difference in suppression of effector T cell proliferation in vitro between T-bet KO vs WT Treg. However, T-bet KO nTreg failed to prevent antigen-specific CD4 T cell proliferation as well as WT nTreg in vivo. Furthermore, T-bet KO nTreg did not prevent antigen-specific CD4 T cell infiltration into grafts and draining lymph nodes (dLN). Wild type nTreg recovered from the islet grafts expressed higher amounts of T-bet than naïve nTreg, suggesting that T-bet expressed by nTreg played an important role in suppression in the graft. Compared with WT, T-bet KO nTreg remained within the graft and failed to traffic to the dLN from the graft afferent lymphatics. In the graft, T-bet KO nTreg expressed more CCR4 and CD103 than WT nTreg, and their cognate ligands CCL22, CCL17 and E-cadherin were also highly expressed. With a footpad migration assay, E-cadherin blockade restored T-bet nTreg homing to the dLN. Fluorescence imaging analysis showed that T-bet KO nTreg remained associated with interstitial ligands and failed to access afferent lymph vessels. WT nTreg recovered from the graft expressed much more Foxp3 and the suppressor effector molecules IL-10, CTLA4, CD73 and CD39 than T-bet KO nTreg. More T-bet nTreg lost Foxp3 expression and became ex-Treg than WT nTreg.
Conclusion: T-bet regulates nTreg distribution through adhesion and migration molecules, resulting in graft retention that ultimately affects Treg stability, which is essential for in vivo suppression to protect islet allografts. These results demonstrate a novel and unique regulation of Treg migration, genetic stability and suppression. These functional and molecular interactions are foci for therapeutic interventions in immunity and tolerance.

427.11

Monocyte-derived dendritic cells promote Th polarization, whereas conventional dendritic cells promote Th proliferation

Kevin V. Chow[1,2,3], Andrew M. Lew[1,2], Robyn M. Sutherland[1,2], Yifan Zhan[1,2].
[1]Immunology, Walter and Eliza Hall Institute of Medical Research, Parkville, Australia; [2]Medical Biology, University of Melbourne, Parkville, Australia; [3]Nephrology, Royal Melbourne Hospital, Parkville, Australia.

Monocyte derived dendritic cells (moDCs) dramatically increase in numbers upon infection and inflammation. We found that this also occurs in response to allogeneic encounters, such as those that occur in the setting of organ transplantation. Despite their prominence in these settings, how emergent moDCs and resident conventional DCs (cDCs) divide their labor as APCs remain undefined. Since, unlike nominal antigens, transplant antigens can be recognised by direct and indirect presentation, we compared both direct and indirect antigen presentation by murine moDCs versus cDCs. We found that, despite having equivalent MHC-II expression and in vitro survival, moDCs were 20-fold less efficient than cDCs at inducing CD4+ T cell proliferation through both direct and indirect Ag presentation. Despite this, moDCs were more potent at inducing Th1 and Th17 differentiation (e.g. 8-fold higher IFN-γ, 2-fold higher IL-17A in T cell co-cultures) whereas cDCs induced 10-fold higher IL-2 production. Intriguingly, moDCs potently reduced the ability of cDCs to stimulate T cell proliferation in vitro and in vivo, partially through nitric oxide production. We surmise that such division of labor between moDCs and cDCs has implications for their respective roles in the immune response.



427.12

Urinary levels of chemokines and growth factors in renal transplant patients

Ali Raza[1], Sadaf Firasat[1], Shagufta Khaliq[1], Tahir Aziz[2], Mohammad Mubarak[3], Anwar Naqvi[4], Adib Rizvi[4], Syed Qasim Mehdi[1], Aiysha Abid[1].
[1]CHGMM, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [2]Nephrology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [3]Pathology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [4]Urology and Transplantation, Sindh Institute of Urology and Transplantation, Karachi, Pakistan.

Background: Immuno-regulatory molecules such as chemokines and growth factors have a major role in renal allograft rejection. Determination of these chemokines and growth factors in urine could be used as early indicators for renal dysfunction [1]. In this study, urinary expression of chemokines (CXCL10, CXCL9, CCL2 and CCL5) and growth factors (VEGF-α and TGF-β1) was assessed for significant changes in their levels among renal transplant patients at different time intervals and histological conditions.
Methodology: This study is comprised of 511 live-related renal transplant patients. Urine samples were collected at(a) 10th, 30th, 90th & 365 days post-transplantation;
(b) Prior to graft biopsy (n=654), categorized as Rejection (REJ; n=267), non-rejection (NonREJ; n=262) and normal histology (NAD; n=125).(c) Non-biopsy stable functioning graft (SG; n=45)The samples were quantified for biomarkers using the ELISA and analyzed through SPSS software.
Results: (a) Urinary levels of biomarkers at 10th, 30th, 90th and 365 days:We have found raised levels of the CCL2 during the initial post-transplantation phase (i.e. at 10th vs. 90th day). CCL5 also showed significant raised in levels between 10th day and other time intervals (30th, 90th and 365th; P<0.005). Levels of other chemokines CXCL9 and CXCL10 and growth factors (VEGF and TGF-β1) did not differ significantly at different time intervals among renal transplant patients.
(b) Urinary levels among REJ, NonREJ, SG and NAD group:All chemokines (CXCL10, CXCL9, CCL2 and CCL5) and growth factor (TGF-β1) levels were raised significantly in the rejection group compared to the non-rejection except VEGF-A. Raised levels of these biomarkers were also found between rejection and NAD group except TGF-β1 and CXCL9. CCL5 and CXCL10 also showed raised levels in the rejection group compared to SG group.
Conclusion: Raised urinary levels of CCL2 and CCL5 indicate the involvement of these chemokines during the initial post-transplant period and rejection and CXCL9 in later phases of post-transplant duration. These chemokines might reflect the initial renal injury associated either with rejection or surgical complication after post-transplantation.

427.13

Positive association of Tcell hyporesponsiveness and age of kidney transplant in patients attending Inkosi Albert Luthuli Central Hospital (Durban, South Africa)

Alain Assounga, Saleha Omarjee, Zama Ngema.
Dept de Nephrology, University of KwaZulu-Natal, Durban, South Africa.

Background: We and others have previously reported a significant reduction in the proliferation of peripheral blood mononuclear cells (PBMC) from transplant patients treated by cyclosporin, tacrolimus or sirolimus based immunosuppressive regimens. Despite the reduction in immunosuppressive dose transplant patients are still subject to infections and malignancies.
Objective: To assess T cell response of kidney transplant recipients according to transplant age.
Method: PBMC were obtained from 38 transplant patients (aged from 0-20 years) by means of density gradient centrifugation of blood samples. Fifty thousand PBMC per well, were incubated overnight (15-18 hours) in triplicate with 5µg/ml of a mitogen, Phytohemagglutinin (PHA). The Promega CellTitre-Glo Luminescent Cell Viability assay which signals the presence of intracellular ATP by means of the luciferin/luciferase. Turner Biosystem luminometer were used to measure ATP in relative lights units (RLU). Results were analysed according to the age of the transplant.
Results: The Chi square test was employed to assess group differences. T cells from transplant patients were less responsive to PHA stimulation as transplant time increased, p=0.0107. (Table 1 below). Furthermore, linear regression analysis indicated that the T cell hyporesponsiveness is not associated with a change in immmunosupressive blood levels (p=0.939).
Conclusion: T cell response of our patients decreased with the age of kidney transplant despite stable blood of levels of immunosuppressive drugs. This may correspond to a progression toward an immune tolerance state. This observation warrant further studies to evaluate factors involved in T cell suppression such as T regulatory cells.

Table 1.

Table 1. PHA cell stimulation of T cells expressed as ATP concentration according to age

	All patients n=38	0-5y transp n=8	6-10y transp n=18	11-15y transp n=7	16-20y transp n=5	P value
Mean [ATP] ±SEM (ng/ml)	265.74 ±25.75	386.25 ±79.14	228.72 ±30.44	259.57 ±47.97	214.80 ±48.60	p=0.0107

428.1

Development of novel ferroptosis inhibitors for the treatment of ischemia/reperfusion injuries

Bettina Proneth[1], Elke Eggenhofer[2], Edward K.Geissler[2], Marc Weidenbusch[3], Hans-Joachim Anders[3], Marcus Conrad[1].
[1]Institute of Developmental Genetics, Helmholtz Zentrum München, Neuherberg, Germany; [2]Department of Surgery, University Medical Center of Regensburg, Regensburg, Germany; [3]Medizinische Klinik und Poliklinik IV, Klinikum der Universität München, München, Germany.

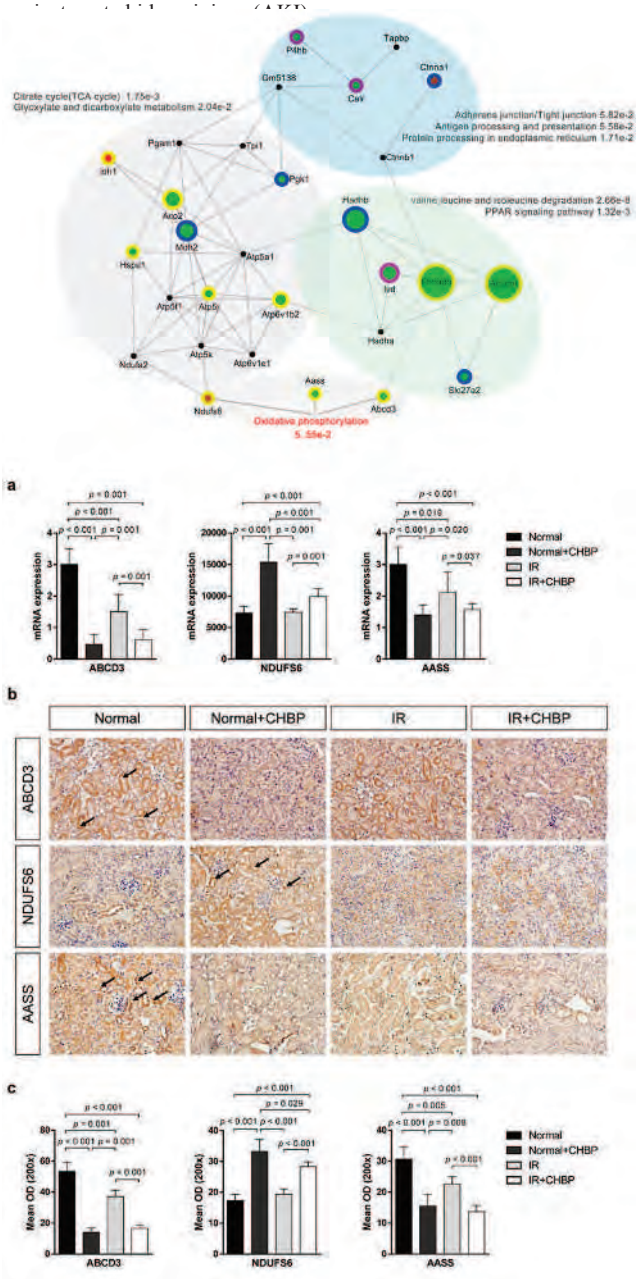
Introduction: Ischemia/reperfusion injuries (IRI) frequently occur upon organ transplantation and surgical interventions. However, despite the enormous impact on patient outcome, only limited therapeutic options exist. Ferroptosis is a newly discovered regulated cell death routine[1] clearly distinct from apoptosis and necroptosis and when impeded preserves cellular function and organ integrity during tissue IRI[2]. Recent evidence has been provided that ferroptosis plays a pivotal role in the pathological context of liver[3], kidney[4] and cardiac[5] IRI. Therefore, the development of novel ferroptosis inhibitors holds high promise for the management of IRI upon solid organ transplantation.
Materials and Methods: In attempts to develop new and highly efficacious ferroptosis inhibitors, we screened a diverse set of small molecule libraries using an in-house phenotypic cell based high-throughput platform. Subsequently, hit-to-lead, lead optimization and DMPK (drug metabolism and pharmacokinetics) profiling was performed to select compounds with a high therapeutic window, favorable physicochemical properties and promising pharmacokinetic profiles. In vivo proof-of concept was generated in mouse models of hepatic and renal (bilateral) IRI with ischemia times of 90 and 30 min, respectively. Hereby, compounds were administered after (liver) or both, prior and after (kidney) the ischemic period. Tissue protective effects were analyzed by clinical chemistry and histopathology 24 hours after reperfusion.
Results and Discussion: Phenotypic screening for drug-like small molecule compounds identified Liproxstatin-1 as a highly efficacious and specific inhibitor against ferroptotic cell death[3]. Medicinal chemistry and DMPK studies of approximately 200 Liproxstatin analogues not only increased efficacy of Liproxstatin-1 but also yielded substantially improved compounds with increased stability in vitro and in vivo. In addition, Liproxstatins were found to be tissue protective in preclinical mouse models of liver and kidney IRI as measured by significant decreases in markers of liver (ALT/AST) and kidney (creatinine/BUN/KIM-1/NGAL) injury, compared to vehicle treated animals. In addition, histopathologic evaluation of treated tissue samples showed a marked reduction of IRI induced tissue damage.
Conclusion: Inhibition of ferroptotic cell death is a viable approach to protect tissues from undergoing degeneration after IRI upon solid organ transplantation, amenable to pharmacological intervention. Further development of improved next-generation Liproxstatins offers new opportunities to ameliorate or prevent IRI by treating organ recipients, with the potential to significantly improve patient outcome after transplantation.
References:
[1] Dixon, S.J., Lemberg, K.M., Lamprecht, M.R., Skouta, R., Zaitsev, E.M., Gleason, C.E., Patel, D.N., Bauer, A.J., Cantley, A.M., Yang, W.S., et al. (2012). Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell 149, 1060-1072.
[2] Conrad, M., Angeli, J.P., Vandenabeele, P., and Stockwell, B.R. (2016). Regulated necrosis: disease relevance and therapeutic opportunities. Nature Reviews Drug Discovery.
[3] Friedmann Angeli, J.P., Schneider, M., Proneth, B., Tyurina, Y.Y., Tyurin, V.A., Hammond, V.J., Herbach, N., Aichler, M., Walch, A., Eggenhofer, E., et al. (2014). Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. Nat Cell Biol 16, 1180-1191.
[4] Linkermann, A., Skouta, R., Himmerkus, N., Mulay, S.R., Dewitz, C., De Zen, F., Prokai, A., Zuchtriegel, G., Krombach, F., Welz, P.S., et al. (2014). Synchronized renal tubular cell death involves ferroptosis. Proc Natl Acad Sci U S A 111, 16836-16841.
[5] Gao, M., Monian, P., Quadri, N., Ramasamy, R., and Jiang, X. (2015). Glutaminolysis and Transferrin Regulate Ferroptosis. Molecular Cell 59, 298-308.

428.2

Proteome analysis of renoprotection mediated by a novel cyclic helix B peptide in kidney ischemia reperfusion injury

Cheng Yang[1,3], Junjun Liu[2], Long Li[1,3], Shangfeng Liu[2], Ruiming Rong[1,3].
[1]Urology, Zhongshan Hospital, Fudan University, Shanghai, People's Republic of China; [2]Stomatology, Huashan Hospital, Fudan University, Shanghai, People's Republic of China; [3]Shanghai Key Laboratory of Organ Transplantation, Shanghai, People's Republic of China.

We developed a novel, erythropoietin-derived, non-erythropoiesis, cyclic helix B peptide (CHBP) that displays potent renoprotection



To determine the mechanism of CHBP-mediated protection, we investigated the proteomic profile of mice treated with CHBP in a kidney ischemia-reperfusion (IR) injury model.

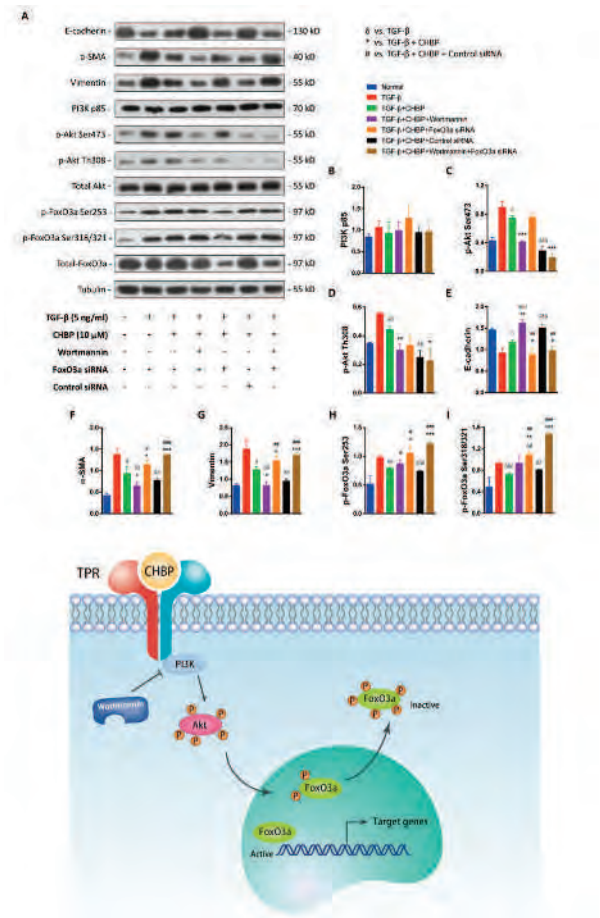
The isobaric tags for relative and absolute quantitation (iTRAQ)-labeled samples were analyzed using a QSTAR XL LC/MS system. In total, 38 differentially expressed proteins (DEPs) were shared by all experimental groups, while 3 DEPs were detected specifically in the IR + CHBP group. Eight significant pathways were identified, and oxidative phosphorylation was shown to be the most important pathway in CHBP-mediated renoprotection. The significant DEPs in the oxidative phosphorylation pathway elicited by CHBP are NADH-ubiquinone oxidoreductase Fe-S protein 6 (NDUFS6), alpha-aminoadipic semialdehyde synthase (AASS) and ATP-binding cassette sub-family D member 3 (ABCD3). The DEPs mentioned above were verified by RT-qPCR and immunostaining in mouse kidneys. We tested 6 DEPs in human biopsy samples from kidney transplant recipients. The trend of differential expression was consistent with that in the murine model. In conclusion, this study helps to elucidate the pharmacological mechanisms of CHBP before clinical translation.

428.3

Cyclic helix B peptide inhibits ischemia reperfusion-induced renal fibrosis via the PI3K/Akt/FoxO3a pathway

Cheng Yang, Tongyu Zhu.
Zhongshan Hospital, Fudan University, Shanghai, People's Republic of China.

Renal fibrosis is a main cause of end-stage renal disease. Clinically, there is no beneficial treatment that can effectively reverse the progressive loss of renal function. We recently synthesized a novel proteolysis-resistant cyclic helix B peptide (CHBP) that exhibits promising renoprotective effects. In this study, we evaluated the effect of CHBP on renal fibrosis in an in vivo ischemia reperfusion injury (IRI) model and in vitro TGF-β-stimulated tubular epithelial cells (TCMK-1 and HK-2) model. In the IRI in vivo model, mice were randomly divided into sham (sham operation), IR and IR+CHBP groups (n = 6). CHBP (8 nmol/kg) was administered intraperitoneally at the onset of reperfusion, and renal fibrosis was evaluated at 12 weeks post-reperfusion. Our results showed that CHBP markedly attenuated the IRI-induced deposition of collagen I and vimentin. In the in vitro model, CHBP reversed the TGF-β-induced down-regulation of E-cadherin and up-regulation of α-SMA and vimentin. Furthermore, CHBP inhibited the phosphorylation of Akt and Forkhead box O 3a (FoxO3a), whose anti-fibrotic effect could be reversed by the 3-phosphoinositide-dependent kinase-1 (PI3K) inhibitor wortmannin as well as FoxO3a siRNA. These findings demonstrate that CHBP attenuates renal fibrosis and the epithelial-mesenchymal transition of tubular cells, possibly through suppression of the PI3K/Akt pathway and thereby the inhibition FoxO3a activity.



428.4

Outcomes after DCD heart normothermic machine perfusion using banked blood in a porcine model

Hong Chee Chew[1], Jeanette Villaneuva[1], Ling Gao[1], Mark Hicks[1], Andrew Jabbour[1,2], Kumud Dhital[1,2], Peter MacDonald[1,2].
[1]Transplant Laboratory, Victor Chang Cardiac Research Institute, Sydney, Australia; [2]Heart Lung Clinic, St Vincent's Hospital, Sydney, Australia.

Aim: To investigate the functional outcomes of porcine heart recovery during normothermic machine perfusion (NMP) when comparing banked blood to donor blood reperfusion and the metabolic disturbances in the early stages of NMP.
Method: Landrace pigs (n=11) were subjected to circulatory arrest to mimic clinical donation after circulatory death (DCD) conditions. Group 1 (n=6), 1-1.2 litres of blood was collected after cessation of circulation for reperfusion of heart on ex-vivo device; Group 2 (n=5), 1-1.2 litres of banked blood was used to reperfuse the heart on the same device. Baseline metabolic features of the blood were established in each group using handheld i-Stat system, and corrected prior to installation of heart for reperfusion. Functional outcomes measured include aortic flow (AF), coronary flow (CF) and mean aortic pressure (MAP). In both groups, reperfusion of organ was sustained for at least 4 hours unless unfavourable lactate changes.
Results: Metabolic profile of donor blood compared to banked blood reveals significant acidosis (pH 7.53 ± 0.04 vs 6.53 ± 0.04) and electrolyte derangement (K+ 4 ± 0.5 vs 8.9 ± 0.3; Ca2+ 1.32 ± 0.03 vs <0.25; HCO3- 33.8 ± 1.3 vs 0) which requires vigorous correction prior to installation of heart for NMP. Functional outcomes show a significantly higher MAP and AF in banked blood group to achieve a similar coronary flow (MAP 62.3 ± 7.6 vs 98.2 ± 30.5mmHg; AF 0.78 vs. 0.86L/min; CF 0.75 vs 0.71L/min). Lactate profile in both groups remain favourable. (Fig 1) However duration of NMP in the banked blood group is shorter (t= 224 ± 65mins vs 305 ± 46mins).

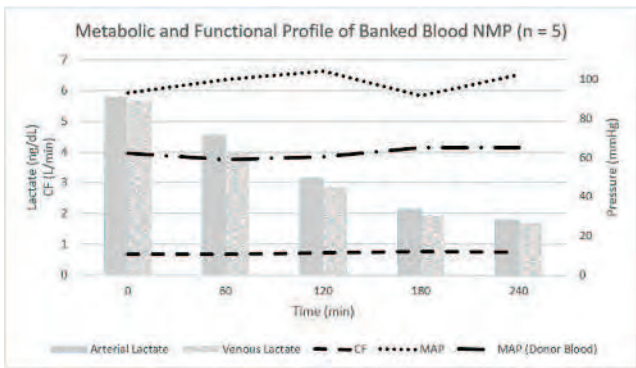


Figure 1. Metabolic and Functional profile of banked blood NMP. MAP in donor blood group is added as comparison.
Conclusion: Donor blood remains superior for the reperfusion of hearts in NMP, however, recovery is achievable using banked blood with vigorous correction of metabolic disturbances. It appears however, components within banked blood induce coronary vasoconstriction during NMP.

428.5

Treatment with tetrahydrobiopterin protects from transplant vasculopathy via prevention of eNOS uncoupling

Rupert Oberhuber[1], Gregor Riede [1], Benno Cardini[1], David Bernhard[4], Barbara Messner[4], Katrin Watschinger[3], Christina Steger[1], Johann Pratschke[1], Gerald Brandacher[1], Ernst Werner[3], Manuel Maglione[1].
[1]Visceral, Transplant and Thoracic Surgery, Innsbruck Medical University, Innsbruck, Austria; [2]Plastic and Reconstructive Surgery, Johns Hopkins University School of Medicine, Baltimore, United States; [3]Biological Chemistry, Biocenter, Innsbruck Medical University, Innsbruck, Austria; [4]Surgery, Vienna Medical University, Vienna, Austria.

Transplant vasculopathy represents a major obstacle to long-term graft survival. Treatment strategies aiming at preventing it are still lacking. Herein we present that the occurrence of transplant vasculopathy crucially depends on oxidative stress formation during organ retrieval. A fully MHC mismatched (BALB/c to C57BL/6) mouse aortic transplantation model was used. Before organ recovery donor animals received either saline (group I - IV) or 50mg/kg b.w. tetrahydrobiopterin i.m. (group V - VIII). Aortic grafts were analysed at 4 different time points: (a) immediately following recovery (group I + V), (b) following 24 hours cold ischemia time (CIT; group II + VI), (c) following 24 hours CIT and 45 min anastomosis time (group III + VII), (d) following 4 weeks graft reperfusion (group IV + VIII). Aortic tetrahydrobiopterin tissue levels were analysed by HPLC, oxidised proteins were measured by the Oxyblot procedure. Monomere/Dimere eNOS ratios were determined using western blot. Transplant vasculopathy was diagnosed by histopathology and immunohistochemistry.
24 hours CIT and 45 min anastomosis time resulted in strong neointima formation and α-smooth muscle actin expression, which could be prevented by donor pre-treatment with tetrahydrobiopterin ($p = 0.008$ and $p = 0.01$, respectively). Similarly, endothelial expression of P-selectin was significantly decreased in the pre-treatment group ($p=0.01$). Interestingly, grafts from pre-treated donor mice showed significantly less oxidised proteins than non-treated grafts already at the time of organ retrieval and following 24 hours CIT, but not at later time points ($p = 0.003$, $p = 0.02$ and p = ns, respectively). Importantly eNOS monomerisation was prevented at the time of organ retrieval by donor BH4 pretreatment.
These data indicate an important role of oxidative stress already during organ recovery, initiating chronic inflammation, which then leads to chronic rejection. Tetrahydrobiopterin protects from early oxidative stress via prevention of eNOS uncoupling.

428.6

Atg5/Atg7-independent macroautophagy mediates hepatic sinusoidal endothelial cells' response to ischemia-reperfusion injury

Xiaobing Liu, Xin LI, Alice Yuenyuen MA, Changxian LI, Hui Liu, Jiang Liu, Kevin Tak-Pan Ng, Chung-Mau Lo, Kwan Man.
Surgery, Hong Kong University, Hong Kong, Hong Kong.

Introduction: Macroautophagy is a basic biological process that degrades and recycles intracellular organelles and proteins to maintain energy homeostasis under physiological or pathological conditions. In the past few years, the role of macroautophagy has been implicated in a variety of liver disorders yet contradictory results were reported. Since liver is a multicelluar organ of great complexity, this discordance in data highlights the necessity of dissecting the roles of individual cell type rather than only focusing on parenchymal cells such as hepatocytes. In this study, we examined whether macroautophagy was involved in the response of one non-parenchymal cell—hepatic sinusoidal endothelial cells (HSECs) to ischemia-reperfusion injury (IRI) and explored the underlying mechanisms.
Materials and Methods: Simulated cellular model of IRI was established with HSECs. The cells were exposed to ischemic condition for 3 hr and collected at 0hr, 2hr, 6hr, 24 hr respectively after replenishing with normal medium. Cellular morphological changes were analyzed with transmitting electron microscopy (TEM) or immunofluorescent staining. Kinetic expression of autophagic markers and cell damage were assessed by either qRT-PCR, western blot or flow cytometry. Validation study was further conducted in mouse IRI model and human post-transplantation liver biopsy specimens.
Results: During simulated IRI in HSECs in vitro, TNFa and ROS production increased within the first 6 hours after reperfusion with concurrent reorganization of actin stress fibers in cytoplasmic compartments. Examination with TEM revealed these changes were accompanied with enhanced autophagosome formation peaked at 2 hrs.Meanwhile, involvement of endosomes in autophagosome membrane construction and mitochondria clearance was also identified. Interference assay with macroautophagy inducer rapamycin further showed that heightened macroautophagy suppressed ROS production during IRI. Additionally, gene expression assessment demonstrated that the levels of several autophagic molecules such as ULK1, Fip200, Rab9 were elevated in response to IRI, yet the levels of essential conventional autophagic markers including LC3II, Atg5, Atg7 remained largely unchanged.Emergence of autophagosomes in the HESCs of mouse liver after ischemia-reperfusion was consistent with that of the cellular IRI model. In human living donor liver grafts 2 hr post-transplantation, interestingly, increased autophagosome formation together with augmented apoptosis was observed in HESCs. This may indicate that autophagic machinery was activated yet not being able to resolve the imbalance incurred by injury in living donor liver transplantation.

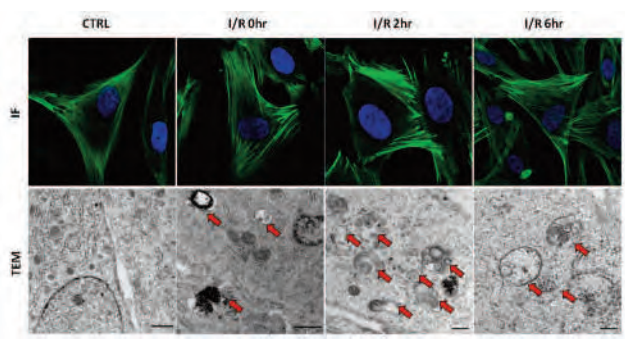


Figure 1. HSECs' response induced by IRI in vitro. Disassembly of stress fibers (upper, phalloidin staining) and increased autophagosome formation (lower) in HSECs after IRI.

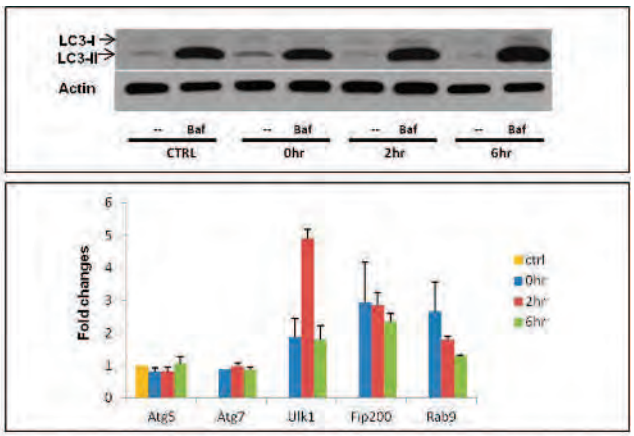


Figure 2. Expression of autophagic markers in HSECs during IRI. The expression levels of conventional autophagic markers including LC3II (upper, without or with bafilomycin interference) , Atg 5 and Atg7 (lower, mRNA) remained unchanged, while levels of ULK1, Fip200, Rab9 increased (lower, mRNA).

Conclusion: Our study showed that activation of macroautophagy in HSECs during IRI shared features of Atg5/Atg7-independent pathway and exerted a protective role. Further investigation on the detailed mechanism may help establish novel therapeutic strategies to improve graft survival.

References:

[1] Peralta, C., M.B. Jimenez-Castro, and J. Gracia-Sancho.Hepatic ischemia and reperfusion injury: Effects on the liver sinusoidal milieu. J Hepatol, 2013. 59(5): p. 1094-106.
[2] DeLeve, L.D., Liver sinusoidal endothelial cells and liver regeneration. J Clin Invest, 2013. 123(5): p. 1861-6.
[3] Nishida, Y., et al., Discovery of Atg5/Atg7-independent alternative macroautophagy. Nature, 2009. 461(7264): p. 654-8.
[4] Tooze, S.A. and T. Yoshimori, The origin of the autophagosomal membrane. Nat Cell Biol, 2010. 12(9): p. 831-5.

428.7

Donor liver transplant rejuvenation: From bench-to-bedside

Ali Zarrinpar[1], Bibo Ke[1], Haofeng Ji[1], Elaine Reed[2], Ronald Busuttil[1], Jerzy Kupiec-Weglinski[1].
[1]Surgery, UCLA, Los Angeles, CA, United States; [2]Pathology and Lab Medicine, UCLA, Los Angeles, CA, United States.

The organ shortage has prompted the use of marginal livers, which are particularly susceptible to ischemia-reperfusion injury (IRI). In the experimental arm of this study, we discovered that Nrf2 deficiency in the donor liver, a master regulator of intracellular redox homeostasis, exacerbated IRI in mouse orthotopic liver transplantation (OLT; BL6 BL6; 20h of cold storage). However, IRI was mitigated if donor livers were pre-conditioned with bone marrow cells overexpressing anti-oxidant HO-1. The success of donor liver rejuvenation was lost after abrogation of SIRT1 activity, a NAD+-dependent type III protein deacetylase with key roles in stress resistance. Thus, SIRT1 signaling dictates rejuvenation outcomes in the mouse liver, in which Nrf2 expression represents a denominator of donor tissue quality. In the clinical arm of the study, we enrolled 30 liver transplant patients in which portal blood and liver perfusate are collected pre-op; during; and at reperfusion. The severity of liver damage is assessed by a new biopsy pathology scoring system, i.e., minimal/mild IRI (score 1-2) vs. moderate/severe IRI (score 3-4), based on incidence of cholestasis, inflammation, steatosis, ballooning/necrosis.

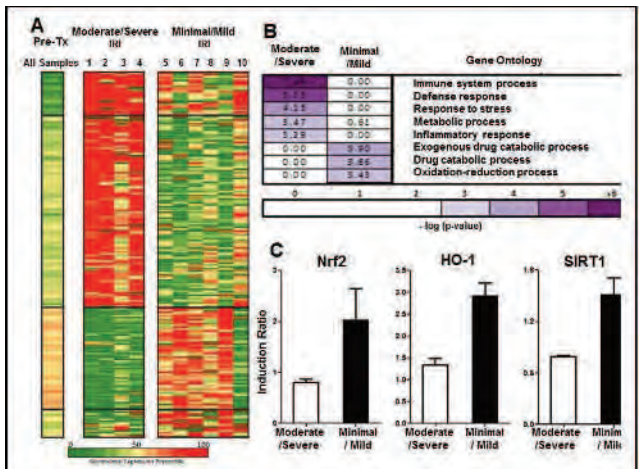


Fig. 1: (A) Human liver transplant transcriptome vs. biopsy-based severity of hepatic IRI; **(B)** Gene Ontology analysis; **(C)** Nrf2, HO-1 and SIRT1 gene induction ratios in human liver transplants (pre-transplant / at reperfusion; mean±SD; n=3-5/gr).

Fig. 1A shows a human liver transplant transcriptome (mRNA pool), as indicated by the heat-map at the bottom. The first column is of the average gene expression in pre-transplant biopsies (n=10). The first sample grouped column (#1-4) represents moderate/severe IRI; the next column (#5-10) identifies minimal/mild IRI. The Gene Ontology analysis (Fig. 1B) revealed the severe IRI cases associate with higher expression of genes with function in the immune system, defense/stress responses, and metabolic processes. Strikingly, and in agreement with our murine OLT functional data, human livers suffering from severe IRI showed ca. 2.5-fold lower induction of Nrf2, HO-1 and SIRT1 genes, compared to IRI-resistant grafts. Conclusion: These findings provide rationale for innovative donor interventions to improve quality and size of the current liver organ supply.

428.8

Dendritic cell phenotype and function modification with targeted porous silicon nanoparticles

Sebastian Stead[1], Steven McInnes[2], Svjetlana Kireta[3], Shilpanjali Jesudason[3,4], Shane Grey[5], Darling Rojas-Canales[3,4], Robert Carroll[3,4], Nico Voelcker[2], Patrick T.Coates[1,3,4].
[1]Medicine, University of Adealide, Adealide, Australia; [2]Biomaterials Engineering & Nanomedicine, University of South Australia, Adelaide, Australia; [3]Transplantation, The Centre for Clinical and Experimental Transplantation , Adelaide, Australia; [4]Renal, Royal Adelaide Hospital, Adelaide, Australia; [5]Immunology, Garvan Institute of Medical Research, Sydney, Australia.

Aims: Dendritic cells (DC) are the most potent antigen-presenting cell and are fundamental in the establishment of transplant tolerance. Targeting DC via the DC-SIGN receptor is a potential target for cell specific therapy. Porous silicon nanoparticles (pSiNP) loaded with immunosuppressant rapamycin (RAPA-pSiNP) provides a unique platform to target and modify DC *in vivo*. The aim of this study was to conjugate monoclonal antibody anti-DC-SIGN on to the surface of RAPA-pSiNP and determine the effects on targeted DC phenotype and stimulatory capacity *in vitro*.
Methods: Fluorescein isothiocyanate (FITC)-labelled pSiNP conjugated to either anti-DC-SIGN or isotype control were cultured with whole blood samples *in vitro* to assess specific targeting to DC. Uptake was determined via flow cytometry and transmission electron microscopy (figure 1). Rapamycin loading of pSiNP was confirmed with ultraviolet visualisation and inferred spectrometry. DC were co-cultured with rapamycin loaded pSiNP (RAPA-pSiNP) for 2 days (± LPS), irradiated and co-cultured with CFSE stained allogeneic T-cells.
Results: Anti-DC-SIGN pSiNP favourably targeted and were phagocytised by myeloid DC in whole blood samples in a time and dose dependent manner. Anti-DC-SIGN conjugation increased DC targeting. Myoloid DC were 42% positive for Anti-DC-SIGN functionalised NP compared to only 10% for Isotype control and 5% for unfunctionalised Np. Compared to mature DC, dendritic cells preconditioning with RAPA-pSiNP resulted in a maturation resistant phenotype and significantly suppress allogeneic $CD3^+$ T-cell proliferation by 28.6 ± 1.9% ($p<0.0001$).
Conclusions: RAPA-pSiNP conjugated to anti-DC-SIGN actively targets and modifies DC function and may serve as a novel therapy to target DC *in vivo*.

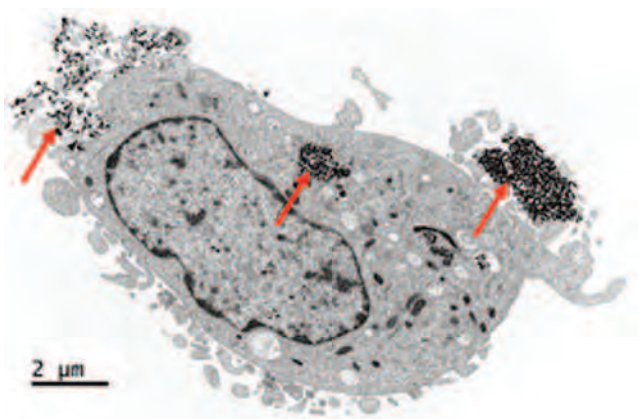


Figure 1: Transmission electron micrograph of human monocyte-derived DC treated with anti-DC-SIGN monoclonal antibody labelled pSiNPs for 2 hours. Arrows show pSiNPs inside the lysosome and on the surface of the DC.

428.9

Follistatin modulates ischemia-reperfusion-induced renal fibrosis in mice

Doreen Fang[1,2], Bo Lu[1], Susan Hayward[4], David de Kretser[3,4], Peter Cowan[1,2], Karen Dwyer[2].
[1]Immunology Research Centre, St. Vincent's Hospital, Melbourne, Australia; [2]Department of Medicine, The University of Melbourne, Melbourne, Australia; [3]Faculty of Medicine, Monash University, Melbourne, Australia; [4]Centre for Reproductive Health, Hudson Institute of Medical Research, Melbourne, Australia.

Introduction: Activins, members of the TGF-β superfamily, are key drivers of fibrosis and hence thought to play a significant role in chronic allograft dysfunction which is the predominant cause of renal graft loss. Ischemia-reperfusion injury (IRI), an inherent process in renal transplantation, is a known trigger for development of chronic allograft dysfunction. Follistatin (FS) is a protein that binds activins and inhibits their activity. Intramuscular injection of a recombinant adeno-associated viral vector carrying a FS gene (rAAV-FS) results in a sustained elevation of circulating FS in mice. This study examined the impact of increased FS levels on renal fibrosis in a mouse model of renal IRI.
Materials and Methods: Mice were injected with rAAV-FS (1.2×10^{10} vector genomes) or empty vector (rAAV-empty) 4 weeks before renal IRI surgery (left renal pedicle was clamped for 20 min) to allow for transduction. Sham mice underwent a midline laparotomy only without IRI surgery. A right nephrectomy was performed 48 h before mice were sacrificed at 4 weeks post IRI. Serum activin A, activin B and FS levels, serum creatinine, and pro-fibrotic markers were assessed.
Results and Discussion: At 4 weeks post IRI, rAAV-empty/IRI mice demonstrated increased mRNA expression of the pro-fibrotic markers TGFβ-1 ($p<0.05$), connective tissue growth factor (CTGF) ($p<0.05$), type I collagen ($p<0.05$), and type IV collagen ($p<0.01$). These were accompanied by impaired kidney function (increased serum creatinine 39.25 ± 2.92 µmol/L, sham = 22.0 ± 1.82 µmol/L, $p<0.01$), and reduced kidney weight (141.40 ± 3.42 mg, sham = 183.30 ± 10.06 mg, $p<0.001$). However, there were no significant differences in serum activin A or B levels between the rAAV-empty/IRI and sham mice. This may be due to fibrosis development being a local process in the kidney hence further analysis to assess activin A and B levels in the kidney is required. Serum FS was significantly increased in rAAV-FS/IRI mice ($p<0.0001$), confirming successful transduction. This increase was associated with significantly lower serum activin A and B ($p<0.001$), suggesting binding of activins to FS and subsequent clearance. Expression of pro-fibrotic markers in rAAV-FS/IRI mice was reduced (TGFβ-1, $p<0.05$; CTGF, $p<0.05$; type I collagen, $p=0.05$; type IV collagen, $p=0.05$), accompanied by improved renal function (reduced serum creatinine 29.29 ± 2.20 µmol/L, $p<0.05$), and preserved kidney weight (176.10 ± 8.11 mg, $p<0.01$). Further analysis to assess the extent of fibrosis on histology is required.
Conclusion: We propose that activin A and B contribute to the development of renal fibrosis induced by IRI. Sustained elevation of FS confers protection against renal fibrosis development, suggesting a potential therapeutic application in renal transplantation to prevent chronic allograft dysfunction and improve graft survival.
Jacquot Research Entry Scholarship; National Health and Medical Research Council of Australia (NHMRC); Oxford Brookes University; Dr Paul Gregorevic, Baker IDI Heart and Diabetes Institute; Ms Sylwia Glowacka, Department of Medicine, The University of Melbourne; Staff at the Immunology Research Centre, St. Vincent's Hospital Melbourne; Staff at the Bioresources Centre, St. Vincent's Hospital Melbourne

428.10

Liver specific activating transcription factor 5 (ATF5) regulates liver ischemia/reperfusion injury

Gong-Qing Shen[2], Miwa Morita[1], John J. Fung[2], Lina Lu[1,2], Shiguang Qian[1,2].
[1]Immunology, Lerner Reaerch Institute, Cleveland Clinic, Cleveland, OH, United States; [2]General Surgery, Transplant Center, Digestive Disease Institute, Cleveland Clinic, Cleveland, OH, United States.

Activating transcription factor 5 (ATF5), a member of the ATF/cAMP response element-binding proteins, caught our attention because it extremely highly expresses in the liver in both mice and humans, but its functions are largely unknown, although several recent studies suggested that ATF5 participates in hepatic stress process. The aim of this study was to investigate the effect of ATF5 on regulating liver ischemia/reperfusion (I/R) injury, for which the ATF5 knockout mice were developed. However, homozygous ($ATF5^{-/-}$) pups had ~85% perinatal mortality (died in 24 hours). It turned out to be difficult to obtain sufficient $ATF5^{-/-}$ mice for studies. We then alternatively generated the conditional ATF5 knockout ($cATF5^{-/-}$) mice using an albumin-Cre-LoxP approach. The $cATF5^{-/-}$ mice were identified via genotyping ($Neo^{+}Cre^{+}Loxp^{+}$), and confirmed by null expression of ATF5 in hepatocytes (qPCR and western). A nonlethal model of segmental (30%) hepatic warm ischemia (60 min) followed by 6h reperfusion (an optimal condition based on blood ALT test) was used for I/R study. Deficiency in ATF5 resulted in marked increases in blood ALT levels (U/L) in a dose dependent manner: 3320±124 (WT), 5875±884 ($ATF5^{+/-}$) and 9096±133 ($ATF5^{-/-}$) ($n=3$, $p<0.05$). Histological I/R scores were well correlated with ALT changes, indicating that ATF5 is a crucial transcription factor in regulating liver I/R injury. Surprisingly, blood ALT levels in $cATF5^{-/-}$ group (3370±116, $n=3$) were comparable to WT ($p>0.05$), markedly lower than $ATF5^{+/-}$ and $ATF5^{-/-}$ groups ($p<0.05$). Conditional knockout status of those $cATF5^{-/-}$ mice were reexamined and confirmed by qPCR and western blotting. The possible explanation is that liver I/R injury is not directly regulated by ATF5 in hepatocytes, rather regulated by non-parenchymal cells (NPC). To determine what type of liver NPC mediate the transcription regulation of liver I/R injury, the expression of ATF5 in hepatocytes, $CD45^{+}$ and $CD45^{-}$ NPC from WT mice was examined by qPCR, showing that NPC expressed minimum amount of ATF5 (only ~1/50 of hepatocytes). Whereas, when ATF5 was knocked out in hepatocytes ($cATF5^{-/-}$), expression of ATF5 in NPC was dramatically increased, implicating an important role of ATF5 in the liver. Our lab is now intensively investigating the functions of ATF5 in NPC in regulating I/R injury and the underling molecular pathways.

428.11

Low expression in preimplantation biopsies of FABP3, a gene that codes for fatty acid binding protein, is a marker for delayed kidney graft function

Tuíla B.Mourão[1], Karina L.Mine[1], Érika F.Campos[1], José O.Medina-Pestana[2,3], Hélio Tedesco-Silva[2], Maria Gerbase-DeLima[1].
[1]Immunogenetics Institute, AFIP - Associação Fundo de Incentivo à Pesquisa, São Paulo , Brazil; [2]Hospital de Rim e Hipertensão , Fundação Oswaldo Ramos, São Paulo, Brazil; [3]Nephrology, Universidade Federal de São Paulo , São Paulo, Brazil.

Introduction: The purpose of this study was to investigate markers for prediction of delayed graft function (DGF) in kidney transplantation (Tx).
Material and Methods: We evaluated, in pre-implantation biopsies (PIB), the expression of five genes selected from a previous large scale gene expression study in PIB performed at our laboratory: ACSL4 (acyl-CoA synthetase long-chain family member 4), CUBN (cubilin), DEFB1 (defensin, beta 1), FABP3 (fatty acid binding protein), and GK (glycerol kinase). Gene expression (real-time TaqMan PCR) was measured in 43 PIBs, corresponding to 22 Tx with DGF and 21 Tx without DGF. Three reference genes (POLR2K, HPRT1, GUSB) were used for gene expression normalization. Lower expression levels of DEFB1 ($p=0.004$), FABP3 ($p=0.001$), and GK ($p=0.016$) were observed in cases with DGF, whereas there were no significant differences concerning ACSL4 or CUBN. In a multivariate analysis, including also clinical variables, only FABP3 expression remained independently associated with DGF. FABP3 expression lower than -1.32 log2 transformed units of relative expression conferred an odds ratio for DGF of 41.1.
Discussion and Conclusions: The lower expression of DEFB1 in Tx with DGF could be reflecting the decrease in the number of tubular cells due to ischemia-induced cell death, since this gene encodes an antimicrobial peptide of urogenital tissues constitutively produced by renal tubular cells (Zhao C et al, 1996; Valore EV et al, 1998); the lower FABP3 expression could be associated with a decreased protection against ischemic damage, as fatty acid-binding proteins have been shown to minimize the tissue damage caused by ischemia by reducing the effects of oxidative stress through the binding of fatty acids and products of lipid peroxidation (Kamijo-Ikemori A et al, 2006; Yamamoto T et al, 2007); an explanation for the association of low GK expression with DGF could be that low levels of glycerol kinase compromise the phosphorylation of glycerol, resulting in increased glycerol levels which were shown to induce acute kidney injury in mice (Homsi E et al, 2006; Rahib L et al, 2007).
Conclusion: This study revealed FABP3 expression in PIB as a very promising marker for DGF, and disclosed new genes possibly involved in the pathogenesis of DGF.

428.12

Using intravoxel incoherent motion MR imaging and blood oxygen level-dependent MR imaging to evaluate rat acute allograft rejection model

Song Zeng[1], Lu Liang[2], Qiang Zhang[1], Yue Xu[1], Wenrui Xue[3], Zijian Zhang[1], Wei Wang[1], Xiaodong Zhang[1], Tao Jiang[2], Xiaopeng Hu[1].
[1]Urology, Chao-Yang Hospital, Beijing, People's Republic of China; [2]Radiology, Chao-Yang Hospital, Beijing, People's Republic of China; [3]Urology, You-An Hospital, Beijing, People's Republic of China.

Objectives: To combine the intravoxel incoherent motion (IVIM) imaging and blood oxygen level-dependent MR imaging (BOLD) for assessment of the renal allograft pathological process and differentia between acute rejection (AR) and acute tubular necrosis (ATN) at the early stage after renal transplantation in rat model.
Materials and Methods: AR was induced by transplantation of Dark Agouti (DA) donor kidneys into Lewis recipients (allogenic ktx) (n=12). Lewis rat that received Lewis kidneys (isogenic ktx) (n=12) served as controls. ATN rat model was caused by ischemia/reperfusion injury (IRI) in Lewis rats (n=12). Six rats of each group were imaged longitudinally at postoperative day (POD) 1, 4 and 7, respectively; two rats were randomly chosen from the rest for histological studies. The apparent diffusion coefficient (ADC), the diffusion coefficient of microcirculation or perfusion (D*), the diffusion coefficient of pure molecular diffusion (D), and perfusion fraction (f) were calculated from IVIM, and R2* was obtained from BOLD. R2* is a measure of the rate of signal loss in a specific region and is related to the amount of deoxyhemoglobin present. Histological changes in the kidney were evaluated according to Banff criteria. Immunofluorescence was used to quantify cell infiltrates and fibrosis in kidney.
Results: After allogenic transplantation, animals developed acute allograft rejection which was confirmed by histological analysis and the severity of AR increased progressively over time. D, f value of the acute rejection group was significantly lower ($p<0.05$) than that in the native kidney and other groups at POD 1, 4, 7 while a progressive reduction in D* was observed at POD 4, 7 in renal cortex. Mean medullary R2* values in AR group were lower than in the isogenic ktx group and ATN group ($p<0.05$) at POD 7. In addition, f and D* were strongly correlated with R2*. **Conclusions:** IVIM imaging coupled with BOLD MR imaging is an effective tool for monitoring progress of AR and differentiating AR from ATN in rat models. Otherwise,it may help elucidate the underlying mechanisms of acute rejection and ATN.
National Natural Science Foundation of China (No.81270837); Beijing Natural Science Foundation (No. 7132107); Beijing Municipal Administration of Hospitals Clinical Medicine Development of Special Funding Support (ZYLX201408); Beijing Municipal health system high level health technology personnel training program (2013-3-015)

428.13

Immune-modifying nanoparticle therapy reduces kidney ischemia reperfusion injury

Huiling Wu[1], Daniel Getts[2], Jin Ma[1], Xiaochen Chen[1], Caryn Van Vreden[2], Nicholas King[2], Steven J.Chadban[1].
[1]Renal Medicine and Kidney Node Laboratory, The Charles Perkins Centre, Royal Prince Alfred Hospital and The University of Sydney, Sydney, Australia; [2]The Discipline of Pathology, Sydney Medical School, The University of Sydney, Sydney, Australia.

Inflammatory monocyte-derived effector cells play a significant role in the pathogenesis of numerous inflammatory diseases including kidney ischemia-reperfusion injury. However, specific therapeutic agents to target these cells are not available. Recently, we reported the capacity of immune modifying nanoparticles (IMPs) to bind to circulating inflammatory monocytes/macrophages (MΦ) via the specific scavenger receptor MARCO, thereby causing inflammatory MΦ removal in spleen with subsequent protection in models of infection, autoimmunity and ischemia injury. Here we investigated the therapeutic potential of IMPs to target MΦ in kidney ischemia-reperfusion injury.
Methods: Kidney ischemia was induced for 22 minutes followed by reperfusion. C57BL/6 mice were randomized to receive 300ul (1.46x1010 particles/ml, 500nm in diameter) of negatively charged IMPs or neutral nanoparticles as a control by tail vein injection 2 hours after ischemia and continued daily for 3 days. Samples were collected at days 1 and 5 after reperfusion.
Results: Mice treated with IMPs were protected against kidney ischemia-reperfusion injury, with lower serum creatinine and less tubular damage versus control mice at days 1 and 5 ($p<0.05$-0.01). Tubulo-interstitial accumulation of neutrophils (day1, $p<0.05$) and CD68+ macrophages (day 5, $p<0.05$) was markedly less in mice treated with negatively charged IMPs versus controls. IFNg expression in ischemia-reperfusion injury kidney at day 1 was significantly reduced while TGFb expression in ischemia-reperfusion injury kidney was increased at day 5 by negatively charged IMPs treatment ($p<0.05$).
Conclusion: IMP infusion affords significant protection from kidney ischemia-reperfusion injury in mice, which may be associated with inhibition of inflammatory MΦ migration and function.

429.1

Knock-in of anti-CD2 and human thrombomodulin transgenes into pig GGTA1 using CRISPR/Cas9

Evelyn Salvaris[1], Nella Fisicaro[1], Ivan Vasiliev[2], Stephen McIlfatrick[2], Mark Nottle[2], Jamie Brady[3], Andrew Lew[3], Peter Cowan[1,4].
[1]Immunology Research Centre, St Vincent's Hospital, Melbourne, Australia; [2]Obstetrics and Gynaecology, University of Adelaide, Adelaide, Australia; [3]Immunology Unit, Walter and Eliza Hall Institute, Melbourne, Australia; [4]Department of Medicine, University of Melbourne, Melbourne, Australia.

Introduction: Successful pig-to-human organ xenotransplantation will require genetic modification of the donor pig to attenuate antibody, coagulation and T cell-mediated immune responses. The CRISPR/Cas9 genome editing system offers a unique opportunity to streamline this process by allowing efficient integration of protective transgenes into detrimental porcine genes such as *GGTA1*, which specifies expression of the major xenoantigen alphaGal. Although a CRISPR-mediated 4.2-kb transgene knock-in pig was reported recently[1], it was generated using the original Cas9, which has a relatively high rate of off-target events, and targeted a 'neutral' locus with minimal relevance for xenotransplantation. In this study, we investigated whether a high fidelity CRISPR system employing the modified nuclease *Fok*I-dCas9 could be used to knock larger transgenes into the pig *GGTA1* locus.
Aim: To separately integrate two 10-kb transgenes, expressing either a chimeric anti-CD2 monoclonal antibody (CD2hb11) or human thrombomodulin (hTBM), into *GGTA1* in the pig genome.
Materials and Methods: Wild type pig fetal fibroblasts (PFF) were transfected with (i) expression constructs for *Fok*I-dCas9 and two guide RNAs targeting exon 9 of *GGTA1*, plus (ii) a neomycin-resistant (Neo^R) H2Kb promoter-driven CD2hb11 or hTBM knock-in construct. Stable Neo^R clones were screened by FACS either directly to detect Gal or hTBM expression, or indirectly (culture supernatant incubated with human T cells) to detect CD2hb11 expression. Suitable clones were used for somatic cell nuclear transfer (SCNT).
Results and Discussion: In an initial small-scale experiment using the CD2hb11 construct, 12 Neo^R clones were isolated, of which one was Gal-negative and secreted the antibody. Using the hTBM construct, 240 Neo^R clones were isolated, of which 11 were Gal-negative and hTBM-positive. PCR and sequencing confirmed correct transgene integration in both CD2hb11 and hTBM clones. Interestingly, molecular analysis of several clones indicated knock-in of the transgene by homology-directed recombination (HDR) at one allele of *GGTA1* and knockout of the second allele by non-homologous end joining (NHEJ). This is consistent with previous studies indicating that NHEJ out-competes HDR at Cas9-mediated double-stranded DNA breaks[1]. Five rounds of SCNT using the CD2hb11 knock-in clone have resulted in 2 pregnancies, with one currently at 11 weeks.
Conclusion: We have used the high fidelity CRISPR/*Fok*I-dCas9 system to efficiently knock two separate transgenes into *GGTA1* in pig cells. The modified cells are being/will be used for SCNT to generate donors for our preclinical pig-to-baboon kidney and islet xenograft models. This is the first report of CRISPR-mediated transgene knock-in in pig cells for xenotransplantation purposes.
References:
[1] Ruan J et al. Highly efficient CRISPR/Cas9-mediated transgene knockin at the H11 locus in pigs. *Sci Rep*. 2015; 5: 14253.

429.2

CD39 plays an important role in the suppressive capacity of xenoantigen stimulated and expanded non-human primate regulatory T cells

Dandan Huang, Ya Wang, Wayne J. Hawthorne, Joanne Hawkes, Heather Burns, Yi Vee Chew, Sussan Davies, Yuanfei Zhao, Min Hu, Shounan Yi, Philip O'Connell.
Centre for Transplant & Renal Research, The Westmead Institute for Medical Research, University of Sydney, Sydney, Australia.

Introduction: We have previously shown that porcine xenoantigen stimulated and expanded baboon $CD4^+CD25^+$ regulatory T cells (Treg) acquired effector Treg phenotype with enhanced suppressive capacity in the xenogeneic response in vivo. However the mechanisms involved are yet to be identified. Since CD39 has been shown to be important for effector Treg to induce allograft tolerance by regulating the metabolism of extracellular ATP, we investigated in this study whether CD39 played a role in the enhanced suppressive function of xenoantigen expanded Treg.
Materials and Methods: Baboon Treg were ex vivo expanded with irradiated porcine peripheral blood mononuclear cells (PBMC) for four weeks. Freshly isolated and expanded Treg were assessed by phenotyping and xenogeneic mixed lymphocyte reaction (MLR) plus and minus CD39 activity inhibitor polyoxometalate-1 (POM-1). In vivo suppressive function of ex vivo expanded Treg was evaluated in NOD-SCID IL-$2Rr^{-/-}$ recipient mice of neonatal porcine islet clusters (NICC) transferred with baboon PBMC alone or plus autologous Treg. NICC xenograft survival, the existence of splenic $CD39^+$ baboon Treg and intragraft gene expression of Treg function markers Foxp3 and CD39 were analysed at predetermined time points after baboon cell transfer.
Results: In addition to upregulated levels of activated markers HLA-DR and ICOS, ex vivo expanded baboon Treg exhibited a $Foxp3^+CD39^+$ phenotype (87.5% of $Foxp3^+CD39^+$ cells) and were more potent in xeno MLR, when compared to freshly isolated Treg (6.5% of $Foxp3^+CD39^+$ cells). The suppressive capacity of expanded Treg in the xenogeneic response was substantially impaired by including POM-1 into the MLR. While baboon PBMC alone reconstituted recipient mice rejected their xenografts completely within 35 days after baboon cell transfer, NICC xenografts remained intact with insulin secretion function in recipients over 100 days after cotransfer with baboon PBMC and expanded Treg. Furthermore, a significant proportion of splenic baboon $CD39^+$ Treg and upregulated levels of intragraft gene expression of baboon Treg function markers Foxp3 and CD39 were detected in the cotransferred recipients.
Discussion: Ex vivo expanded baboon Treg with porcine xenoantigen stimulation acquire effector Treg phenotype with high levels of Foxp3 and CD39 expression, which leads to their enhanced capacity to inhibit the xenogeneic response in vitro. The suppressive capacity of $Foxp3^+CD39^+$ baboon Treg in vitro could be inhibited by CD39 activity inhibitor POM-1. Adoptive transfer of ex vivo expanded baboon Treg protected against porcine islet xenograft rejection in a primatized mouse model and the prolonged xenograft survival was associated with the existence of $CD39^+$ baboon Treg in baboon PBMC and expanded Treg cotransferred recipients.
Conclusion: Ex vivo expanded baboon Treg are capable of suppressing the xenogeneic responses both in vitro and in vivo via at least in part, CD39 involved mechanism(s).

429.3

Long-term islet xenograft survival requires effective early control of xenogeneic IL-17 response but late control of xenogeneic IFN-γ response

Hee Kap Kang[1], Shusen Wang[2], Xiaomin Zhang[3], Amar Singh[5], Lei Zhang[1], Wilma Suarez-Pinzon[5], Stephen D.Miller[4], Bernhard J.Hering[5], Xunrong Luo[1,3,4].
[1]Division of Nephrology and Hypertension, Northwestern University Feinberg School of Medicine, Chicago, IL, United States; [2]Tianjin Union Medical Center, Nancy University Affiliated Hospital, Tianjin, People's Republic of China; [3]Comprehensive Transplant Center, Northwestern University Feinberg School of Medicine, Chicago, IL, United States; [4]Department of Microbiology and Immunology, Northwestern University Feinberg School of Medicine, Chicago, IL, United States; [5]Schulze Diabetes Institute, University of Minnesota, Minneapolis, MN, United States.

Introduction: Tolerance induction for xenogeneic islet transplant remains a challenge. We have previously shown that peri-transplant infusion of donor cells treated with 1-ethyl-3-(3'-dimethylaminopropyl)-carbodiimide (ECDI) is an effective treatment for inducing long-term donor-specific allograft protection. The current study tested the efficacy of this therapy in islet xenogneic transplantation and examined mechanisms of protection against early rejection and causes of late rejection.
Method: ECDI-treated porcine splenocytes (SP) were infused to B6 mice at day -7, +1. Anti-CD20 (250 ug) was given on day -10, +1. Rapamycin (1mg/kg daily) was given from day -1 to +9. 3,000 IEQ of porcine islets were transplanted in B6 mice on day 0.
Results: In this model, we found that donor-stimulated IL-17 production played a prominent role in the early rejection of porcine islet xenograft. The anti-donor IL-17 response was induced by both direct and indirect donor stimulation, and was seen in both CD4 and CD8 compartments. Recipient treatment with donor porcine ECDI-SP significantly reduced the anti-donor IL-17 and anti-donor DTH response, however by itself did not provide significant protection to the transplanted porcine islets (MST = 12 days). Interestingly, rejecting porcine islet xenografts were heavily infiltrated with recipient B220+ B cells, and depletion of B cells with anti-CD20 mAb at the time of donor ECDI-SP infusion further extended islet xenograft survival (MST = 45 days). When we further added a short course of peri-transplant rapamycin (rapa, 1mg/kg from day -8 to day +10) to the dual therapy, the resulting triple therapy (pig ECDI-SP + anti-CD20 + rapa) led to further porcine islet xenograft protection and long-term (> 100 days) graft survival in ~65% recipients. The protection provided by the triple therapy correlated with sustained inhibition of anti-donor IL-17 response. However, recipients with islet xenograft survival >100 days experienced late rejection between 100 – 200 days post transplant. The late rejection coincided with the emergence of robust anti-donor IFN-g response and extensive B cell infiltration in the graft, although a complete absence of anti-xenogeneic antibodies.
Conclusions: Early rejection of discordant islet xenografts is predominantly driven by anti-donor IL-17 response, whereas late rejection in triple therapy treated recipients is driven predominantly by anti-donor IFN-g response and possibly graft-infiltrating B cells exerting a direct graft effect beyond that of their capacity in xenogeneic antibody production.

429.4

Human regulatory macrophages are more potent in suppression of the xeno- than the allo-response

Fei Guo[1], Dandan Huang[1], Wayne J. Hawthorne[1], Min Hu[1], Yuangfei Zhao[1], Joanne Hawkes[1], Heather Burns[1], Benjamin Heng[2], Gilles Guillemin[2], Chia K.Lim[2], Shounan Yi[1], Philip O'Connell[1].
[1]Centre for Transplant and Renal Research, The Westmead Institute for Medical Research, Westmead, Australia; [2]The Australian School of Advanced Medicine, Macquarie University, Sydney, Australia.

Introduction: Regulatory macrophages (Mreg) have been shown to suppress polyclonal T cell proliferation in vitro and prolong allograft survival in a mouse model, and are being currently under evaluation in a clinical trial for living donor renal transplantation (The One Study). However whether they are capable of suppressing the xenogeneic response remain to be investigated. In this study the suppressive capacity of human Mreg in the xenogeneic response was evaluated in a parallel comparison with their suppressive potency in the allogeneic response in vitro.
Materials and Methods: CD14+ monocytes selected from human peripheral blood mononuclear cells (PBMC) were cultured with macrophage colony-stimulating factor (M-CSF) for 7 day with IFN-γ added at day 6 for Mreg induction. Mreg suppressive function was assessed by mixed-lymphocyte reaction (MLR) using irradiated pig and human PBMC as xenogeneic and allogeneic stimulator cells respectively, human PBMC as responder cells and autologous Mreg as suppressor cells. mRNA expression of IDO, IL-10, IFN-γ, iNOS and TGF-β by Mreg after MLR was measured by real-time PCR. Supernatants collected from MLR cultures were used for IDO activity assay by HPLC.
Results: Both proliferating allo and xeno reactive human responder cells were inhibited significantly by Mreg with stronger suppressive potency in the xenogeneic response even at a 1:32 ratio of Mreg: responder cells. While they expressed similar levels of IL-10 and TGF-b, and extremely low level of IFN-γ and iNOS gene expression, Mreg harvested from xeno but not allo MLR cultures demonstrated remarkably high levels of IDO mRNA detected by real-time PCR. Consistently, a stronger IDO activity measured by kynurenine/tryptophan ratio was revealed in the supernatants collected from xeno MLR cultures when compared to that from allo MLR cultures.
Discussion: Human Mreg showed a more potent suppression in the xeno-response compared to that they did in the allo-response. This stronger suppressive characteristic in the xeno-response may be attributedat least in part, to the higher levels of their IDO expression and activity, one of reported main mechanisms used by Mreg to regulate immune responses.
Conclusion: Human Mreg are more suppressive in vitro with IDO-involved mechanisms in the xeno- than in the allo-response, suggesting their potential in immunomodulation of the xenogeneic response.

429.5

SCR-4 is required for CD55-induced suppression on macrophage-mediated cytotoxicity

Akira Maeda[1], Thuy-vy Choi[2], Pei-Chi Lo[1], Rieko Sakai[1], Mayumi Asada[1], Kazuaki Yamanaka[1], Kengo Nakahata[1], Rei Matsuura[1], Hiroshi Eguchi[1], Hiroomi Okuyama[1], Shuji Miyagawa[1].
[1]Department of Surgery, Osaka University Graduate School of Medicine, Suita, Japan; [2]School of Biological Sciences, University of Calfornia, Irvine, CA, United States.

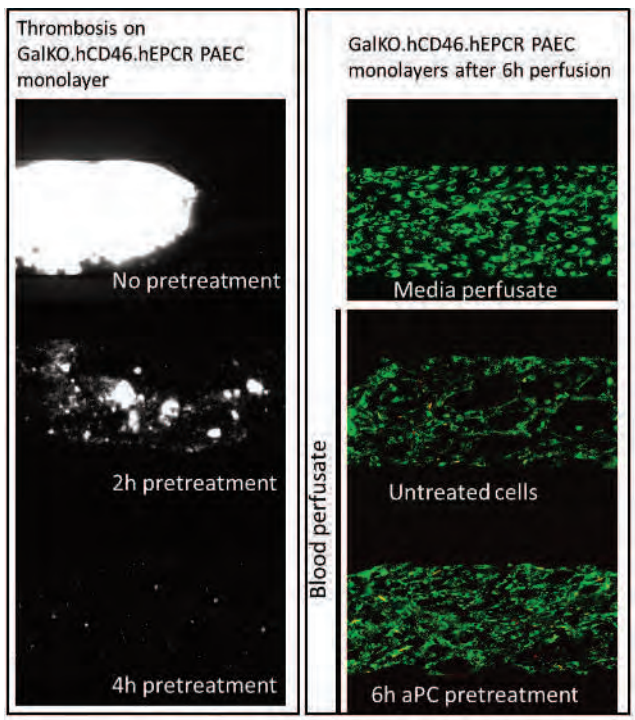
Human CD55 is well-known to bind and inactivate complement C3b. Thus, transgenic expression of human CD55 on porcine cells may be a very attractive strategy to reduce xenogeneic complementary rejection. Transgenic expression of human complement regulatory proteins has been found to reduce the frequency of hyper acute rejection (HAR) in xenotransplantation. After a strategy to handle HAR was developed, the delayed-type rejection, which is mediated by monocytes, macrophages, and NK cells, became a critical problem. We previously reported that human CD55 suppresses not only complement-mediated rejection but also macrophage-mediated xenogeneic cytotoxicity. In the present study, we investigated the mechanisms of human CD55-induced suppression in macrophage-mediated xenogeneic cytotoxicity. The amelioration of macrophage-mediated cytotoxicity by various deletion mutants was studied using WST-8 assay. Naïve swine endothelial cells (SEC) and SEC/hCD55 were co-cultured with human monocyte-like cells, THP-1, which were differentiated to macrophages with 200nM PMA. 24hours after co-culture, cytotoxicity by THP-1 cells was measured by WST-8 assay. While differentiated THP-1 cells induced significant cytotoxicity against SEC (%cytotoxicity: 29.3±17.7%), human CD55 significantly reduced the THP-1 mediated cytotoxicity (%cytotoxicity: 15.2±27.8%, $p<0.05$ vs SEC). Furthermore, delta-short consensus repeats (SCR) 4-CD55 did not show any suppression of macrophage-mediated cytotoxicity (%cytotoxicity: 26.1±18.2%, $p<0.05$ vs SEC/hCD55), indicating that SCR4 is responsible for the inhibitory function of human DAF in macrophage-mediated cytotoxicity. Next, we studied the function of hCD55 in phagocytosis by macrophages. CFSE-labeled SEC or SEC transfectants were co-cultured with LPS-stimulated monocytes for 24hours. Subsequently, cells were harvested and stained with APC conjugated anti-CD14. The percentage of phagocytosis was calculated as [(number of CFSE+CD14+ cells)/ (number of CD14+ cells)] x100. While the phagocytosis of SEC/hCD55 and SEC/hCD55 delta-SCR1 was significantly suppressed compared to that of naïve SEC, hCD55 delta-SCR4 failed to suppress the phagocytosis by macrophages. These findings indicate that CD55 might suppress macrophage-mediated rejection via binding of SCR4 to an inhibitory receptor on macrophages.

429.6

Pretreatment of transgenic porcine endothelium expressing human endothelial protein C receptor with activated protein C attenuates endothelial damage and thrombosis during human blood perfusion

Christopher Laird[1], Beth M.French[1], Donald Harris[1], Xiangfei Chang[1], David Ayares[2], Richard NPierson[1], Agnes M.Azimzadeh[1].
[1]Surgery, University of Maryland School of Medicine, Baltimore, United States; [2]Revivicor, Inc., Blacksburg, United States.

Introduction: In spite of extensive genetic modification, porcine lung xenografts still exhibit acute dysfunction following transplant into non-human primates. This dysfunction is associated with endothelial damage, inflammation, platelet activation, and thrombosis. Activated Protein C (aPC), acting primarily through the human endothelial protein C receptor (hEPCR), mediates anti-thrombotic and anti-inflammatory cytoprotective effects in humans. We hypothesized that treatment of hEPCR expressing porcine endothelial cells (ECs) with recombinant aPC would attenuate endothelial cell injury in a xenotransplant model. **Materials and Methods:** Confluent GalKO.hCD46 and GalKO.hCD46.hEPCR porcine aortic EC (PAEC) monolayers in microfluidic channels were pretreated with 0.02μg/mL recombinant human aPC (raPC) several hours prior to perfusion with heparinized human blood. Blocking anti-EPCR antibody and nonblocking IgG1 antibodies were used to confirm that effects were caused by addition of raPC. Thrombosis, viability, and endothelial surface area coverage were calculated by fluorescent analysis using image processing software.



Results and Discussion: raPC pretreatment of ECs reduced platelet adhesion (45.6±16.2 % surface area (SA) coverage in untreated hEPCR+ cells) to 27.3±10.2% after 2 h exposure to aPC ($p=.085$ vs. untreated), 6.6±1.6% after 4h ($p=.003$), and 5.3±1.8% after 6h ($p=0.0025$). Relative to untreated monolayers (63.45±30 au), platelet aggregation also decreased with increasing duration of aPC Rx from 2h (34.6±16.1 au, $p=0.13$) to 6h (26±8.2 au, $p=0.05$). When perfused with human blood, untreated GalKO.hCD46.hEPCR PAEC monolayers showed cellular damage (7.4±1.8 propidium iodide (PI) positive

cells/hpf, vs. none with cell culture medium) and reduced surface area coverage (75±3.5%, vs. 100% with cell culture medium). Six hour pretreatment with raPC was associated with significantly fewer PI+ cells (3±1.23 cells/hpf, p=0.0003 vs. untreated EC), and significantly higher EC surface coverage (87.3±2.9 %, p<0.0001). Prevention of increased permeability of hEPCR+ PAEC after human thrombin exposure, measured by electrical impedance (xCELLigence), correlated directly with increasing duration of EC pretreatment with aPC. Effects of aPC pretreatment were only seen with pAECs that express hEPCR, and these protective effects disappeared with addition of blocking anti-EPCR antibodies.
Conclusions: aPC pretreatment of hEPCR-expressing PAEC monolayers significantly reduced thrombotic potential and pEC damage after simulated perfusion with human blood. This strategy may be useful to mitigate dysfunction of hEPCR-expressing pig lung xenografts following transplantation by inhibiting clot initiation, thrombus propagation, and endothelial cell injury.

429.7

Reducing the inflammatory response by expressing human thrombomodulin in pigs

Hidetaka Hara[1], Hayato Iwase[1], Yuko Miyagawa[1], Whayoung Lee[1], Cassandra Long[1], Carol Phelps[2], David Ayares[2], David K.C.Cooper[1]. [1]Surgery, Thomas E. Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA, United States; [2]Revivicor, Inc., Blacksburg, VA, United States.

Introduction: Regulation of inflammation is an important target in xenotransplantation. The expression of thrombomodulin (TBM), which reduces coagulation, has been shown to be down-regulated by inflammatory cytokines, such as TNF-α. Although pigs transgenic for human (h)TBM have been developed to reduce coagulation dysfunction, the role of the anti-inflammatory effect of hTBM pigs is unknown. The aim of this study was to investigate in vitro (i) whether hTNF-α stimulated cells increase human platelet aggregation, (ii) whether cells from hTBM pigs could regulate the inflammatory reaction through a coagulation-independent assay.
Materials and Methods: Human aortic endothelial cells (hAECs) and AECs from α1,3-galactosyltransferase gene-knockout pigs transgenic for hCD46 (GTKO/CD46) and from GTKO/CD46/hTBM pigs (p) were used for this study. To investigate the inflammatory response in AECs after hTNF-α stimulation, the expression of NFk-B, TBM, E/P-selection, VCAM-1, and MCP-1 on hAECs and pAECs was measured by either flow cytometry or real-time PCR. Using platelet-aggregometry, human whole blood platelet aggregation was evaluated after co-incubation with hTNF-α stimulated AEC.
Results: After hTNF-α stimulation, there were significant reductions (60% hAECs, 90% GTKO/CD46 pAECs, p<0.05) in the expression of TBM on hAECs and GTKO/CD46 pAECs, and significant increases (50%, p<0.05) of NFk-B signal, E/P-selectin and VCAM-1 expression. In contrast, hTBM expression on the GTKO/CD46/hTBM pAECs was not affected by hTNF-α stimulation whereas the expression of pTBM mRNA was significantly reduced (by 90%, p<0.05). After stimulation, there was significant suppression of the expression of E-selection (30% reduction, p<0.05), VCAM-1(70% reduction, p<0.05), and MCP-1 (50% reduction, p<0.05) on GTKO/CD46/hTBM pAECs compared to GTKO/CD46 pAECs. There was a significant increase in platelet aggregation following incubation with hTNF-α stimulated hAECs or pAECs, which was suppressed by hTNF-α or NFk-B inhibitors.
Conclusions: (i)It is important to suppress the inflammatory reaction to prevent the down-regulation of TBM expression in both the recipient and the pig graft because TBM can have an anti-inflammatory effect (possibly independent of its effect on coagulation). (ii) Stable expression of hTBM in hTBM transgenic pigs may locally regulate the inflammatory reaction, in addition to its anticoagulant effect.

429.8

Growth hormone receptor knockout on porcine endothelial cells: effect on complement and coagulation

Riccardo Sfriso[1], Nikolai Klymiuk[2], Joerg D.Seebach[3], Eckhard Wolf[2], Robert Rieben[1].
[1]Departement Klinische Forschung, Universität Bern, Bern, Switzerland; [2]Institute of Molecular Animal Breeding and Biotechnology, Ludwig-Maximilian University, Munich, Germany; [3]Division of Immunology and Allergology, University Hospital and Medical Faculty, Geneva, Switzerland.

Background: Growth hormone insensitivity (GHI) is a group of inherited disorders characterized by a reduction or absence of signaling triggered by growth hormone (GH). Laron syndrome - a classical example of GHI - is caused by mutations in the growth hormone receptor (GHR) gene and it is characterized by dwarfism. GHR knockout (GHRKO) pigs have already been produced and due to the smaller size of their organs they might be suitable candidates for xenotransplantation. Furthermore, Laron syndrome affected people are protected from vascular diseases such as atherosclerosis and type II diabetes. For that reason this study aims to screen whether the GHRKO phenotype may help to prevent vascular disorders in the context of delayed xenograft rejection.
Methods: An immortalized porcine aortic endothelial cell line lacking αGal expression (PED GalTKO) was further modified by knocking out the GHR gene. The resulting cell line, PED GalTKO/GHRKO, was used in vitro to assess the effect of GHR deletion in a xenotransplantation setting. After transformation, cells were characterized by immunofluorescence (IF) for expression of endothelial cell markers such as CD31, VE-cadherin and von Willebrand Factor (vWF). Cell ELISA was carried out to screen the effect of the GHR deletion on deposition of complement (C3b/c and C4b/c) and immunoglobulins after incubation with pooled normal human serum. In order to evaluate whether GHRKO affects coagulation, a whole blood clotting assay was performed using endothelial cell-coated microcarriers.
Results: PED GalTKO/GHRKO present a clear endothelial phenotype, undistinguishable from the original PED GalTKO. A strong expression of CD31, vWF, VE-cadherin, and heparan sulfate proteoglycans was observed by immunofluorescence. Cell ELISA after incubation with pooled normal human serum showed significantly lower IgM, IgG and C3b/c deposition compared to wild type porcine endothelial cells. Binding of immunoglobulins and complement were similar to the PED GalTKO cell line. Furthermore, PED GalTKO/GHRKO cells grown on microbeads significantly delayed the clotting time of freshly drawn, whole, non anticoagulated human blood as compared to wild type porcine endothelial cells (53 ± 21 min vs. 19.8 ± 7 min). These data are similar to the what was observed for the original PED GalTKO cell line (50 ± 31.8 min).
Conclusions: Our data show that GHR deficiency on porcine endothelial cells with GalTKO background preserved the endothelial phenotype. No differences in immunoglobulin binding, complement deposition or anticoagulant properties were observed as compared with the original PED GalTKO cell line. The used in vitro tests did not reveal a significant benefit of the growth hormone receptor knockout with respect to the anticoagulant properties of the endothelial cells and in vivo experiments may be needed to provide a better understanding of the long term effects of this phenotype on coagulation.

429.9

Surface modification of porcine aortic endothelial cells with Corline Heparin Conjugate (CHC) protects against xenogeneic thrombosis and inflammation

Anjan Bongoni[1], Evelyn Salvaris[1], Bo Lu[1], Bo Nilsson[2], Sofia Nordling[2], Peetra U.Magnusson[2], Peter J.Cowan[1,3].
[1]Immunology Research Centre, St. Vincent's Hospital Melbourne, Melbourne, Australia; [2]Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden; [3]Department of Medicine, University of Melbourne, Melbourne, Australia.

Background: Genetic modification of the donor pig to remove xenoantigens and to correct molecular incompatibilities has demonstrably improved the survival of porcine xenografts in preclinical models. However, it does not fully address the problem of transplant-associated endothelial activation and shedding of the glycocalyx, a layer of heparan sulfate proteoglycans on the luminal surface of the endothelium. This process contributes to the development of a pro-inflammatory and pro-coagulant environment at the vascular surface of xenograft endothelium. Corline Heparin Conjugate (CHC), a macromolecular branched conjugate comprising multiple heparin molecules, can be used to coat living cells with a glycocalyx-like layer without compromising their biological function. In this project, we investigated in an in vitro model whether CHC coating of pig endothelial cells protects them from activation and injury mediated by human blood.
Aim: To demonstrate the feasibility of using CHC to protect vascular endothelial cells in an in vitro pig-to-human xenotransplantation setting.
Methods: Binding of CHC to wild-type porcine aortic endothelial cells (PAEC) was measured using FITC-labeled CHC. To test the effect of CHC on complement activation, C3b/c deposition on PAEC was measured after incubation with normal human serum (NHS) in the presence of CHC. To test the effect of CHC on hemocompatibility, PAEC were grown on microcarrier beads, coated with CHC under starvation conditions, and incubated with freshly drawn non-anticoagulated whole human blood in a model that closely mimics the in vivo small vessel endothelial surface-to-blood volume ratio. Uncoated PAEC were used as controls.
Results: Confocal microscopy demonstrated a uniform coating of CHC on the surface of PAEC. Treatment of PAEC with NHS (1:10) in the presence of CHC (50-200µg/ml) resulted in a significant reduction of C3b/c deposition, in a dose-dependent manner ($p=0.05$ to 0.001). In the whole blood coagulation assay, surface coating of CHC significantly improved the human blood compatibility of PAEC, as shown by increased clotting time (CHC 50.8±8.1 min vs. untreated 29.9±6.4 min, $p<0.001$) and reduced CD41-positive platelet deposition (CHC 58.5±4.0% vs. untreated 100%, $p<0.01$). EDTA-plasma was collected from the coagulation assays at 20 min (i.e. before the onset of clotting) and analyzed by ELISA. Soluble markers of complement activation (sC5b-9) ($p<0.0001$), coagulation activation (thrombin-antithrombin complex and D-dimer) (both $p<0.0001$) and inhibition of fibrinolysis (tPA/PAI-1) ($p<0.0001$) were all significantly lower with CHC-PAEC than with untreated PAEC.
Conclusion: Our results indicate that surface immobilization of the multi-arm heparin conjugate CHC protects PAEC from the pro-coagulant and pro-inflammatory environment induced by xenotransplantation. This strategy provides a possible therapeutic option to attenuate thromboinflammation in xenotransplantation.

429.10

Inhibition of xenogeneic platelet activation and aggregation by surface conjugation of apyrase to porcine aortic endothelial cells

Anjan Bongoni[1], Evelyn Salvaris[1], Yuji Teramura[2], Sana Asif[3], Bo Nilsson[3], Peter J.Cowan[1,4].
[1]Immunology Research Centre, St. Vincent's Hospital Melbourne, Melbourne, Australia; [2]Department of Bioengineering, University of Tokyo, Tokyo, Japan; [3]Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden; [4]Department of Medicine, University of Melbourne, Melbourne, Australia.

Background: Activation and aggregation of recipient platelets is a key feature of the dysregulated coagulation that is frequently observed in pig-to-primate xenotransplantation. Platelet activation is associated with complement and contact system activation, which in turn leads to inflammation. The platelet agonist ADP is an obvious target for platelet inhibition. Apyrase is a soluble enzyme that, like CD39, hydrolyzes extracellular ATP and ADP. In this study, we hypothesized that coating porcine aortic endothelial cells (PAEC) with apyrase will inhibit xenogeneic platelet activation.
Aim: To investigate the impact of surface-conjugated apyrase on PAEC in an in vitro model of xenograft-induced thrombosis and inflammation.
Methods: PAEC from wild-type pigs were cultured to confluence on Biosilon microcarrier beads. Maleimide-PEG-lipid was used to immobilize thiolated apyrase (25µg/ml) on the PAEC surface. Apyrase activity was measured using an ATPDase assay. Apyrase-PAEC on beads were tested in an in vitro coagulation assay by incubation with non-anticoagulated whole human blood in a ratio that mimics that in small vessels in vivo. Uncoated and maleimide-PEG-lipid coated PAEC were used as controls. EDTA-plasma samples collected from the assay were used to measure complement, coagulation and fibrinolytic cascade activation markers by ELISA. Platelet-white blood cell aggregation was analyzed by FACS.
Results: Immobilization of apyrase on PAEC was confirmed by confocal microscopy. Beads coated with apyrase-PAEC degraded exogenous ATP in a dose-dependent manner (ATPDase activity: 9.9±1.1 nmol/20min at 10µg/ml apyrase; 17.7±0.6 at 25µg/ml). Immobilized apyrase retained approximately 81% of the activity of the native form. Apyrase-PAEC significantly prolonged clotting of human blood (65.2±14.4 min vs. untreated PAEC 29.9±6.4 min, $p<0.001$, n=5). Deposition of CD41-positive platelets was significantly lower on apyrase-PAEC (27.0±3.6% vs. untreated 100%, $p<0.0001$). Aggregation of CD42a-positive platelets with CD14-positive monocytes was significantly reduced by apyrase-PAEC (36.4±2.7% vs. untreated 58.1±5.9%, $p<0.05$). In addition, the concentration of markers of complement activation (sC5b-9) ($p=0.015$), coagulation activation (thrombin-antithrombin complex and D-dimer) (both $p<0.0001$) and inhibition of fibrinolysis (tPA/PAI-1) ($p<0.0001$) was lower with apyrase-PAEC than untreated PAEC.
Conclusion: Apyrase immobilization on PAEC inhibits xenogeneic platelet activation and aggregation and suppresses coagulation in a whole blood assay. Therefore, this approach, like transgenic expression of CD39, is a potential therapy to inhibit xenotransplantation-associated thrombotic inflammation. Furthermore, this assay can be used to evaluate the potential of novel therapeutic substances not only in xenotransplantation but also in allotransplantation and ischemia/reperfusion injury.

429.11

Foxp3+ regulatory T cells are essential for the induction and maintenance of tolerance to neonatal porcine islet cell cluster xenografts induced by co-stimulatory blockade

Yi Wen Qian[1], Wayne Hawthorne[1], Jingjing Wu[1], David Liuwantara[1], Heather Burns[1], Yi Vee Chew[1], Stephen Alexander[2], Philip O'Connell[1], Min Hu[1].
[1]Centre for Transplant and Renal Research (CTRR), Westmead Millennium Institute, Sydney, Australia; [2]Centre for Kidney Research, Children's Hospital at Westmead, Sydney, Australia.

Introduction: CTLA-4 Fc and MR-1 (anti-CD154) antibodies inhibit B7-CD28 pathway and CD40-CD154 pathway respectively, and are able to induce tolerance towards porcine islet cell cluster (NICC) xenografts in mice. We previously reported that depletion of Foxp3+ Tregs break tolerance in a mouse kidney allotransplantation tolerant model, however the role of Foxp3+ regulatory T cells (Tregs) in NICC xenografts tolerance is not clear.
Aim: To investigate the role of Tregs in a NICC xenotransplantation mouse model, and whether Foxp3+ Treg are essential for the induction and maintenance of tolerance to NICC xenografts.
Methods: C57BL/6-DEREG mice were transplanted with 2000 to 4000 NICC under the renal capsule. DEREG mice have diphtheria toxin receptor-eGFP attached to their Foxp3 gene, therefore Foxp3+ Tregs can be selectively depleted with diphtheria toxin (DT). Recipient mice were treated IP with CTLA-4 Fc (500µg) at day 0, and MR-1 (500µg) at day 0, 2, 4, and 6. Tregs were depleted by DT (12ng/gram/mouse) during the induction phase (3 days pre-transplantation), or maintenance phase (80 days post-transplantation) for 21 days. Treg population in the lymphoid tissues were quantified with FACS (CD3+CD4+CD25+FoxP3+) at day 8-12, 20, 60-80 and 100. Rejection was determined by histology and insulin/glucagon staining, and function was determined by porcine c-peptide concentration. The expression of FoxP3, IFNγ, TGFβ, CTLA-4, granzyme A, Blimp-1, IL-10 and CD39 was assessed for graft and lymphoid tissues on day 8 and day 100 using RT-PCR.
Results: Without DT, tolerance to NICC xenografts was achieved in DEREG mice with histology at 100 days, showing xenografts with positive insulin staining and elevated porcine C-peptide (mean=480 pmol/L, n=6). Large numbers of Foxp3+ Treg were observed in NICC xenografts in tolerant mice on day 8, fewer on day 20, then at a greater number on day 100. DT treated mice during both the induction and maintenance phases have massive cell infiltration, no insulin or glucagon staining were present in their NICC grafts, and no porcine C-peptide in their serum. Treg proportion of CD4+ T cells were higher in the draining lymph nodes (DLN) in the tolerant mice when compared to their peripheral lymph nodes on day 60-80. FoxP3 was expressed significantly higher in the DLN of tolerant mice on both day 8 ($P=0.0010$) and 100 ($P=0.0128$) when compared to the control group. Tolerant grafts also expressed higher CTLA-4 ($P=0.0022$) on day 8, and IL-10 ($P=0.0042$) and CD39 ($P=0.0047$) on day 100. These data suggest Foxp3 Tregs are expanded within the xenografts and DLN, and xenografts tolerance induced by co-stimulation is dependent upon Foxp3+ Tregs.
Conclusion: Foxp3+ Tregs are essential for both the induction and maintenance of tolerance to islet xenografts after co-stimulation blockade by CTLA-4 Fc and anti-CD154 antibodies.

429.12

Comparison of post transplant inflammatory response from 6-gene vs. 3-gene donor pigs in heterotopic cardiac xenotransplantation

Avneesh Singh[1], Joshua Chan[1], Philip Corcoran[1], Marvin Thomas[2], Billeta Lewis[2], David Ayares[3], Keith Horvath[1], Muhammad Mohiuddin[1].
[1]Cardiothoracic Surgery Research Program, NHLBI /NIH, Bethesda, MD, United States; [2]Division of Veterinary Resources, NIH, Bethesda, MD, United States; [3]Revivicor, Inc., Blacksburg, VA, United States.

Introduction: We have recently reported xenograft survival of more than 2.5 years in a pig to baboon heterotopic cardiac xenograft model. Some of the animals exhibited a local and systemic inflammatory response to the xenograft, which aided in graft rejection. We report the benefits of using donor organs expressing 5 human transgenes along with one gene knock out and the ability of multi-transgenics to improve graft survival and suppress the inflammatory response.
Methods: Six young (8-12Kg) specific pathogen free baboons received heterotopic cardiac xenografts from either Group 1 (GTKO.CD46.EPCR.DAF.TFPI.CD47) (n=2) or (GTKO.CD46.EPCR.DAF.CD39.TBM) (n=1) and Group 2 (GTKO.CD46.TBM) (n=3) pigs. Recipient baboons were treated with a modified immunosuppression protocol developed at the NHLBI. Graft survival was monitored with continuous telemetry, periodic echocardiography and manual palpation. Cytokines (e.g. IFNg, IL-6, TNFa, IL-4, IL-10 and IL-13) in baboon recipient serum was measured weekly or biweekly during three months after cardiac xenotransplantation by Affymetrix Luminex assay. Statistical t test and F test were used for determining the differences between the groups.
Results: Heterotopic cardiac xenografts from both 6-gene and 3-gene donor pigs on average survived more than 6 months (ranging from 46 to 945 days) in baboons. One of the grafts in Group 1 (6-gene pigs) ceased contracting on day 46 and was explanted. The remaining grafts are still surviving for 142 and 207 days. No signs of any inflammation or adhesions were observed during laparotomy for graft evaluation and biopsy procedures. However, while the Group 2 xenografts (3-gene pigs) survived longer (ranging from 156 to 945 days), some adhesions and inflammation were observed after transplantation. Florescent intensity (FI) measured by Luminex for inflammatory cytokines (e.g. IFNg (5.2±0.2 vs. 6.6±0.3; $p<0.01$), TNFa (14.4±0.3 vs. 15.4±0.3; $p<0.05$)) and IL-6 (22.8±8.8 vs. 55.9±25.5; $p=0.09$) were significantly inhibited in recipient serum receiving xenografts from 6-gene vs. 3-gene donor pigs. Anti-inflammatory (IL-4, IL-10 and IL-13) cytokines were also slightly inhibited but the difference was not significant.
Conclusion: The expression of multiple human genes prevented xenograft rejection for an extended period of time and also helped in avoiding inflammatory reactions. While further evaluation is required, the presence of additional human transgenes on the xenografts might have reduced immunogenicity that may have contributed to avoiding inflammation.

429.13

Human CD200 suppresses the M0 macrophage-mediated cytotoxicity in xenotransplantation

Akira Maeda[1], Pei-Chi Lo[1], Thuy-vy Choi[2], Rieko Sakai[1], Mayumi Asada[1], Kazuaki Yamanaka[1], Kengo Nakahata[1], Rei Matsuura[1], Hiroshi Eguchi[1], Hiroomi Okuyama[1], Shuji Miyagawa[1].
[1]Department of Surgery, Osaka University Graduate School of Medicine, Suita, Japan; [2]School of Biological Sciences, University of Calfornia, Irvine, CA, United States.

A number of approaches, such as generation of alpha-1,3-Galactosyltransferase knocked-out pigs and CD55 transgenic pigs, have been investigated to inhibit pig to human xenogeneic rejection. Our mission in the future is to develop the strategies to hurdle not only hyper acute rejection (HAR) but also delayed type rejection (DXR). Although macrophages have been well known to play a critical role in DXR, monocyte/macrophage-mediated xenogeneic rejection has not been well studied. In this study, we evaluated the effect of CD200 in xenogeneic rejection by macrophages. CD200 is a member of the Ig supergene family (IgSF) and relatively ubiquitously expressed. The suppressive function of CD200 was suggested to depend upon engagement with a receptor (CD200R). CD200 is also an IgSF protein, with an inhibitory intracellular NPXY signaling motif. CD200R is mainly detected on cells of the myeloid lineage. Therefore, we checked the expression of CD200R on monocyte-like cells, THP-1, and peripheral blood monocyte-derived macrophages, which were generated by M-CSF (M0 macrophage), by flow cytometry. While the expression level of CD200R on THP-1 was faint, M0 macrophages expressed a significant amount of CD200R. The effect of human CD200 in macrophage-mediated cytotoxicity was studied using WST-8 assay. Naïve swine endothelial cells (SEC) and SEC/CD200 were co-cultured with THP-1 or M0 macrophages. 24hours after co-culture, cytotoxicity was measured by WST-8 assay. While CD200 failed to suppress a significant amount of cytotoxicity against SEC by THP-1 (%cytotoxicity: SEC 46.3%±7.1%, SEC/CD200 43.8±7.1%, n=3), M0 macrophage mediated cytotoxicity was significantly suppressed by human CD200 (%cytotoxicity: SEC 24.9±11.2%, SEC/CD200 18.4±13.9%, $p<0.05$, n=9). Furthermore, to test the phagocytosis of SEC by M0 macrophages, CFSE-labeled SEC and SEC/CD200 were co-cultured with M0 macrophages for 24hours. Subsequently, cells were stained with APC-conjugated anti-CD14 and analysis by flow cytometry revealed that human CD200 suppresses M0 macrophage-mediated cytotoxicity (%phagocytosis; SEC 70.7%, SEC/CD200 60.8%, n=1). Our findings indicate that human CD200 suppresses the xenogeneic rejection by CD200R+ macrophages and the generation of hCD200 transgenic pigs are very attractive for use in xenografts to prevent delayed type rejection.

445.3

A clinical trial of cell therapy-based tolerance induction in living donor liver transplantation: Long-term follow-up results

Kenichiro Yamashita[1], Masaaki Zaitsu[2], Ryoichi Goto[2], Akihisa Nagatsu[2], Tetsu Oura[2], Masaaki Watanabe[2], Takeshi Aoyagi[2], Tomomi Suzuki[2], Tsuyoshi Shimamura[3], Toshiya Kamiyama[2], Norihiro Sato[4], Junichi Sugita[5], Kanako Hatanaka[6], Hisashi Bashuda[7], Ko Okumura[7], Satoru Todo[8].
[1]Transplant Surgery, Hokkaido University, Sapporo, Japan; [2]Gastroenterological Surgery 1, Hokkaido University, Sapporo, Japan; [3]Organ Transplantation, Hokkaido University Hospital, Sapporo, Japan; [4]Clinical Research & Medical Innovation Center, Hokkaido University Hospital, Sapporo, Japan; [5]Hematology, Hokkaido University Hospital, Sapporo, Japan; [6]Pathology, Hokkaido University Hospital, Sapporo, Japan; [7]Immunology, Juntendo University, Tokyo, Japan; [8]Research Institute, St. Mary's Hospital, Kurume, Japan.

Background and Aim: Induction of tolerance is an ultimate goal in organ transplantation. We conducted a clinical study aiming to induce operational tolerance in living donor liver transplantation (LDLT) patients by applying the cell-therapy using donor antigen-pulsed regulatory T cells (Tregs). Herein, we report long-term follow-up results of the ongoing clinical trial.
Patients and Methods: Ten adult liver transplant recipients (HBV; 1, HCV; 1, Alcoholic: 2, NASH; 3, PBC; 2, PSC; 1) received a left-lobe liver allograft from living donors. After LDLT, immunosuppression (IS) was initiated with steroid, MMF and calcineurin inhibitor (CNI). Cyclophosphamide was given on day 4. Donor antigen-pulsed Tregs were generated *ex-vivo* by co-culturing recipient-PBMCs (+splenocytes) and irradiated donor-PBMCs under αCD80+αCD86 mAbs for 2-weeks, and were infused on day 13. Steroid and MMF was stopped within a month. CNI was space-weaned every 3 months starting from 6 months post-LT, and was finally stopped.
Results: After ex vivo cell culture, the $CD4^+CD25^+Foxp3^+$ T cells increased from 6.7±3.8% to 28.1±17.7%. The mean infused $CD4^+CD25^+Foxp3^+$ T cell number was $2.3x10^8$ cells/body. No adverse event was noted related to the cell infusion. The mean follow-up time after LDLT was 53 (42-61) months. IS was successfully withdrawn in 7 (70%) LT recipients, whereas a mild ACR occurred in the other 3, who are maintained with a minimally required IS. All IS-free patients are maintaining a good liver graft function without an episode of rejection for 34.6 (24-41) months. Four cases are IS-free for over 3 years. Among seven IS-free patients, 3 developed DSA, while latest graft histology did not show any sign of rejection, fibrosis progression or C4d deposition. Immunological assays revealed a hyporesponsiveness state against the donor-antigens.
Conclusion: The cell therapy using the *ex-vivo* generated donor antigen-pulsed Tregs allows early IS withdrawal, and promotes operational tolerance in LDLT.

445.4

Donor-specific anti-HLA antibody mediated rejection in liver transplant recipients

Tomasz Dziodzio[1], Nils Lachmann[2], Sinem Ünlü[1,2], Sascha Weiss[1], Sascha Chopra[1], Safak Gül[1], Undine Gerlach[1], Christian Denecke[1], Matthias Biebl[1], Constanze Schönemann[2], Johann Pratschke[1], Robert Öllinger[1].
[1]Department of General, Visceral and Transplantation Surgery, Charité-Universitätsmedizin Berlin, Berlin, Germany; [2]Center of Tumor Medicine, HLA-Laboratory Campus Virchow-Klinikum, Charité-Universitätsmedizin Berlin, Berlin, Germany.

Introduction: The clinical role of donor-specific anti-HLA antibodies (DSAs) in liver transplantation (LT) is not clearly established. We investigated the impact of antibody-mediated rejection (AMR) on the clinical course, morbidity and mortality of LT recipients.
Materials and Methods: Between 2008 and 2015 649 LTs were performed at our institute. DSAs were determined by the Luminex® assay for all patients. Patients with more than two positive DSA results were included in the study. The mean fluorescence intensity (MFI) was used to quantify DSAs and was correlated with the patient courses and the success of AMR therapy.
Results: Thirty-three patients with class-I and/or class-II DSAs were identified. Of those, 19 patients (57.6%) showed clinical signs of AMR. In 17 patients (51.5%) an additional cellular rejection was observed. Sixteen patients (48.5%) were treated with steroids, 11 (33.3%) with plasmapheresis and immunoglobulins and 6 patients (18.2%) received anti-thymocyte globuline. Six patients (18.2%) showed a decay of rejection (MFI value reduction of >84%) and improvement of clinical parameters. Six patients needed a re-transplantation. Overall mortality was 21.2% with sepsis being the leading cause of death (5 patients). Fourteen patients (12.1%) with persistent elevated MFI values showed no clinical signs of AMR .
Conclusion: AMR after LT is relatively rare, however DSAs can cause fulminant rejections with fatal courses. Current therapy concepts were only successful in a quarter of patients. Interestingly, sepsis was the leading cause of death. Further, nearly half of the patients with positive DSAs showed no signs of AMR. Thus, future investigations need to focus on the balancing of AMR treatment and its side-effects, as well as the causes of heterogeneous susceptibility of LT recipients to AMR.

450.1

Seven year follow-up of a phase 2 clinical trial to induce tolerance in living donor renal transplant recipients

Joseph R. Leventhal[1], John Galvin[1], Dianne Stare[1], Lorenzo Gallon[1], Joshua Miller [1], James M. Mathew[1], Michael M. Abecassis[1], Suzanne T. Ildstad[2].
[1]Northwestern University, Chicago, IL, United States; [2]Institute for Cellular Therapeutics, University of Louisville, Louisville, KY, United States.

Introduction: 31 subjects have been transplanted in a phase 2 protocol (IDE 13947) to induce tolerance in recipients of living donor renal allografts (KTx).
Materials, Methods, Results and Discussion: The protocol is based upon tolerogenic CD8+/TCR-facilitating cells (FCRx) and 200 cGy TBI-based nonmyeloablative conditioning, with fludarabine (30mg/kg/dose, days -5,-4,-3), cyclophosphamide (50mg/kg/dose, day-3 and+3), 200 cGy TBI (day-1) followed by KTx (day0). A G-CSF mobilized peripheral blood mononuclear cell product was apheresed from the donor >2 weeks pre-KTx, processed to remove graft-versus-host disease (GVHD)-producing cells yet retain CD34 + cells and FC, and cryopreserved until administration day+1 post-KTx. Subjects ranged in age from 18-65 years and were from 6/6 HLA matched related to 0/6 matched unrelated. 12 subjects had unrelated and 19 had related donors. 30 of 31 subjects exhibited donor chimerism at one month post KTx. The one subject without chimerism (NW9) was highly sensitized (PRA>50%). MMF and tacrolimus based immunosuppression (IS) was weaned and discontinued at 1 year if chimerism, normal renal function and normal KTx biopsy were noted. There was a learning curve in the early phases of this study. Subjects NW1 and NW4 received a suboptimal cell dose and were only transiently chimeric (< 6 months). Another subject (NW11) with a high PRA (33%, maximum historic 64%) developed transient chimerism. Transiently chimeric subjects resumed endogenous hematopoiesis and are on low-dose IS with stable renal fcn. 1 subject (NW27) exhibited grade 1-2 GVHD that was successfully treated with corticosteroids. A second subject (NW33) developed concomitant Grade 2-3 GVHD and CMV disease and is undergoing treatment. There have been 2 KTx losses related to infections post-Tx. 1 and 5yr Pt survival is 100%. High levels of chimerism allowing for full IS withdrawal developed in 19 subjects (time off IS ranging from 3 – 65 months). 16 subjects had "full" (> 98% donor) chimerism, and three subjects mixed chimerism. All stable chimeric subjects retained chimerism after removal of IS and remain rejection-free, as demonstrated by protocol biopsy, while 3 of 5 who were transiently chimeric had subclinical rejection. None of the durably chimeric subjects has developed recurrence of autoimmune disease while two of the transiently chimeric subjects have.
Conclusion: In summary, high levels of chimerism and tolerance with low incidence of GVHD have been achieved in mismatched related/unrelated recipients of combined FCRx and living donor KTx.

450.2

Relationship between mixed chimerism and clinical tolerance after combined kidney and hematopoietic cell transplantation using total lymphoid irradiation and antithymocyte globulin conditioning

Stephan Busque, John D. Scandling, Judith Shizuru, Robert Lowsky, Asha Shori, Kent Jensen, Edgar Engleman, Samuel Strober.
Stanford University School of Medicine, Stanford, CA, United States.

Combined living donor kidney and hematopoietic cell transplants were given to 22 fully HLA matched recipients who received posttransplant conditioning with 10 doses of total lymphoid irradiation (each dose 120 cGy) and 5 doses of rabbit antithymocyte globulin, and an infusion of $1x10^6$ T cells/kg and $4\text{-}17x10^6$ CD34+ cells/kg from G-CSF "mobilized" donor apheresis products. Eighteen of 22 recipients developed mixed chimerism that persisted for least 12 months, and these patients were withdrawn from all maintenance immunosuppressive drug therapy. Mycophenolate mofetil was discontinued after 1 month and cyclosporine was discontinued by 6 to 14 months. Nine of the 18 patients continued with mixed chimerism after drug withdrawal and were without evidence of rejection at the last observation point during followup of up to 7 years off drugs. The remaining 9 patients withdrawn from immunosuppressive drugs had a loss of mixed chimerism during the second year posttransplant. Eight of these 9 had no subsequent evidence of kidney graft rejection, up to 7 years off drugs. One of these 9 lost tolerance and developed a Banff1A rejection episode about 4 years after the withdrawal of cyclosporine. Rejection rapidly resolved with treatment. Thus, after immunosuppressive drug withdrawal, tolerance has been maintained in all (9 of 9) HLA matched patients with persistent donor mixed chimerism and in the majority of recipients (8 of 9) who had a late (after 1 year) loss of donor hematopoietic cells. In further study, 13 HLA haplotype-matched patients were given combined transplants. Mixed chimerism persisted for at least 10 to 21 months in 5 patients given the highest doses (up to $50x10^6$/kg) of T cells and CD34+cells/kg (up to $22x10^6$cells/kg). Mixed chimerism was lost rapidly in the first 2 of these mismatched patients when mycophenolate mofetil and tacrolimus withdrawal was completed at one year posttransplant. In contrast to the fully matched patients, the latter HLA mismatched patients developed rejection episodes shortly after losing chimerism. Rejection rapidly resolved with treatment. In conclusion, the loss of chimerism after immunosuppressive drug withdrawal may be a biomarker of rejection in the HLA mismatched patients and a biomarker of increased risk of losing tolerance after several years in fully matched patients.

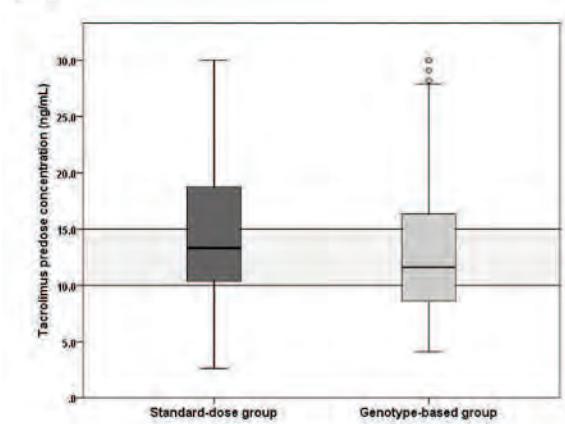
450.3

A randomized-controlled trial comparing the efficacy of CYP3A5 genotype-based with bodyweight-based tacrolimus dosing after living donor kidney transplantation

Nauras Shuker[1,2], Rachida Bouamar[2], Ron H. Nvan Schaik [3], Marian C. Clahsen -van Groningen [4], Jeffrey Damman[5], Carla C. Baan[1], Jacqueline van de Wetering[1], Ajda T. Rowshani[1], Willem Weimar[1], Teun van Gelder[1,2], Dennis A. Hesselink[1].
[1]Internal Medicine , Erasmus MC, Rotterdam, Netherlands; [2]Hospital Pharmacy , Erasmus MC, Rotterdam, Netherlands; [3]Clinical Chemistry, Erasmus MC, Rotterdam, Netherlands; [4]Pathology , Erasmus MC, Rotterdam , Netherlands; [5]Pathology , Academic Medical Centre, Amsterdam, Amsterdam , Netherlands.

Background: The expression of the metabolizing enzyme cytochrome P450 (CYP) 3A5 is determined by polymorphisms in the CYP3A5 gene[1]. Renal transplant recipients (RTRs) carrying the CYP3A5*1 allele require a higher dose of the immunosuppressant tacrolimus to achieve therapeutic exposure[2].
Methods: We did a randomized-controlled, parallel-group, single-center trial with the primary aim to study whether a tacrolimus starting dose based on a patient's CYP3A5 genotype leads to a higher proportion of patients achieving the target tacrolimus predose concentration (C_0) on day 3 after kidney transplantation compared with a standard tacrolimus dose based on bodyweight. Between November 10, 2010 and September 30, 2013, 240 recipients of a living donor kidney were randomly assigned to either arm. Randomization was performed by use of sealed, opaque, sequentially-numbered envelopes containing treatment allocation. Secondary, outcome measures included the incidence of acute rejection.
Results: Of the 237 RTRs who were available for the intention-to-treat analysis, 119 and 118 were assigned to the standard-dose and genotype-based groups, respectively. On day 3, 37.4% (95% CI: 28.5 – 47.0%) and 35.6% (95% CI: 27.0 – 45.0%) of the patients in the standard-dose and genotype-based groups, respectively, were within the target tacrolimus C_0 range ($p = 0.79$). The proportion of patients with a sub-therapeutic (i.e. <10 ng/mL) or a supra-therapeutic (i.e. >15 ng/mL) Tac C0 in the two groups was also not significantly different (Figure 1).The incidence of acute rejection was comparable between both groups ($p = 0.82$).

Figure 1.



	Standard-dose group	Genotype-based group	p
	n = 99	n = 104	
Supra-therapeutic concentration	39 (39.4%)	31 (29.8%)	0.20
Therapeutic concentration	37 (37.4%)	37 (35.6%)	0.79
Sub-therapeutic concentration	23 (23.2%)	36 (34.6%)	0.10

Conclusion: Pharmacogenetic adaptation of the tacrolimus starting dose does not lead to a higher proportion of patients reaching the target tacrolimus C_0 early after transplantation and does not lead to improved clinical outcome. Therefore, routinely genotyping RTRs for CYP3A5 cannot be recommended.

References:

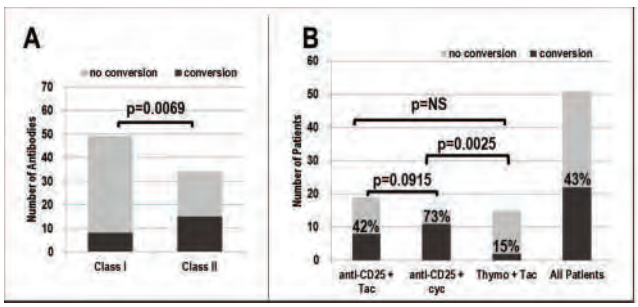
[1] van Gelder T. Practicability of Pharmacogenetics in Transplantation Medicine. *Clin Pharmacol Ther*. 2014; 95:262-264.
[2] Hesselink D.A. Genetic polymorphisms of the CYP3A4, CYP3A5, and MDR-1 genes and pharmacokinetics of the calcineurin inhibitors cyclosporine and tacrolimus. *Clin Pharmacol Ther*. 2003; 74:245-254.

450.4

Impact of induction and maintenance immunosuppression on pre-transplant IgM DSA to post-transplant IgG DSA conversion

Anh Nguyen[1], Lorita M.Rebellato[2], Carl E.Haisch[2], Kimberly P.Briley[2], Paul Bolin[2], Scott A.Kendrick[3], Angela Q.Maldonado[4], Claire Morgan[2], Matthew Everly[1].
[1]Terasaki Foundation Laboratory, Los Angeles, CA, United States; [2]East Carolina University, Greenville, NC, United States; [3]Eastern Nephrology Associates, Greenville, NC, United States; [4]Vidant Medical Center, Greenville, NC, United States.

Body: Considering the impact that post-tx IgG donor specific anti-HLA antibodies (DSA) has on late graft loss, it may be important to understand why and how many pre-tx IgM convert to IgG DSA post-tx. Our aim was to learn more about the IgM-IgG conversion post-tx and determine if immunosuppression affected this conversion.
Methods: We retrospectively analyzed 218 consecutive kidney tx patients at a single center, transplanted between 1/06 and 12/10, with a minimum 3 year follow-up. We excluded 20 patients from the analysis due to HLA-matched tx, insufficient patient information, or lack of patient sera. Sera sampling occurred pre-tx, 1, 3, 6, 9, 12 months post-tx and annually thereafter. Antibody identification was determined by LABScreen single antigen beads.
Results: 26% (51/198) of patients had pre-tx IgM DSA but were negative for IgG DSA. Of the 51 patients, 22 patients (43%) converted pre-tx IgM to IgG DSA post-tx of the same specificity.IgM to IgG DSA conversion was most common (54%) within the 1st post-transplant year. Interestingly, significantly more Class II antibodies (44%) converted as compared to Class I antibodies (16%), Fig. A. Also, IgM DQ DSA was >2 times more likely to convert to IgG DSA (of the same specificity) when compared to other HLA loci. Patients on an induction + main immunosuppression combination of Thymoglobulin (Thymo) + Tacrolimus (Tac) had the least conversion (15%) as compared to patients on anti-CD25 + Tac (42%) and anti-CD25 + cyclosporine (73%), Fig. B. Delayed graft function, acute rejection, nonadherence, and BK virus positivity was not shown to affect pre-tx IgM to post-tx IgG conversion.



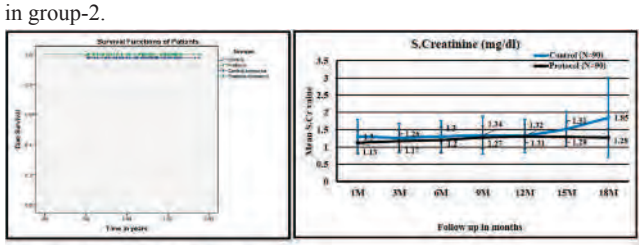
Conclusion: Significantly more Class II Abs convert post-tx. Induction with Thymo combined with Tac as the main immunosuppression may be most effective in preventing post-tx IgG DSA conversion from pre-tx IgM. More studies are needed to further understand the conversion and confirm the effect immunosuppression has on the process.

450.5

T-regulatory cells support stem cells in safe minimization of immunosuppression in renal transplantation

Hargovind L.Trivedi[1,3], ArunaV. Vanikar[2,3], Himanshu V.Patel[1].
[1]Department of Nephrology and Transplantation Medicine, Institute of Kidney Diseases & Research Centre- Institute of Transplantation Sciences, Ahmedabad, India; [2]Department of Pathology, Laboratory Medicine, Transfusion Services and Immunohematology, Institute of Kidney Diseases & Research Centre- Institute of Transplantation Sciences, Ahmedabad, India; [3]Department of Regenerative Medicine and Stem Cell Therapy, Institute of Kidney Diseases & Research Centre- Institute of Transplantation Sciences, Ahmedabad, India.

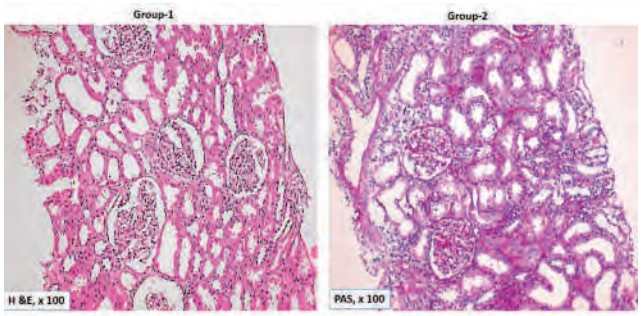
Background: Trials for safe, effective immunosuppression (IS) minimization using deletional agents and stem cell (SC) therapy are ongoing. We report interim results of portal and intrathymic infusion of donor adipose derived mesenchymal SC (AD-MSC), hematopoietic SC (HSC) and T-regulatory cells (Treg)[CD4+CD25highCD127neg/low] in living donor renal transplantation (LDRT).
Material and Methods: This was a prospective study of demographically well-balanced 2 groups, each with 90 patients, predominantly males with mean age, 30.03 years in group-1 and 33.6 years in group-2. Mean donor HLA-match was 2.72 and 2.6 respectively. After baseline tests of anti-human globulin lymphocyte cross-match(XM) (AHG), T/B-cell flow-XM(T/B-FXM) and donor specific antibody (DSA), group-1 underwent non-myeloablative conditioning of subtotal lymphoid irradiation (200 cGYx4), cyclophosphamide, 20 mg/kgBW to create space[1] followed by portal and intrathymic infusion[2],[3] of cytokine-stimulated donor peripheral blood stem cells 169.66x108/kgBW with mean CD34+ 2.05x106/kgBW and AD-MSC 12.5x106/kgBW with mean CD45-/CD90+/CD73+ 2.18/0.59x106/kgBW and peripheral infusion of 2.5x106/kgBW T-regs. RT was performed after favorable cross-match with perioperative rabbit-antithymoglobulin (r-ATG) (1.5 mg/kgBW). Methylprednisone (MP) 500 mg was given on day-1, 0, +1 of RT. Bortezomib 1.3 mg/m2x4 with MP 125mg was administered every 3rd day after 2nd postoperative day. Group-2 received r-ATG and Bortezomib. Group-1 IS included Tacrolimus, 0.03 mg/kgBW/day +Prednisone, 10 mg/day for 3 months subsequently Tacrolimus was replaced by Sirolimus 1 mg/day or mycofenolate 360 mg BD, prednisone was reduced to 5 mg/day. Group-2 received Tacrolimus 0.05 mg/kgBW/day, Mycofenolate 360 mg TID/QID and Prednisone 20 mg/day tapered to 10 mg/day after 1 month.
Tregs and AD-MSC were generated in vitro from donor adipose tissue and recipient blood by indigenous techniques[4]. Monitoring included serum creatinine (SCr-mg/dL), complete blood counts, urinalysis and liver enzymes. DSA were planned 3-monthly. Biopsy was performed in event of graft dysfunction and treated as per standard guidelines. Protocol biopsy was planned after 1 year in willing patients.
Results and Discussion: Over 18 months follow-up there was 100% graft and patient survival in group-1 and 97.8% patient and death-censored graft survival in group-2 with SCr of 1.28 in group-1 and 1.85 in group-2.



Key: Control= Group-2, Protocol= Group-1

Group-1 had more stable graft function. MSC are tolerogenic and recruit T-regs which exert their immunomodulatory effect by infectious tolerance and linked suppression[5]-[8]. There were 9 episodes of acute

rejection (AR) with Banff score ≤ag1at1av1ai2, PTC score-0 in group-1 and 4 episodes in group-2. Group-2 also had 17 episodes of AR score >ag1at1av1ai2, PTC score-0.



DSA were absent in majority of group-1 patients. Protocol biopsy permission was not granted.

Conclusion: T-regs support SC in safe minimization of IS in LDRT.

Mrs. Priyadarshini Shah, Mrs. Aanal Mehta, Dr. Umang Thakkar

References:

[1] Slavin S, Fuks Z, Kaplan HS, Strober S. Transplantation of allogeneic bone marrow without graft-versus-host disease using total lymphoid irradiation. J Exp med 1978; 147:963-72

[2] Remuzzi G, Perico N, Carpenter C, et al. The thymic way to transplantation tolerance. J Am SocNephrol 1995; 5:1639-46

[3] Gorczynski RM: Immunosuppresion induced by hepatic portal venous immunization spares reactivity in IL-4 producing T lymphocytes. Immunol Lett 1992; 33:67-77

[4] Trivedi HL, Vanikar AV, Patel HV, et al. Regulatory T-cells support stem cell therapy in safe minimization of immunosuppression in living donor renal transplantation. J Stem Cell Res Ther 2014; 4(10):1-8

[5] Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 2005; 105: 1815-22

[6] Qin S, Cobbold SP, Pope H, et al. "Infectious" transplantation tolerance. Science 1993; 259: 974-7

[7] Hoyne GF, Dallman MJ, Lamb JR. T-cell regulation of peripheral tolerance and immunity: the potential role for Notch signalling. Immunology 2000; 100: 281-8

[8] Lal G, Bromberg JS. Epigenetic mechanisms of regulation of Foxp3 expression. Blood 2009; 114: 3727-35

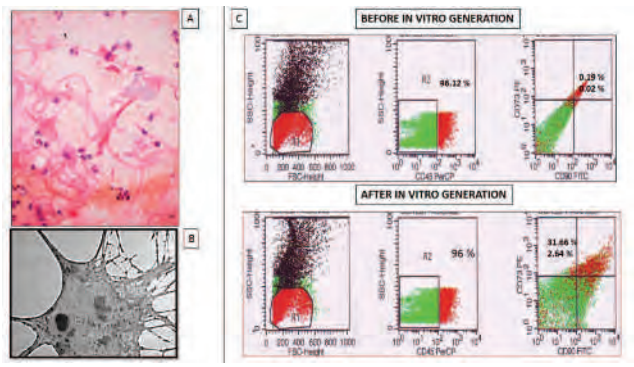
450.6

Tolerance induction in renal transplantation using stem cell therapy: Lessons learned

Aruna Vanikar[1,3], Hargovind L.Trivedi[2,3], Himanshu V.Patel[2].
[1]Department of Pathology, Laboratory Medicine, Transfusion Services and Immunohematology and , IKDRC-Dr.H.L.Trivedi Institute of Transplantation Sciences , Ahmedabad, India; [2]Department of Nephrology and Transplantation Medicine, IKDRC-Dr.H.L.Trivedi Institute of Transplantation Sciences , Ahmedabad, India; [3]Department of Regenerative Medicine and Stem Cell Therapy, IKDRC-Dr.H.L.Trivedi Institute of Transplantation Sciences , Ahmedabad, India.

Background: Strategies for tolerance induction (TI) include chimerism and clonal deletion[1]. We present 5 year experience of TI in living-donor renal transplantation (LDRT) using adipose derived mesenchymal stem cell (AD-MSC) and hematopoietic stem cell (HSC) infusion under non-myeloablative conditioning.

Material and Methods: Twenty patients divided in 2 demographically balanced groups underwent LDRT between Dec'09 and April,'11 under TI protocol consisting of conditioning with Bortezomib, 1.3 mg/m2 in 4 divided doses, rabbit-anti-thymocyte globulin, 1.5 mg/kgBW and Rituximab, 375 mg/m2. Group-1 (7 males, 3 females) with mean age of 31.6 years received mean 27.1 hemodialysis and 2.5 third party transfusions. Mean donor age was 43.6 years with mean HLA-match of 2.33. Group-2 (10 males) with mean age, 33.7 years received mean 26 hemodialysis and 1.4 third party transfusions. Mean donor age was 42.5 years with mean HLA-match of 2.4. Group-1 underwent portal infusion of in vitro generated mean AD-MSC, 19.4×104/kgBW and bone marrow-derived HSC, 0.37×108/kgBW with mean CD34+ 21.8×104/kgBW.



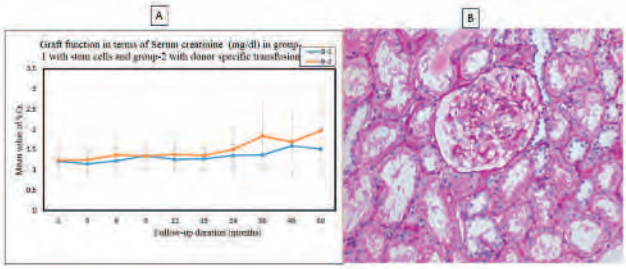
Peripheral blood stem cells, 34.4x108/KgBW were infused in periphery. Group-2 were administered donor specific transfusions (DST) with mean 46.2×103 cells/kgBW. Transplantation was carried out with at least 2 of 3 favorable tests; anti-human globulin lymphocyte cross-match <20%, flow cross-match with T-cells <50 median channel shift (MCS), B-cell <100 MCS and donor specific antibody (DSA) <1000 mean fluorescent intensity. No conventional immunosuppression was to be given. Monitoring included serum creatinine (SCr-mg/dL) and DSA. Protocol biopsies were planned after 100 days and yearly, in willing patients. In event of rejection/rise in DSA, rescue immunosuppression was planned.

Results and Discussion: No adverse effect of conditioning/ SC infusion was reported. Over mean 5.1 year follow-up, there was 70% patient + graft survival in both groups with mean SCr of 1.52 in group-1 and 1.97 in group-2.

There was loss of 2 patients (pneumonia in 1, chronic graft dysfunction in other) and 1 graft to unexplained interstitial fibrosis and tubular atrophy (IF/TA) in group-1. In group-2, 1 patient was lost to de novo focal segmental glomerulosclerosis and 2 grafts were lost to CMV infection and IF/TA respectively. In group-1, 5 patients are on no

conventional immunosuppression, and 2 on mycofenolate + prednisone. In group-2, 4 patients are on no conventional immunosuppression, 2 on mycofenolate+ prednisone, and 1 on Tacrolimus in addition. DSA appeared in 4 patients of group-1 without affecting graft function and 3 of group-2 causing graft dysfunction.

MSC evade immune injury since they do not express HLA-DR[2]. They recruit and generate T-regulatory cells which induce infectious tolerance and linked suppression. HSC help in supporting MSC by engraftment in BM niches. DST are effective in safe minimization of immunosuppression. Portal infusion was selected to exploit the tolerogenicity of liver[3].



Conclusion: To our knowledge this is the first clinical report of 5-year follow-up of tolerance induction in LDRT across HLA barriers where no conventional immunosuppression was used from day zero of transplantation. Immune tolerance induction is possible using stem cells and DST. However stem cells have the added benefit of immunomodulatory properties of AD-MSC.

Dr. Umang Thakkar, Prof. PR Modi, Prof. SJ Rizvi, Prof. RD Patel, Prof. KV Kanodia, Dr. LA Nigam, Dr. KS Suthar and lab staff of IKDRC-ITS, Mrs. Priyadarshini Shah, Aanal Soni

References:

[1] 1. Auchincloss H. In search of the elusive Holy Grail: The mechanisms and prospects for achieving clinical transplantation tolerance. Am J Transplant 2001; 1: 6–12.

[2] 2. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 2005; 105:1815-22

[3] 3. Vanikar AV, Trivedi HL. Stem Cell Transplantation in Living Donor Renal Transplantation for Minimization of Immunosuppression. Transplantation 2012; 94(8): 845-50

450.7

Malignancies contribute to presensitization with alloreactive T-cells in kidney transplant recipients

Thomas Schachtner[1,2], Maik Stein[2], Petra Reinke[1,2].
[1]Nephrology and Internal Intensive Care, Charité Campus Virchow Clinic, Berlin, Germany; [2]Berlin-Brandenburg Center for Regenerative Therapies, Charité Campus Virchow Clinic, Berlin, Germany.

Introduction: Alloreactive T-cells have been suggested to impact allograft outcome due to a higher incidence of acute rejection in the initial period after kidney transplantation. Knowledge on risk factors for the development of preformed and de-novo activated alloreactive T-cells and their impact on patient and allograft outcomes, however, remains scarce.

Material and Methods: Here, we analyzed clinical characteristics and outcomes of 327 kidney transplant recipients (KTRs) of a first kidney allograft transplanted at our center from 2008 to 2013. KTRs were grouped with respect to the presence of alloreactive T-cells pretransplantation. KTRs without alloreactive T-cells pretransplantation were grouped with respect to the development of alloreactive T-cells posttransplantation. Samples were collected pretransplantation, at +1, +2, and +3 months posttransplantation, and alloreactive T-cells were measured using an interferon-γ Elispot assay.

Results and Discussion: Among 327 KTRs, 107 KTRs (33%) showed detectable alloreactive T-cells pretransplantation, whereas 220 KTR (67%) didn't. Risk factors for the presence of preformed alloreactive T-cells included older age, diabetes, and prior cancer ($p<0.05$). Preformed alloreactive T-cells were associated with a higher incidence of delayed graft function ($p=0.017$). Among 220 KTRs without alloreactive T-cells pretransplantation, 31 KTRs (14%) showed detectable alloreactive T-cells posttransplantation, whereas 189 KTR (86%) didn't. Risk factors for the development of de-novo alloreactive T-cells included younger age, female sex, and prior cancer ($p<0.05$). KTRs with preformed/de-novo alloreactive T-cells showed inferior patient survival, allograft survival, and allograft function ($p<0.05$). In addition, those KTRs showed a higher incidence of acute cellular rejection and a higher rate of allograft loss due to acute cellular rejection ($p<0.05$). KTRs with preformed/de-novo alloreactive T-cells showed a higher incidence of septic complications, posttransplant cancer, and death from cancer ($p<0.05$).

Conclusion: The presence of alloreactive T-cells strongly impacts patient and allograft outcomes. Our data highly suggest that cured or undetected cancer at transplantation may lead to presensitization with preformed alloreactive T-cells. Caution should be taken in KTRs with alloreactive T-cells with regards to minimizing immunosuppression.

450.8

The effect of everolimus and tacrolimus exposure levels on renal histology parameters 6 months post-transplantation

F. Vincenti[1], D. Shaffer[2], Y. Qazi[3], F. Shihab[4], K. McCague[5], D. Patel[5], V.R. Peddi[6], S. Yilmaz[7].
[1]University of California San Francisco, San Francisco, CA, United States; [2]Vanderbilt University, Nashville, TN, United States; [3]University of Southern California, Los Angeles, CA, United States; [4]University of Utah, Salt Lake City, UT, United States; [5]Novartis Pharmaceuticals Corporation, East Hanover, NJ, United States; [6]California Pacific Medical Center, San Francisco, CA, United States; [7]University of Calgary, Calgary, AB, Canada.

Introduction: Morphologic changes in renal transplant protocol biopsies provide early evidence of renal damage. Arteriolar hyalinosis (AH) is of particular interest as previous studies have implicated chronic calcineurin inhibitor (CNI) nephrotoxicity in the development of AH following kidney transplantation. We examined renal histology scores, including AH, in renal transplantation patients with different exposure levels of everolimus (EVR) and tacrolimus (TAC) over 6 months.
Methods: Renal transplant patients (N=613) were randomized (1:1) to EVR+low-dose TAC (EVR+LTAC) or mycophenolate mofetil+standard-dose TAC (MMF+STAC). EVR+LTAC received EVR to maintain a trough of 3–8 ng/mL with LTAC (C0h 0–2 Months(M): 4–7 ng/mL, 2–6M: 3–6 ng/mL, 6–12M: 2–5 ng/mL. MMF+STAC received 2 g/day of MMF with STAC (C0h 0–2M: 8–12 ng/mL, 2–6M: 7–10 ng/mL, 6–12M: 5–8 ng/mL). EVR exposure level in the EVR+LTAC group was assessed at 6 months using area-under-curve (AUC) values and patients were ranked and divided into 3 groups (highest to lowest, n=43 in each group). Histological scores, acute and chronic/sclerosing lesion scorings at 6 month protocol biopsies, were compared between the high and low exposure groups using Banff classification. The same analysis was undertaken for TAC exposure level in the EVR+LTAC group.
Results: Histological scores were typically lower for those with higher EVR levels but this effect was not seen with higher TAC levels (Table). AH scores were not correlated with TAC exposure and were similar in patients with upper and lower AUC levels at 6 months (Table).
Discussion: These data show that patients with higher EVR AUC levels had reduced morphological changes associated with renal damage than those with lower EVR AUC levels. While etiological factors in the development of AH in kidney transplant biopsies are not well understood, previous studies have implicated CNI toxicity. Our findings do not support such an association.
Conclusion: Higher EVR levels were more effective at limiting renal damage 6 months post transplantation. TAC treatment was not associated with AH in renal transplant patients 6 months post-transplant. Longer term data are needed to examine these conclusions beyond 6 months.

Table. The effect of everolimus (EVR) and tacrolimus (TAC) exposure levels on renal histology parameters 6 months post-transplantation

	Low vs High EVR area under curve mean (SD)			Low vs High TAC area under curve mean (SD)		
	Low	High	P-value	Low	High	P-value
Area-under-curve	653 (115)	1222 (169)	<0.0001	841 (186)	1041 (259)	<0.0001
Mean trough (ng/ml)	3.76 (0.78)	5.81 (1.03)	<0.0001	5.52 (0.91)	7.47 (1.47)	<0.0001
T-Tubulitis	0.7 (1.1)	0.3 (0.8)	0.0215	0.4 (0.8)	0.5 (1.1)	0.6453
I-Interstitial inflammation	0.6 (1.0)	0.2 (0.6)	0.1869	0.4 (0.8)	0.4 (0.7)	1.0000
I+t-inflammation in unstained and stained	1.1 (1.0)	0.6 (0.9)	0.0251	0.7 (0.8)	0.8 (0.9)	0.8657
Ct Interstitial Fibrosis	0.8 (1.0)	0.4 (0.8)	0.0090	0.5 (0.8)	0.7 (0.9)	0.2887
Ct Tubular atrophy	0.9 (0.8)	0.4 (0.6)	0.0023	0.6 (0.8)	0.7 (0.8)	0.4452
Cv-vascular intimal thickening	1.0 (1.0)	0.6 (0.8)	0.0710	0.8 (0.8)	0.9 (1.0)	0.8258
TI+CI	2.8 (2.8)	1.1 (1.8)	0.0052	1.8 (2.3)	2.0 (2.5)	0.7258
CI+CtCV	2.8 (2.8)	1.4 (1.8)	0.0055	2.0 (2.0)	2.2 (2.4)	0.5469
TI+TI+CI+CI+Cv	5.2 (5.2)	2.8 (3.0)	0.0058	3.6 (3.5)	4.0 (4.4)	0.6148
CADI (Chronic allograft damage index)	4.0 (3.6)	2.5 (2.4)	0.0260	2.9 (3.0)	3.3 (3.0)	0.5828
Arteriolar hyalinosis	0.4 (0.6)	0.4 (0.7)	0.9429	0.3 (0.6)	0.4 (0.7)	0.8057

450.9

Everolimus plus reduced-exposure cyclosporin versus mycophenolic acid plus cyclosporin: long-term follow-up of Australia and New Zealand kidney transplant recipients in the A2309 randomised controlled trial

Steven Chadban[1], Helen Pilmore[2], Graeme Russ[3], Kanellis John[4], Scott Campbell[5], Philip O'Connell[6], Wai H. Lim[7], Mark Lutherborrow[8], Nicols Kurstjens[8], Rowan Walker[9].
[1]Royal Prince Alfred Hospital, Sydney, Australia; [2]Auckland City Hospital, Sydney, Australia; [3]3Central Northern Adelaide Renal and Transplantation Service, Royal Adelaide Hospital, Adelaide, Australia; [4]Monash Medical Centre, Melbourne, Australia; [5]Princess Alexandra Hospital, Brisbane, Australia; [6]Westmead Hospital, Sydney, Australia; [7]Sir Charles Gairdner Hospital, Perth, Australia; [8]Novartis Pharmaceuticals Australia, Sydney, Australia; [9]The Alfred Hospital, Melbourne, Australia.

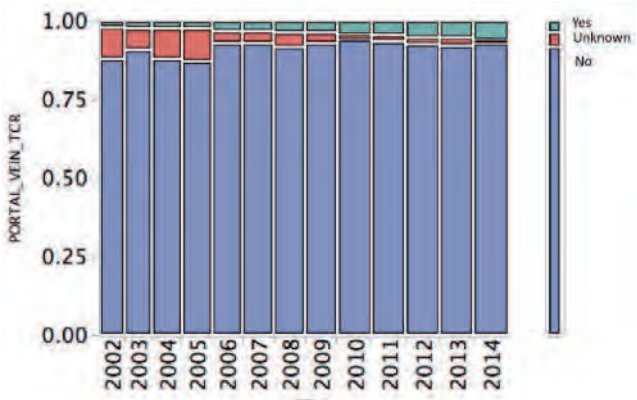
Background: The A2309 was a 2-year, phase III randomized controlled trial evaluating the effect on graft and patient outcomes of three treatment groups:1.5mg everolimus plus reduced exposure cyclosporin (EVR1.5), 3.0mg everolimus plus reduced exposure cyclosporin (EVR3.0) or 1.44g mycophenolic acid plus standard cyclosporin (MPA). The aim of this study was to evaluate the long-term clinical outcomes of 95 Australia and New Zealand (ANZ) participants in the A2309 study.
Methods: Seven-year data for 95 ANZ A2309 participants were extracted from the Australia and New Zealand Dialysis and Transplant (ANZDATA) registry. Associations between treatment and outcomes, including rejection, estimated glomerular filtration rate (eGFR), graft loss, death and cancer incidence were examined using adjusted generalized linear regression or Cox regression analyses using an intention-to-treat analysis. Adverse events and discontinuation of study drugs (up to 2-years) were compared between treatment groups using data from Novartis.
Results: Of the 95 participants, 66 (75.2%) were randomized to EVR1.5 or EVR3.0 and 29 (24.8%) to MPA. Compared to MPA, the combined everolimus treatment groups were associated with adjusted hazard ratios of 0.34 (95%CI 0.13, 0.91), 0.35 (0.09, 1.25) and 0.32 (0.15, 0.71) for non-melanoma skin cancers (NMSC), non-skin cancers and any cancers respectively, independent of age, gender and waiting time. The adjusted mean (95%CI) difference in eGFR at 2 and 5-years were numerically (but not statistically significant) higher in the EVR1.5 group compared to MPA group (2 years: 8.66, 95%CI 1.46, 15.85; and 5 years: 4.68, 95%CI -5.37, 14.7mL/min/1.73m2). There were no associations between treatment groups and other clinical outcomes, including rejection, graft loss or death up to 7-years post-transplant. Adverse events and discontinuation rates were similar between treatment groups after 2-years post-transplant.
Conclusions: Compared to standard-dose cyclosporin, everolimus with reduced-exposure cyclosporin was associated with over a 50% reduction in cancer incidence, particularly NMSC. This data supports a class-effect for mammalian target of rapamycin (mTOR)-inhibitors in reducing the burden of cancer after transplantation.

451.1

Broad assessment of the clinical impact of portal vein thrombosis in the waitlist for liver transplantation

Martin Montenovo, Jorge Reyes, James Perkins.
Surgery, University of Washington, Seattle, United States.

Introduction: Portal vein thrombosis (PVT) is quite common in patients with cirrhosis, but its effect on the waitlist outcomes is not well defined. The objective of this study was to determine the impact of PVT on the waitlist outcomes.
Methods: We conducted a retrospective cohort analysis using the United Network for Organ Sharing database from January 2002 through June 2014, including adults (³18 years old) who were listed for primary OLT. We looked at rate of PVT during the study period, the waiting time from listing until transplant and the rate and cause of drop-off of the waiting list among those patients with PVT at listing vs those without PVT. Logistic regression was used to adjust for recipient characteristics in order to estimate the risk factors associated with being removed from the waitlist.
Results: A total of 134,109 patients listed for primary OLT were identified. The rate of PVT at listing has increased over the course of the years (fig 1).



Patients with PVT at listing have a shorter waiting time (350 ±558 days vs 439±668 days p<.0001); are transplanted at a higher rate (52% vs 49% p<.003); are more likely to be removed from the waitlist for being too sick (11% vs 9% p<.0001) and have a higher lab MELD (23 vs 21 p<.0001). On the multivariate analysis PVT at listing was an independent risk factor of falling off the waitlist (table 1).

Table 1. Logistic Regression Analysis of Factors Predicting Falling off the Waitlist.

Variable	Removal from Waitlist	
	HR	95% CI
PVT at listing	1.12	1.01-1.23
Recipient age	1.03	1.02-1.03
Life support	1.37	1.25-1.51
Previous abdominal surgery	1.06	1.01-1.11
Albumin at time of tx	0.94	0.91-0.96
Ascitis	1.08	1.03-1.13
MELD at time of tx	12.6	10.4-15.3
HD at time of tx	1.31	1.24-1.4
Diabetes	1.11	1.06-1.16
HCC	2.68	2.41-2.97

Conclusions: PVT at listing has significantly increased over the course of the years representing a quite significant burden in the waitlist for liver transplantation. PVT at listing is an independent predictor of being removed from the waitlist. Guidelines for the management of PVT in cirrhosis are needed to improve the access to these patients to liver transplantation.

451.2

Clinical and immunological significance of controlling portal vein pressure in living donor liver transplantation

Kazuhiro Taguchi[1,2], Takashi Onoe[1,2], Shinji Hashimoto[2], Yuka Tanaka[2], Hideki Ohdan[2].
[1]Institute for Clinical Research, National Hospital Organization Kure Medical Center and Chugoku Cancer Center, Kure City, Hiroshima, Japan; [2]Department of Gastroenterological and Transplant Surgery, Hiroshima University, Hiroshima, Japan.

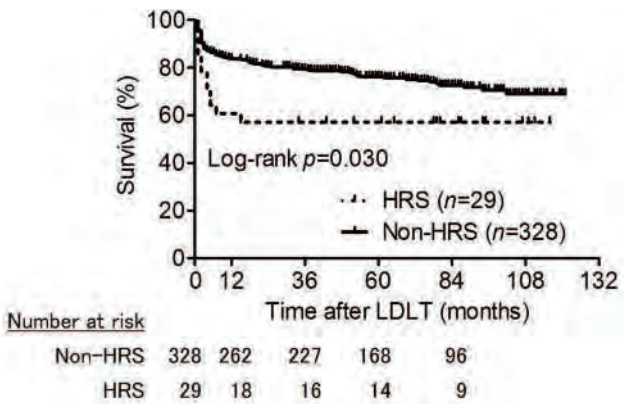
Recently, an importance of controlling portal vein pressure (PVP) in living donor liver transplantation (LDLT) has attracted more attention. Nonetheless, clinical significance of portal hypertension in LDLT as risk factor is still controversial. Furthermore, the immunological impact of portal hypertension remains unclear. In this study, we investigated significance of portal hypertension for graft survival and anti-donor immune response.
First, we investigated a significance of portal hypertension as the risk factors for graft survival. In this study, 158 consecutive adult patients undergoing LDLT were included. We categorized the patient into low (<15 mmHg) and high (≥15 mmHg) PVP groups, based on the level of PVP at the end of surgery. The Kaplan-Meier and log-rank test revealed that the 2-year graft survival rate was significantly lower in high PVP group than that in low PVP group (P <0.05). Next, we performed univariate and multivariate analysis of the variables (recipient factors, donor factors and operative factors) including portal hypertension to determine significant risk factor for 2-years graft survival. Multivariate analysis showed that HCV infection and high PVP were independent risk factors (P = 0.005 and 0.018, respectively). In analysis of high PVP subpopulation, multivariate analysis showed that HCV infection, donor older age and longer operation time were significant risk factors for 2-year graft survival (P = 0.001, 0.019 and 0.026, respectively).
Second, we investigated an impact of portal hypertension on immunological reaction in same cohort. For monitoring the cellular alloreacivity of the patients, multiparameter MLR assays were routinely performed at 2 weeks intervals after LDLT. Patients were divided into low or high PVP groups and immunological outcomes were compared. To overcome bias due to the different distribution of covariates in the 2 groups, propensity score matching was performed. The propensity score analysis revealed that 53 matched pairs were selected and those had the same background characteristics (excluding the PVP level). MLR assay revealed that the stimulation index of CD8+ T cells which indicates the anti-donor proliferative response was significantly higher in high PVP group than that in low PVP group within one month after LDLT (P = 0.005). Furthermore, percentage of CD25+ cells among CD8+ T cells which indicates proportion of activated CD8+ T cells in high PVP group was also significantly higher than that in low PVP group (P = 0.005). In addition, the incidence of acute rejection episode within 3 months after LDLT was significantly higher in high PVP group than that in low PVP group (34% vs 11.3%, respectively, P = 0.01). Those data indicates that immunological anti-donor response was promoted in recipients of high PVP group.
In conclusion, those results show that postoperative portal hypertension has significantly adverse impact on graft survival as well as immunological anti-donor response. Our results might propose a novel concept to prevent an acceleration of alloimmune responses and improve survival by controlling PVP or modulating immunosuppressant in high PVP patients after LDLT, especially in patients with HCV infection or with graft from aged donor.

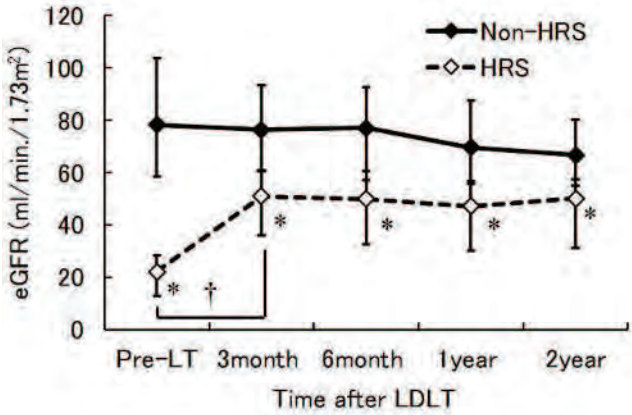
451.3

Impact of hepatorenal syndrome on outcome of living donor liver transplantation: A single-center experience in 357 patients

Yusuke Okamura, Koichiro Hata, Ichiro Tamaki, Yermek Nigmet, Osamu Inamoto, Toyonari Kubota, Hirofumi Hirao, Shintaro Yagi, Atsushi Yoshizawa, Hideaki Okajima, Toshimi Kaido, Shinji Uemoto.
Division of Hepato-Biliary-Pancreatic Surgery and Transplantation, Department of Surgery, Kyoto University, Kyoto, Japan.

Introduction: Hepatorenal syndrome (HRS) is one of the most serious complications in end-stage liver diseases, characterized by renal failure and disrupted splanchnic circulation. However, impact of HRS on the patient outcome after living donor liver transplantation (LDLT) is still unclear. The aim of this study was to evaluate the impact of HRS on the outcome of LDLT.
Methods: We retrospectively analyzed consecutive 357 adult patients who underwent primary LDLT between January 2005 and March 2013 in Kyoto University hospital. The median follow-up period of the population was 60 (IQR, 22-88) months. The outcome of the patients with HRS were compared with those without HRS. The definition of HRS was based on the established diagnostic criteria published in 2007 by the International Ascites Club, as follows: cirrhosis with ascites; serum creatinine>133mmol/L (1.5 mg/dL); no improvement of serum creatinine (decrease to a level of<133mmol/L) after at least 2 days with diuretic withdrawal and volume expansion with albumin; absence of shock; no current or recent treatment with nephrotoxic drugs; and absence of parenchymal kidney disease as indicated by proteinuria>500 mg/dL, microhaematuria and/or abnormal renal ultrasonography.
Results: Twenty nine patients (8%) were diagnosed as HRS (Group-HRS) preoperatively, and the other 328 cases (92%) were not (Group-Non-HRS). In Group-HRS, 15 patients (52%) were classified into type-1 HRS, while 14 (48%) were type-2. Group-HRS had significantly higher MELD scores (32 [IQR, 21-40] vs. 18 [IQR, 14-23], p<0.001), requiring significantly more preoperative renal replacement therapy than in Group-Non-HRS (55% vs. 3%, p<0.001). Patient survival in Group-HRS were significantly worse than that in Group-Non-HRS (Log-rank p=0.030) (Figure 1). The 1-, 3- and 5-year survival of the recipients were 60.7%, 57.1% and 57.1% in Group-HRS, and 83.7%, 79.4% and 76.2% in Group-Non-HRS, respectively. Concomitant HRS significantly elongated post-LDLT hospital stays (75 [IQR, 46-142] vs. 50 [IQR, 38-75] days, p=0.003), as well as significantly predisposed to higher in-hospital mortality (41% vs. 18%, p=0.005). The eGFR in Group-HRS was significantly improved after LDLT (22.1 [IQR, 13.1-28.3] preoperatively vs. 50.9 [IQR, 36.1-61.0] ml/min./1.73m2 at 3 months postoperatively, p<0.001) (Figure 2). The values had then plateaued until 2 years after LDLT. Despite such significant recovery of eGFR in Group-HRS, it was still lower than in Group-Non-HRS throughout the observation period of 2 years.





Conclusions: Recipients' as well as renal outcome in HRS patients after LDLT has been seldom elucidated so far. Here we thus report the largest series of analysis in LDLT than ever, demonstrating not only HRS resulted in significantly worse recipients' survival after LDLT, especially in the early posttransplant period, but also prolonged renal dysfunction after transplant even in the long-term.

451.4

Risk factors for primary sclerosing cholangitis recurrence after othotopic liver transplantation

Pavel Drastich[1], Lukas Bajer[1], Monika Drastichova[3], Eva Sticova[2], Pavel Trunecka[4], Julius Spicak[1].
[1]Hepatogastroenterology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [2]Pathology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [3]Radiology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [4]Transplancentre, Institute for Clinical and Experimental Medicine, Prague, Czech Republic.

Background: Primary sclerosing cholangitis (PSC) is a chronic liver disorder of unknown etiology. The disease may progress to cirrhosis and eventually to liver failure and death. For those who develop end – stage liver disease, orthotopic liver transplantation (OLT) remains the only effective treatment currently available. However, recurrent form of PSC (rPSC) often appears in patients after OLT and may eventually lead to graft loss and liver re - transplantation (re - OLT). The aim of our retrospective study was to identify risk factors for rPSC.
Patients and Methods: A total number of 115 orthotopic liver transplantations for PSC were performed at Institute for Clinical and Experimental Medicine, Prague, Czech Republic between July 1994 and May 2015. The diagnosis of rPSC was based on histology and cholangiographic findings. We retrospectively analyzed medical records of all PSC patients from our computed database. Only patients with a proper record of pre - OLT (≤ 12 months) colonoscopy and those monitored for a time period of ≥ 60 months post - OLT were included.
Results: We analyzed a cohort of 47 patients. 31 were male, 16 female, with median age of 36 (range 15 - 68) and median follow - up 122 months (range 60 - 249) after OLT. In 21/47 (44.7%) patients, rPSC was diagnosed during the follow - up. Two patients underwent re - OLT (after 103 and 116 months respectively, both for rPSC). According to performed univariate analysis, presence of de - novo colitis ($p = 0.0002$; OR 27.50, 95% CI 3.13 – 241.94) and OLT for overlap with autoimmune hepatitis (PSC/AIH) ($p = 0.0133$) were significantly associated with rPSC. Presence of HLA-DRB1*04 in the recipient was identified as protective factor for rPSC ($p = 0.0287$). In case of de - novo colitis, statistical significance was further confirmed by nominal logistic regression analysis ($p = 0.0094$; OR 22.00, 95% CI 2.04 – 591.60). Neither gender, nor age, CMV infection, acute cellular rejection (ACR), corticoresistant ACR, use of OKT3, presence of HLA-DRB1*08, history of cholangiocarcinoma, cold ischemia time and length of corticosteroids use were significantly associated with rPSC.
Conclusion: Recurrent PSC is an important clinical entity with high prevalence in patients after OLT. De - novo colitis is a novel significant risk factor associated with rPSC, along with PSC/AIH overlap, while presence of HLA-DRB1*04 in the recipient appeared to have a protective effect on rPSC.

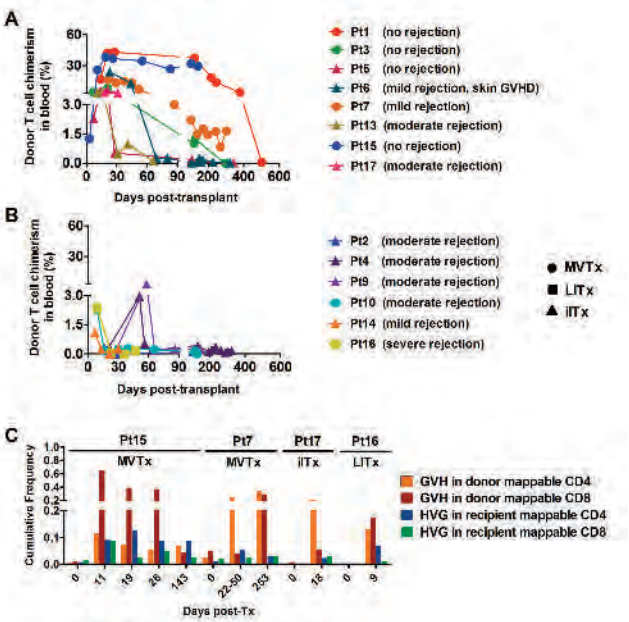
451.5

Generation of naïve donor-derived lymphocytes from graft-resident lymphoid progenitors after human intestinal transplantation

Jianing Fu, Julien Zuber, Brittany A.Shonts, Sai Ping Lau, Aleksandar Obradovic, Thomas M.Savage, Michael Simpson, Suxiao Yang, Michelle Miron, Yufeng Shen, Donna Farber, Mercedes Martinez, Adam Griesemer, Tomoaki Kato, Megan Sykes.
Columbia University Medical Center, New York, NY, United States.

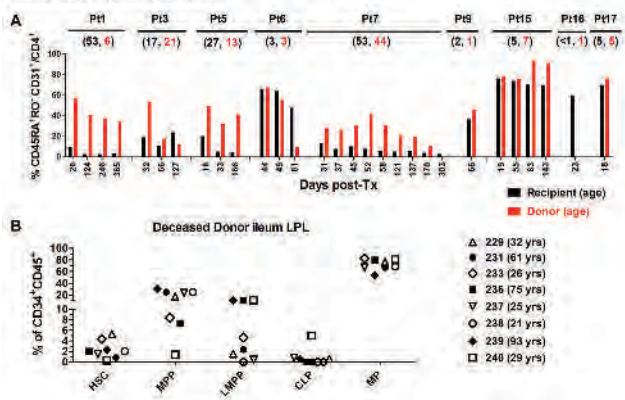
Multivisceral transplantation (MVTx) is associated with a lower rate of severe graft rejection than isolated intestinal transplantation (iITx). We recently demonstrated that mixed T cell macrochimerism (>3% donor cells) often appears in blood (8/14), usually without graft-versus-host disease (GVHD), following ITx, and is greatest in MVTx recipients[1]. However, reasons for the occurrence and persistence of donor T cell macrochimersim in blood are unknown.
We analyzed the phenotype, repertoire, alloreactivity and origin of donor circulating T cells in ITx recipients using flow cytometry, mixed lymphocyte reaction and a high-throughput TCR sequencing to identify and track alloreactive T cells.

Figure 1: (A-B) Donor T cell macrochimerism (>3%) in blood associated with lower rejection rates post-ITx (POD<90). (C) High cumulative frequency (>0.2) of GVH clones in circulation early post-ITx (POD<50) in patients with donor T cell macrochimerism.



The donor T cells among patients with macrochimerism but without GVHD were markedly enriched for the naïve recent thymic emigrant (RTE) phenotype ($CD45RA^{+}CD31^{+}$) and for T-cell receptor excision circles compared with recipient cells and could persist > 1 year. RTE represented 40-95% of donor T cells regardless of donor age (1-44 years old), while the level of recipient-derived RTE usually remained below 25-40%, except in young children less than 6 years old. Surface markers of donor circulating T cells were characteristic of blood ($CD28^{high}CD69^{-}CD103^{-}$), rather than intestinal ($CD28^{-}CD69^{high}CD103^{high}$) T cells. Furthermore, expanded graft-versus-host (GVH) clones were detected early in intestinal biopsies in association with rapid myeloid antigen-presenting cell replacement in the graft by the recipient. GVH-reactive clones were enriched in recipient blood early post-MVTx, even in the absence of GVHD, representing up to 35-65% of donor CD8 cells or 20-25% of donor CD4 cells in early PBMCs (POD<50). GVH clones were later absent in circulating naïve donor-derived T cells (POD>100), consistent with their de novo generation from progenitors. In addition, donor chimerism was identified in circulating T-cell progenitors in 4 MVTx patients. We also detected donor-derived hematopoietic stem cells (HSC) and/or multiple types of progenitors, including multipotent progenitors (MPP), lymphoid-primed multipotent progenitors (LMPP), common lymphoid progenitors (CLP), and mixed myeloid progenitors (MP) in liver and ileum of organ donors, and in perfusates from donor liver, small intestine, and transplanted multivisceral organ blocks.

Figure 2: (A) Circulating donor-derived naive T cells display a recent thymic emigrant (RTE) phenotype. (B) Hematopoietic stem cell and progenitors are detectable in deceased donor ileum lamina propria lymphocytes (LPL).



Together, our findings suggest that GVH-reactive donor T cells expand initially within the graft. These then enter the recipient circulation and may attack host hematopoietic cells, usually without causing GVHD. This lymphohematopoietic GVH response might allow the survival and expansion of donor hematopoietic progenitors from the graft that enter the peripheral blood and then the thymus, resulting in de novo T cell generation, and promoting sustained T cell chimerism. This pathway provides new insights into the underlying mechanism of blood mixed chimerism and may help to reduce graft rejection after ITx.

NIH NIAID Grant P01 AI106697. Research reported in this publication was performed in the Columbia Center for Translational Immunology (CCTI) Flow Cytometry Core, supported in part by the Office of the Director, National Institutes of Health under awards S10RR027050 and S10OD020056

References:

[1] Zuber J, Rosen S, Shonts B, Sprangers B, Savage TM, Richman S, Yang S, Lau SP, DeWolf S, Farber D, Vlad G, Zorn E, Wong W, Emond J, Levin B, Martinez M, Kato T, Sykes M. Macrochimerism in Intestinal Transplantation: Association With Lower Rejection Rates and Multivisceral Transplants, Without GVHD. Am J Transplant. 2015 Oct;15(10):2691-703.

451.6

De novo donor-specific HLA antibodies after combined intestinal and vascularized composite allotransplantation

Georgios Vrakas, Annemarie Weissenbacher, Mian Chen, Srikanth Reddy, Philip Allan, Henk Giele, Anil Vaidya, Sue Fuggle, Peter Friend.
Oxford Transplant Centre, Oxford University Hospitals, Oxford, United Kingdom.

Introduction: The numbers of both, intestinal and VCA transplantations, are small compared to other solid organ transplants. In the young field of VCA as well as in bowel and multivisceral transplantations, the occurrence of DSAs has been described. Our aim was to investigate the incidence and clinical effect of de novo DSAs in our cohort of patients receiving an intestinal transplant together with a full-thickness, vascularised abdominal wall graft (a VCA).
Materials and Methods: This is a single-center, retrospective clinical study. The patient cohort includes all recipients of deceased donor intestinal and VCA transplants performed at the Oxford Transplant Centre between October 2008 and December 2015. Pre-transplant HLA antibody status was available for all patients. Clinical and HLA antibody data were obtained monthly for the first year and 3 monthly thereafter, as well as at the time of clinical events.
Results: Thirty-two intestinal transplants were included. One patient underwent a second small bowel and abdominal wall transplant. All organs were retrieved from DBD donors. There were 8 modified multivisceral transplants (8/32, 25%) and 24 isolated small bowel transplants (24/32, 75%). An abdominal wall VCA (from the same donor) was used in 18 cases (18/32, 56%). All patients received alemutzumab induction and tacrolimus maintenance immunosuppression.
DSAs were detectable in 2/31 (6.5%) recipients before the combined transplant. Twelve (38.7%), of the remaining 29, developed de novo DSAs. Five patients developed de novo DSAs against HLA class-I (15.6%), 4 against class-II (12.5%) and 3 patients (9.4%) against both classes. The mean MFI of DSAs was 8250 ± 6580SD. In the VCA group, a lower percentage developed de novo DSAs compared to the group without VCA; 6/14 (42.9%) vs. 6/18 (33%).
Intestinal rejection was proven in 5/14 (35.7%) cases without VCA and 3/18 (16.7%) cases with the VCA. VCA rejection was seen in 7/18 (38.9%) cases. One was grade 1, 1 grade 2 and 5 grade 3. There were no episodes of intestinal rejection without VCA rejection. The occurrence of de novo DSAs in the absence of clinical rejection, has not led to organ or patient deterioration and has not been treated.
Discussion: The combination of intestinal transplantation with a VCA is immunologically complex: although we have found no evidence that the VCA sensitises the patient, predisposing to rejection of both organs, further experience is needed. The development of DSAs in intestinal transplantation, in line with other organ types, is detrimental to the long-term survival of the graft.
Conclusion: Our data suggest that combining an abdominal wall VCA with an intestinal transplant does not increase the incidence of de novo DSAs.

451.7

Intestinal retransplantation in children: Single center experience

Neslihan Celik[1], Frederick Vyas[1], Geoffrey J.Bond[1], Kyle A.Soltys[1], Jeffrey Rudolph[2], Rakesh Sindhi[1], George V.Mazariegos[1].
[1]Hillman Center for Pediatric Transplantation, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA, United States; [2]Intestinal Care and Rehabilitation Center, Department of Pediatrics, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA, United States.

Aim: To analyze the patient and graft characteristics and improvement in patient outcomes for intestinal retransplantation (Re-ITx) in the era of induction immunosuppression with the comparison of outcomes between 1990-2000 and 2001-2015.
Methods: The data of all children receiving Re-ITx between 1990 and 2015 at our center were reviewed retrospectively.
Results: 242 children received primary intestinal grafts. 23 children (9.5%) were retransplanted with 24 grafts. Causes of graft failure were acute cellular rejection (n=3), liver failure (n=2), chronic rejection (n=11), post-transplant lymphoproliferative disorder (n=3), graft dysmotility or dysfunction (n=2), volvulus (n=1), arterial graft aneurysm (n=1), and aortic graft thrombosis (n=1). Re-ITx was with isolated small bowel in five, liver-small bowel±pancreas in five, multivisceral in thirteen and modified multivisceral in one. Sixteen (69.6%) patients are alive with functioning grafts at a mean current follow-up time of 8.8 years. 5 year patient and graft survival improved to 89% and 79% respectively since 2001. Seven patients died primarily from infectious complications at a mean time of 35.7 months post-Re-ITx. Two patients had allograft enterectomies due to severe acute rejection and chronic rejection. 93% of surviving patients with their retransplanted intestine weaned-off total parenteral nutrition and intravenous fluids.

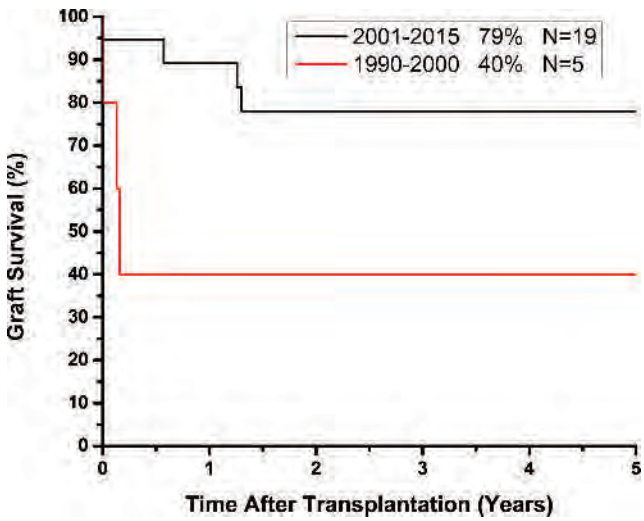


Figure 1. Five year graft survival for Re-ITx by era.
Conclusions: Improved early and intermediate term survival and outcome in pediatric Re-ITx may be attributed to improvements in initial immunosuppression protocols, technical modifications, proper timing, and improved infectious disease monitoring. Careful patient selection and post-transplant management are essential for successful long-term outcome.

451.8

Skin GVHD in paediatric intestinal transplant recipients – experience from a National UK Centre

Suzan Warner[1], Sarah Lawson[2], Malobi Ogboli[3], Arun Alfred[4], Peter Taylor[4], Jane Hartley[1], Girish Gupte[1].
[1]Hepatology , Birmingham Children's Hospital, Birmingham, United Kingdom; [2]Haematology , Birmingham Children's Hospital, Birmingham , United Kingdom; [3]Dermatology, Birmingham Children's Hospital, Birmingham , United Kingdom; [4]Haematology, Rotherham Hospital, Rotherham, United Kingdom.

Introduction: Severe skin GVHD in intestinal transplantation is hard to treat. Various regimens have been tried, with more novel approaches such as extracorporeal photophoresis (ECP) and mesenchymal stem cell infusions (MSC) in recent years. We report our experience in the management of skin GVHD in intestinal transplant patients.
Materials and Methods: Retrospective study by review of clinical case-notes and electronic database of intestinal transplant recipients with skin GVHD from 1993 to 2016.
Results: 12 out of 94 patients had skin GVHD from 1993 to 2016; age range 10mths to 9yrs – median age 24mths. Data from 11 of these patients were obtained (1 medical notes cannot be traced); underlying diagnosis were NEC (n=3), Megacystis microcolon-intestinal hypoperistalsis syndrome (MMIH; n=3), Gastroschisis (n=2), Microvillus inclusion disease (n=2) and malrotation with volvulus (n=1).
Five had combined liver and small bowel, 3 isolated small bowel, 2 multivisceral and 1 modified multivisceral transplants. All transplants were from DBD donors (dead after brain-dead). Median cold ischaemic time was 6hrs 57minutes (range 4hrs 43mins to 9hrs 38mins). Median warm ischaemic time was 33minutes (range 20mins to 1hr 5mins).
Mean onset time from transplant to skin GVHD was 3mths; seven had Grade 4, two Grade 3 and two Grade 1 skin GVHD. 5 patients had mucositis (oral ulceration), 4 ocular GVHD, 4 haemolytic anaemia (bone marrow GVHD), 1 liver GVHD and one had neurological involvement (immune mediated Myasthenia gravis).
Chimerism studies at 4mths post-transplant from our cohort showed mostly CD3+ donor cell mixed chimerism (range 6-67%, mode 17%) and a reduced donor B cell lineage.
All except 3patients had intravenous steroids at diagnosis; 4 had Infliximab and Basiliximab, 1 had Infliximab alone, 2 Dacluzimab, 2 Campath, 1 Methotrexate, 5 ECP and 7 MSC. ECP and MSC became available at our centre from 2010; average time of referral for these treatments from GVHD onset was 1month from 2014 onwards compared to 7mths from 2010 to 2013.
Although 4 patients initially had resolution of their skin GVHD and 5 had chronic skin GVHD, four patients (all Grade 4 skin GVHD) eventually died as a result of complications from the skin GVHD.
Discussion: Skin was the primary organ affected with GVHD in our cohort of patients. Mixed chimersm studies showed higher than expected CD3+ activated donor cells at 4mths and thereafter. This may reflect a correlation with GVHD activity.
Our initial experience of steroid refractory skin GVHD with a combination of interleukin 2 receptor antagonists (Basiliximab and Daclulizimab) and TNFα (Infliximab) were ineffective. Second line therapy with ECP and MSC had more success with clinical improvements, particularly if patients were referred earlier as seen from 2014.
Conclusion: Skin GHVD in intestinal transplant populations can progress rapidly, is associated with high mortality and should be treated aggressively. ECP and MSC should form second line treatment in children with steroid refractory GVHD.
References:
[1] Vrakas G et al. Graft versus host disease after intestinal transplantation. Transplantation, 2014; 98/(869), 0041-1337
[2] Song et al. Prevention of GVHD and graft rejection by a new S1P receptor agonist, W-061, in rat small bowel transplantation. Transplant Immunology, 2012; 26: 163-170
[3] Wu et al. Graft-versus-host disease after intestinal and multivisceral transplantation. Transplantation, 2011; 91/2: 219-24.

451.9

Donor-specific antibodies are associated with rejection after intestinal transplant in pediatric patients

C. Andrew Bonham[1], Thomas Pham[1], Jackie Lee[1], Ricardo Castillo[2], Sarah Conlon[1], Waldo Concepcion[1], Carlos Esquivel[1].
[1]Surgery, Stanford University Medical School, Palo Alto, CA, United States; [2]Pediatrics, Stanford University Medical School, Palo Alto, CA, United States.

Isolated small bowel or multi-visceral transplant is the treatment of choice in children with chronic intestinal failure with or without end stage liver disease. Acute and chronic intestinal allograft rejection negatively impacts long term patient and graft survival. The role of donor specific antibodies (DSAs) and their impact on rejection and graft survival is well documented following kidney and heart transplant but remains unclear in pediatric small bowel transplant. We hypothesize that the presence of DSAs combined with positive C4d staining is a marker for humoral rejection and thus may influence treatment decisions. DSAs were reviewed in fifteen children aged 1 to 18 years undergoing small bowel transplant between the years 2002 to 2014. Ten of these children underwent combined multi-visceral transplants. Six patients had documented clinical acute rejection ranging from mild to severe. There were no graft losses documented. DSAs were analyzed at or soon after the time of rejection and were detectable in 5 of 6 (83%) patients with rejection. The one patient that did not have detectable DSAs had a cumulative panel reactive antibody of 8% and negative C4d staining at the time of rejection. C4d staining ranged from focal/weak to strong in the remaining 5 patients but did not correlate to the severity of rejection, the amount of panel reactive antibodies or the quantity/intensity of DSAs detected. Detectable DSAs persisted for years following rejection episodes, even in the absence of further rejection. The remaining nine patients did not have documented rejection but had DSAs analyzed post-transplant. Only one of them had detectable DSAs but no C4d detected on biopsy. The presence of detectable DSAs was significantly higher in those who had documented rejection (n=5) as compared to those who never had rejection (n=1) (p=0.001). There was no significant difference in rejection (p=0.5) and presence of DSAs (p=0.5) between isolated small bowel and multi-visceral transplants. Following small bowel and multi-visceral transplant, DSAs are associated with rejection and C4d deposition into the graft, which is suggestive of antibody-mediated rejection. The addition of a liver allograft did not translate into a decrease in recipient DSAs. Routine DSA monitoring will help treat rejection more efficiently and possibly prevent future episodes thus prolonging graft and patient survival.

452.1

MicroRNAs in EBV+ B cell lymphomas and secreted exosomes

Vandana Kaul, Aleishia Harris-Arnold, Carlos Esquivel, Olivia M. Martinez, Sheri M. Krams.
Department of Surgery, Division of Abdominal Transplantation, Stanford University School of Medicine, Stanford, CA, United States.

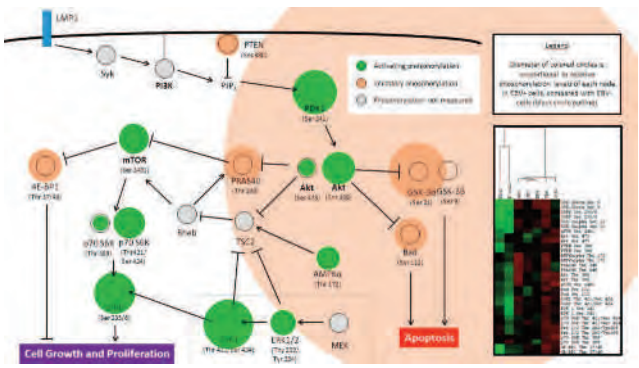
Introduction: Post-transplant lymphoproliferative disorder (PTLD) is a potentially fatal complication of organ transplantation commonly associated with Epstein Barr virus (EBV) infection. EBV viral loads are typically monitored by qPCR but EBV viremia does not always portend PTLD, and EBV$^+$ PTLD can occur in the absence of increased viral levels. Thus, new biomarkers are critical to improving patient outcomes.
Methods: microRNA-microarray profiling (Taqman-TLDA cards) was used to quantitate expression levels of 639 microRNAs in RNA isolated from spontaneously arising EBV$^+$ B lymphoma cell lines from patients with PTLD (SLCL) (n=6), B lymphoblastoid cell lines (LCL) generated in vitro with the B95.8 strain of EBV (n=4), and normal human B cells (n=4). One-way ANOVA analysis was used to identify the microRNAs differentially modulated within the groups. Further, real-time qPCR analysis was used to validate the specific differentially expressed microRNAs identified from microarray profiling. Exosomes are nanometric (30-150nm) membrane vesicles that are released by most cell types. Studies have shown that exosomes contain and transfer microRNAs between cells thus allowing for local and distant intercellular communication. Exosomes were isolated from the culture supernatant of SLCL using ExoQuick-TC and centrifugation. RNA was isolated from exosomes and microRNAs quantitated
Results: 133 microRNAs were significantly modulated by EBV infection in both B lymphoblastoid cell lines (LCL) and SLCL Sixteen microRNAs, 13 increased and three decreased, were uniquely modulated in EBV+ B lymphoma cell lines from patients with PTLD (SLCLs). Real-time qPCR validated a panel of 5 microRNAs (miR-19a, miR-100, miR-106a, miR-422a miR-449b) that were significantly increased in SLCLs as compared to normal EBV$^+$ B cells. Exosomes, which were confirmed by transmission electron microscopy, were present in very high levels in SLCL supernatants. Interestingly, miR-30c, which was down regulated in SLCLs, was increased in SLCL exosomes. Importantly, microRNAs including miR-19a and high levels of miR-106a were readily detected in exosomes from SLCL suggesting that circulating cell-free microRNAs are associated with PTLD.
Conclusion: EBV$^+$ B lymphoma cell lines from PTLD patients produce microRNA-containing exosomes and this may constitute a mechanism of intercellular communication by microRNA transfer to other cells. Importantly, cellular and cell-free microRNAs have great potential as non-invasive biomarkers of PTLD.

452.2

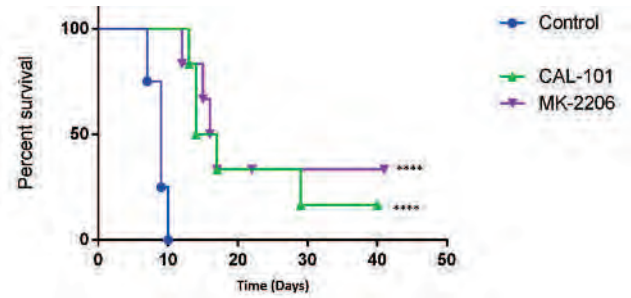
Inhibition of the constitutively active PI3K/Akt/mTOR signaling pathway in epstein-barr virus B cell lymphomas demonstrate both antitumor and antirejection properties

Adam Sang, Geoffrey T.Ivison, Xiumei Qu, Carlos O. Esquivel, Sheri M. Krams, Olivia M. Martinez.
Abdominal Transplantation, Stanford University School of Medicine, Stanford, CA, United States.

Introduction: Epstein-Barr virus (EBV) B cell lymphomas in post-transplant lymphoproliferative disorder (PTLD) is a major problem in organ transplantation. Treatment options for PTLD are limited and may harm the graft. Rationally-targeted antitumor therapies with antirejection properties would be of great importance to PTLD patients. Previous studies have shown that multiple cellular signal transduction pathways, including the PI3K/Akt/mTOR pathway, are dysregulated in EBV+ B cell lymphomas, and that inhibiting mTOR with rapamycin results in partial inhibition of proliferation. We hypothesize that targeting molecules upstream of mTOR would more effectively inhibit EBV+ B cell lymphoma proliferation. Moreover, blockade of this pathway may prolong graft survival, since this pathway is also known to be activated downstream of the T cell receptor.
Materials and Methods: Phosphorylation of the PI3K/Akt/mTOR signaling cascade was determined by pathway protein array on cellular lysates from EBV+ B cell lymphoma lines taken from five patients with PTLD. Cellular lysates from B cells of healthy donors and EBV- Burkitt's lymphoma lines were used as controls. The effect of small-molecule inhibitors (rapamycin (mTOR inhibitor), CAL101 (PI3K inhibitor), and MK2206 (Akt inhibitor)) on cellular proliferation was determined using colorimetric MTS assay.
A mouse heterotopic heart transplant model using Balb/C donors and MHC-mismatched C57Bl/6 recipients was used to assess the effect of these inhibitors (rapamycin, CAL101, MK2206) on transplant graft survival (n=6 in each group). The heart grafts were palpated daily to assess for survival.
Results and Discussion: EBV+ B lymphoma cell lines showed hyperactivation of multiple nodes along the PI3K/Akt/mTOR pathway, including mTOR, 4EBP1, S6K, and GSK3α/β, compared to EBV- cell lines (Fig 1). Hierarchical clustering distinguished the EBV+ B cell lines from EBV- B cells (Fig 1, Inset).



Several signals upstream of mTOR, such as PDK3 and Akt, were also constitutively phosphorylated, offering a possible explanation for the modest effect of rapamycin alone, as well as novel therapeutic targets. Inhibiting upstream PI3K with CAL101 resulted in a dose-dependent decrease in proliferation (x=51.6±6.6%, range 39.8-62.6%) of the EBV+ B cell lymphoma lines (n=3). Likewise, inhibition of Akt with MK2206 also decreased proliferation (x=52.1±3.5%, range 46.5-58.5%).
T cells, which play a central role in graft rejection of MHC-mismatched organs, also use the PI3K/Akt/mTOR pathway for activation. We show that in vivo treatment of cardiac allograft recipients with either CAL101 or MK2206 prolonged allograft survival from 8.75 days (control) to 21.2 (SEM ±4.48) and 20.5 (±4.31) days, respectively (Fig 2).



Conclusion: The inhibition of signaling nodes upstream of mTOR, such as PI3K and Akt, demonstrates antitumor properties and immunosuppressive effects, suggesting this approach may be an attractive therapeutic option for treatment of EBV+ PTLD.
Support was provided by the Ernest and Amelia Gallo Endowed Postdoctoral Fellowship as well as the Child Health Research Institute and the Stanford CTSA (UL1 TR001085)

452.3

Lymphoproliferative disorders after renal transplantation along two decades: a large longitudinal study of 21.546 recipients

Antonio Franco[6], Dolores Burgos[7], Angel Alonso[4], Naroa Maruri[1], Marta Crespo[2], Domingo Hernandez[7].
[1]Nephrology, Hospital Cruces, Bilbao, Spain; [2]Nephrology, Hospital del Mar , Barcelona, Spain; [3]Nephrology, Hospital carlos Haya, Malaga, Spain; [4]Nephrology, Hospital Juan Canalejo, La Coruña, Spain; [5]Nephrology, hospital Ramon Y Cajal, madrid, Spain; [6]Nephrology, Hospital General, Alicante, Spain; [7]Nephrology, Hospital Carlos Haya, Malaga, Spain.

Post-transplant lymphoproliferative disorders (PTLD) are heterogeneous lymphoid proliferations in recipients of solid organs which seem to be related to Epstein Barr Virus (EBV). The use of antilymphocyte antibodies, EBV seronegativity in the recipient, acute rejection and Citomegalovirus infection have been identified as classical risk factors.
We have evaluated in a longuitudinal study the cumulative incidence of PTLD, its relationship with EBV, presence of classical risk factors and outcome in 21546 simple adult renal transplant recipients from cadaveric and living donors, transplanted in 21 hospitals from 1990 to 2009. The follow up was at least of 5 years.
A total of 272 recipients 193 males (70,9%) and 79 females (29,1%) aged 50,6 (14,7) years, developed PTLD (1,2%), 8.18 cases (8.16;8.19)/10.000 patients/year. Cumulative incidence was 1.72 ‰, 5.72 ‰ and 10.5 ‰ at 1, 5 and 10 years post-transplant. Two hundred forty-two (88,9%) were 1st transplant recipients and 266 (97,7%) from cadaveric donors. At diagnosis the recipients received cyclosporine (58,8%) FK (35,4%), Micophenolate (40,3%), azathiprine (22,6%) and steroids (83,1%) . EBV in the tissue was reported in 94 out of the 155 studied recipients (60.6%) and 86.0 % of the proliferations were due to B lymphocytes . PTLD median appearance after transplant were 42.months (p25,75;12,77,5) , 81,7 in recipients with EBV positive (25;129) and 82,8 in negative (41;109) in tissue (p=0,725).One hundred eighty-eight recipients (69.1 %) had any classical risk factor and the use of antilymphocyte antibodies was the most frequent.
During the follow-up , 172 patients died (63,2%) and 100 (36,7%) had a complete remission, but 20 of them lost their grafts (7,3%). The main cause of death was PTLD progression (n=91, 52,9%),followed by sepsis (n=24, 13,9%).
Patient survival after diagnosis was 53%, 46% and 40% after 1st , 2nd and 5 th years, respectively. Finally, overall graft survival was 48%, 39% and 33% at 1, 2 and 5 years.
In conclusion, most of the proliferations are due to B lymphocytes and seem to have a close relationship with EBV .PTLD can develop in the absence of classical risk factors, the use of antilymphocyte antibodies, the most frequent .There is no relationship between presence of EBV and appeareance after transplant .Although PTLD incidence is low , the prognosis is poor at 1 year, mainly due to PTLD progression, but later the survivors can even mantein their grafts.
Astellas Pharma Spain

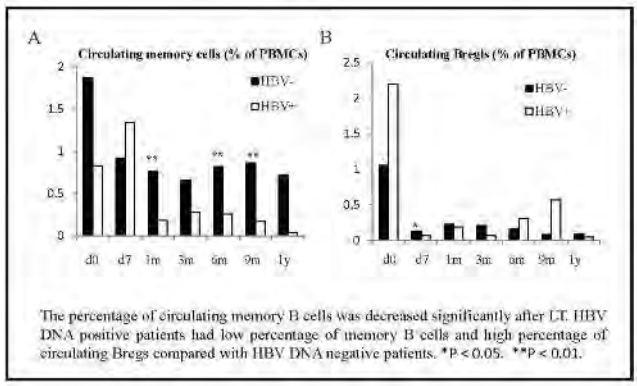
452.4

Stem cell like memory B cells attenuate hepatitis B virus relapse in hepatocellular carcinoma patients post liver transplantation

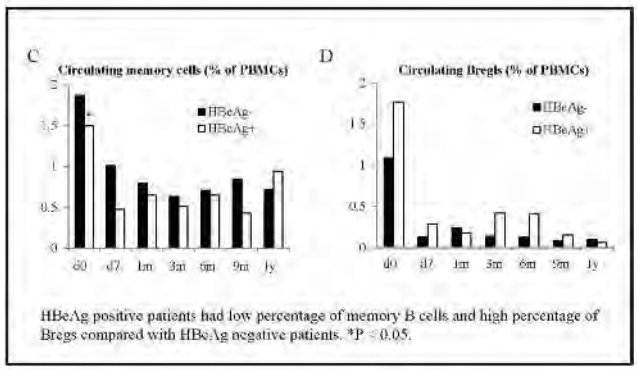
Y Shao, CM Lo, YF Lam, QS Liu, KTP Ng, K Man.
Surgery, The University of Hong Kong, Hong Kong, Hong Kong.

Background and Objective: Memory B cells play an important role in the clearance of hepatitis B virus (HBV). Stem like memory B cells with the ability of self-renewal and multipotency might enhance the clearance of HBV and anti-tumor specifically. We previously reported that the relevance of HBV genotype and HBV-specific CD4 T cell immunity is not responsible for viral clearance after liver transplantation (LT) under antiviral prophylaxis[1]. Here, we aimed to identify stem like memory B cells and investigate their role in HBV relapse in hepatocellular carcinoma (HCC) patients after LT.
Methods: The correlation of circulating B cell subsets and clinical-pathological data were analyzed in 53 HCC recipients before and after LT. The plasma levels of PD1 were detected by ELISA. HBV in liver tissues were detected with Orcein stain. A stem-like memory B cell subset (CD80+PDL2+) was isolated from human bloods and identified by self-renewal and multipotency in vitro.

Fig. 1.



The percentage of circulating memory B cells was decreased significantly after LT. HBV DNA positive patients had low percentage of memory B cells and high percentage of circulating Bregs compared with HBV DNA negative patients. *P < 0.05. **P < 0.01.



HBeAg positive patients had low percentage of memory B cells and high percentage of Bregs compared with HBeAg negative patients. *P < 0.05.

Results: Clinically, there was a close correlation between HBV DNA and the numbers of circulating memory B cells (CD19+CD24+CD38-) (p=0.038). HBV DNA positive patients had less memory B cells (after LT, 1 month: P=0.004, 6 month: P=0.0002 and 9 month: P=0.001) (Fig1. A). Consistently, HBeAg positive patients had less memory B cells compared with HBeAg negative patients before LT (1.5(0.32-4.15) Vs. 1.875 (0.11-16.21) %, P=0.028) (Fig1. C). The percentage of circulating memory B cells was negatively correlated with HBV DNA after LT. The positive association between memory B cells and serum HBV specific IgG level was confirmed by ELISA. In addition, high circulating regulatory B cells (Bregs: CD19+CD24+CD38+) tended to correlate with HBV DNA or HBeAg status (Fig1. B and D). Stem like

memory B enhanced proliferative and survival capacities compared with conventional memory B cells. HBV pulsed memory B cells enhanced CD8+ T cell cytotoxicity, while Bregs suppressed it. Blockade of PD-1/PDL pathways increased memory B-cell activation, proliferation, the production of inflammatory cytokines and IgG production.

Conclusion: Memory B cells may play an important role in attenuation of HBV relapse by switching into HBV-specific plasma cells and regulating CD8+ T cell immunity. Stem memory B cell may prevent HBV recurrence though PD1-PDL interaction.

References:

[1] Y. Luo, C.M. Lo,et al, Hepatitis B virus-specific CD4 T cell immunity after liver transplantation for chronic hepatitis B, Liver Transpl 15 (2009) 292-299.

452.5

Sequential therapy and risk-stratification in PTLD: Pooled analysis of 222 patients treated in the prospective, international, multicentric PTLD-1 trials

Ralf Trappe[1,2,4], Daan Dierickx[3], Heiner Zimmermann[1], Franck Morschhauser[5], Peter Mollee[6], Jan M.Zaucha[7], Martin Dreyling[8], Ulrich Dührsen[9], Petra Reinke[10], Gregor Verhoef[3], Marion Subklewe[8], Andreas Hüttmann[9], Thomas Tousseyn[11], Gilles Salles[12], Volker Kliem[13], Ingeborg A.Hauser[14], Corrado Tarella[15], Eric Van Den Neste[16], Ioannis Anagnostopoulos[17], Hanno Riess[2], Veronique Leblond[18], Sylvain Choquet[18].

[1]Department of Hematology and Oncology, DIAKO Ev. Diakonie-Krankenhaus, Bremen, Germany; [2]Department of Hematology and Oncology, Campus Virchow-Klinikum, Charité - Universitätsmedizin Berlin, Berlin, Germany; [3]Department of Hematology, University Hospital Gasthuisberg Leuven, Leuven, Belgium; [4]Department of Internal Medicine II: Hematology and Oncology, University Medical Center Schleswig-Holstein, Campus Kiel, Kiel, Germany; [5]Département d'Hématologie, Hôpital Claude Huriez, Lille, France; [6]Dept. of Haematology, Pathology Qld, Princess Alexandra Hospital, Brisbane, Australia; [7]Department of Hematology, Medical University of Gdansk, Gdansk, Poland; [8]Department of Internal Medicine III, University of Munich - Campus Großhadern, Munich, Germany; [9]Department of Hematology, Essen University Hospital, University of Duisburg-Essen, Essen, Germany; [10]Department of Nephrology and Intensive Care, Campus Virchow-Klinikum, Charité - Universitätsmedizin Berlin, Berlin, Germany; [11]Department of Pathology, Catholic University Leuven, Leuven, Belgium; [12]Hospices Civils de Lyon, Université de Lyon, Pierre-Bénite, France; [13]Department of Internal Medicine/Nephrology, Nephrological Center Lower Saxony, Hann. Münden, Germany; [14]Department of Internal Medicine III, J.W. Goethe University Hospital, Frankfurt/Main, Germany; [15]Department of Hematology, A.O. Mauriziano, University of Torino, Turino, Italy; [16]Hematology Department, Cliniques Universitaires UCL Saint-Luc, Brussels, Belgium; [17]Department of Pathology, Campus Mitte, Charité - Universitätsmedizin Berlin, Berlin, Germany; [18]Département d'Hématologie, Hopital Pitie-Salpêtriere, Université Pierre et Marie Curie, Paris, Germany.

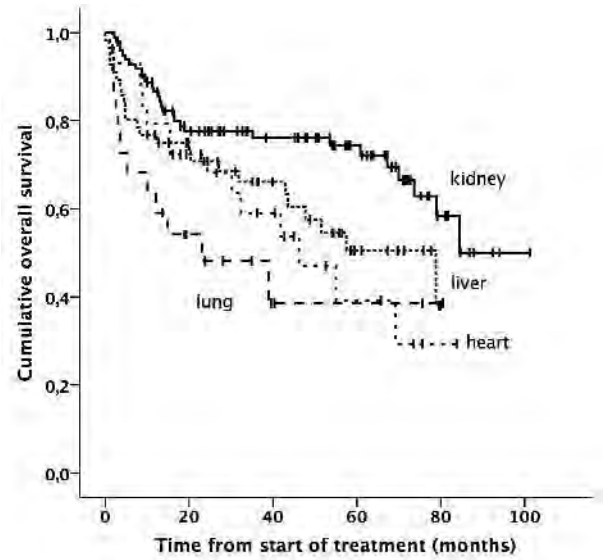
Background: The PTLD-1 trials have tested sequential treatment with rituximab and CHOP-based chemotherapy in CD20-positive post-transplant lymphoproliferative disorder (PTLD). Based on the hypothesis that rituximab consolidation might be sufficient treatment for patients already in a complete response (CR) after rituximab induction, trial treatment was changed in 2007 through a protocol amendment introducing risk-stratified sequential treatment (RSST): rituximab consolidation for patients in CR after rituximab induction and R-CHOP-21 consolidation for all others.

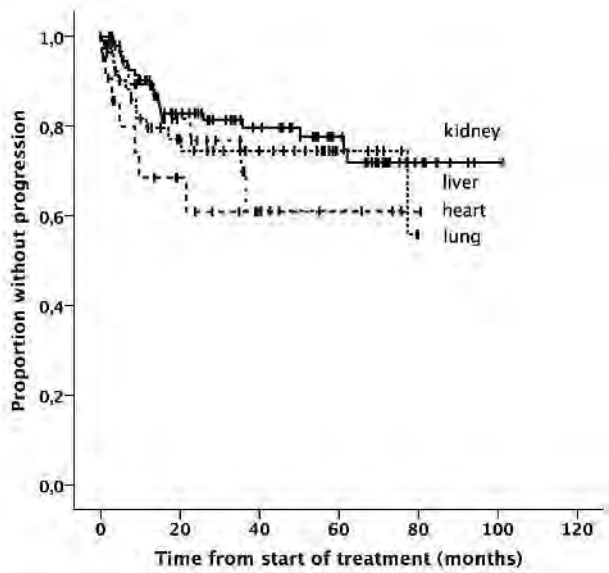
Methods: This is a pooled analysis of the patients treated in both international, multicenter, phase II trial protocols (NCT01458548 and NCT00590447). Treatment-naïve adult SOT recipients diagnosed with CD20-positive PTLD were treated with rituximab (375 mg/m2 IV) on days 1, 8, 15 and 22. In the original PTLD-1 protocol (NCT01458548), all patients continued with four cycles of CHOP21 + GCSF. In RSST (NCT00590447), patients in CR after restaging continued with four three-weekly courses of rituximab monotherapy while all others received 4 cycles of R-CHOP-21 + G-CSF. Inclusion criteria and follow-up schedule were identical; there were no significant differences in the transplant- and lymphoma-related baseline factors. Primary endpoint was treatment efficacy measured as response rates and response duration. Analysis was by intention to treat. 70 patients were treated in NCT01458548 and 152 in NCT00590447.

Results: 162/222 patients were male. 98/222 were kidney, 56 liver, 29 heart, 22 lung, 7 kidney/pancreas, 5 heart/kidney, and 4 heart/lung transplant recipients. Median age at diagnosis was 55 years. PTLD was late (> 1 year after transplant) in 173/222. 96/210 PTLD were EBV-

associated. 169/222 patients (76%) had monomorphic DLBCL-like PTLD, 23 polymorphic and 2 early lesion PTLD. The overall response rate (ORR) was 164/185 (89 %, CR: 128/185 [69 %]). Median follow-up was 4.6 years. Median duration of remission (DR) was not reached; the 3-year Kaplan-Meier estimate was 79%. Median time to progression (TTP) was not reached either. The 3-year Kaplan-Meier estimate was 75%. Median OS was 6.6 years with a 3-year estimate of 67%. There was no significant difference in ORR, DR or OS between EBV-positive and EBV-negative PTLD. On the other hand, response to 4 applications of rituximab was a highly significant predictor of overall survival, time to progression and progression-free survival despite introduction of treatment stratification in the 3rd amendment (all $p<0.001$). In an analysis limited to the most common transplanted organs (liver, kidney, heart, lung), there was a significant difference in OS ($p=0.004$) but not in TTP ($p=0.256$).

Conclusions: This pooled analysis of the two largest ever trial cohorts in PTLD to date demonstrates that sequential treatment strategies in PTLD are feasible, safe and effective in both EBV-positive and negative PTLD and across a range of transplanted organs. Nowadays many patients can be cured and treatment toxicity considerably is reduced by sequential treatment and risk stratification.





452.6

Cancer-related incidence and progression to mortality among kidney allograft recipients in England: a population-cohort analysis between 2003 and 2013

Francesca Jackson-Spence[1], Holly Gillott[1], Sanna Tahir[1], Jay Nath[3], Felicity Evison[2], Jemma Mytton[2], Adnan Sharif[3].
[1]Medical School, University of Birmingham, Birmingham, United Kingdom; [2]Department of Medical Informatics, Queen Elizabeth Hospital, Birmingham, United Kingdom; [3]Department of Nephrology and Transplantation, Queen Elizabeth Hospital, Birmingham, United Kingdom.

Background: Cancer-related incidence and mortality for kidney allograft recipients are raised compared the general population due to the burden of immunosuppression. While our understanding of cancer-related epidemiology has increased over the last decade, no study has ever documented the risk of cancer-incidence post kidney transplantation progressing to cancer-related mortality within the same cohort. In this population-based cohort study, we sought to understand the risk of cancer developing post-transplantation and how this relates to the risk of cancer progression to mortality (both all-cause and cancer-related deaths).

Methods: We obtained data from every kidney-alone transplant procedure performed in England between 2003 and 2013, collecting patient demographics that included age, gender, donor type (living or deceased), socioeconomic deprivation, transplant year, medical co-morbidities (using ICD-10 linked cancer codes) and ethnicity. Data was extracted from Hospital Episode Statistics, with linkage to the Office for National Statistics for mortality data (including causality from death certification).

Results: Data was available for 21,371 kidney allograft recipients who received a transplant in England between 2003 and 2013, with median follow up 6.3 years post-transplantation. After exclusions due to missing demographic data and patients who received combined transplants, we had 18,493 patients over the age of 18 for analysis. The mean age of the cohort was 47.6, 61.5% were male, 78.5% were of white ethnicity, 22.3% lived in the most socio-economically deprived area, 36.3% received a living-donor kidney, 2.8% were on a repeat transplant and pre-transplant cancer was diagnosed in 1.8% of patients. Post-transplant admissions with a cancer diagnosis occurred in 12.3% (n=2,273) of kidney allograft recipient. In total, all-cause (including cancer) and cancer-specific mortality occurred in 2,461 and 444 of kidney allograft recipients respectively at median follow up (up to October 2015). For patients with a cancer admission post kidney transplantation, by median follow up 19.5% had died from cancer-related deaths (n=444) and 10.6% had died from non-cancer related deaths (n=240. Skin cancer was the commonest occurring cancer (43.4% of all cancers), but was associated with mortality in only 4.9% of cancer-related deaths. Cancer sites with the greatest of site-specific deaths within our cohort were lung, digestive and mesothelial/soft tissue (all >40% risk of death by median follow up).

Conclusion: To our knowledge, this study is the first to highlight cancer occurrence post kidney transplantation and risk of progression to all-cause or cancer-related mortality. It demonstrates the competing risks for death for kidney allograft recipients after development of cancer and reinforces the need to further our understanding of cancer epidemiology post-transplantation to optimise care.

Kidney Research UK

452.7

Impact of pulse pressure on patient and graft survival in renal transplant recipients: results from the Collaborative Transplant Study

Bernd Krüger[1], Bernd Döhler[2], Gerhard Opelz[2], Bernhard K.Krämer[1], Caner Süsal[2].
[1]Vth Medical Department, University Medical Center Mannheim, Mannheim, Germany; [2]Transplantation Immunology, Institute of Immunology, University of Heidelberg, Heidelberg, Germany.

Background: In kidney transplant patients, as well as in the general population, systolic blood pressure (SBP) and diastolic blood pressure (DBP) display important predictors for patient, and in the transplant population, graft survival. In addition, pulse pressure (PP), the difference between systolic and diastolic pressure, has been associated with cardiovascular and renal morbidity in the general population. Therefore we conducted the current analysis to test whether increased pulse pressure is associated with patient or graft survival in renal transplant recipients.
Methods: We included all adult patients receiving their first renal transplant from a deceased donor between 1995 and 2014 with both a functioning graft and an available blood pressure reading at year 1, in our analysis for graft and patient survival.
Results: We included 38,411 renal transplant recipients showing that a higher PP at year 1 was significantly associated with inferior death-censored graft survival. The extent of this association was comparable to the association of DBP and SBP with death-censored graft survival. In accordance lower patient survival was significantly associated with a higher PP in a linear fashion. However, in comparison to DBP or SBP this association was stronger for PP. If restricted to "death to cardiovascular disease", the analysis showed similar results for patient survival. Furthermore, we found no additional effect on death-censored graft survival for the different categories of PP when patients presented with a SBP >160 mmHg. In contrast, in patients presented with a SBP >160 mmHg as well as a PP >70 mmHg at year 1, an inferior death-censored graft survival could be shown.
Conclusions: In this large retrospective analysis of first renal transplant recipients receiving a deceased organ, based on prospectively collected data from CTS, we found convincing evidence that PP 1-year post transplant is a superior predictor of patient survival and a good predictor of death-censored graft survival.

452.8

Cardiac biomarkers correlate with changes in left ventricular mass index and diastolic dysfunction following renal transplantation.

Amy Crosthwaite[1,2], Matthew Roberts[3], Rosemary Masterson[4], Adam Hedley[1], Ruth Lim[5], Omar Farouque[2,6], Frank Ierino[1,2].
[1]Nephrology, Austin Health, Heidelberg, Australia; [2]Medicine, University of Melbourne, Melbourne, Australia; [3]Renal Medicine, Eastern Health Clinical School, Monash University, Melbourne, Australia; [4]Nephrology, Melbourne Health, Parkville, Australia; [5]Radiology, Austin Health, Heidelberg, Australia; [6]Cardiology, Austin Health, Heidelberg, Australia.

Background: Chronic and end-stage kidney disease (CKD/ESKD) is associated with profound increase in burden of Cardiovascular disease (CVD). This improves following kidney transplantation but remains a leading cause of death with a functioning graft. Asymptomatic elevation of cardiac biomarkers (high-sensitivity cardiac troponin T (hscTnT) and N-terminal proBNP (NT-proBNP)) and cardiac abnormalities detected by cardiac magnetic resonance imaging (cMRI) and transthoracic echocardiography (TTE) predict patient outcomes[1]. Early identification of abnormal cardiac biomarkers and imaging following transplantation may assist detection of early cardiac pathology and direct therapeutic strategies.
Aim: To define temporal changes in cardiac pathology demonstrated by cMRI, TTE, high-sensitivity cardiac troponin T (hs-cTnT) and NT-proBNP in incident renal transplant recipients (RTR).
Methods: cMRI and TTE were performed at enrolment and after 12 months in incident RTR (n=22). Cardiac imaging parameters measured were; Left ventricular mass index (LVMI), left ventricular ejection fraction (%EF) left atrial area (cMRI) and lateral Ee/e' (TTE). Serum was collected at 18 time points. hs-TnT and NT-proBNP (ECLIA Roche) were measured on a Roche Cobas e602 analyser. Skewed variables were log-transformed and the geometric mean compared using a paired t-test. Non-parametric variables were analysed by the Wilcoxon signed-rank test.

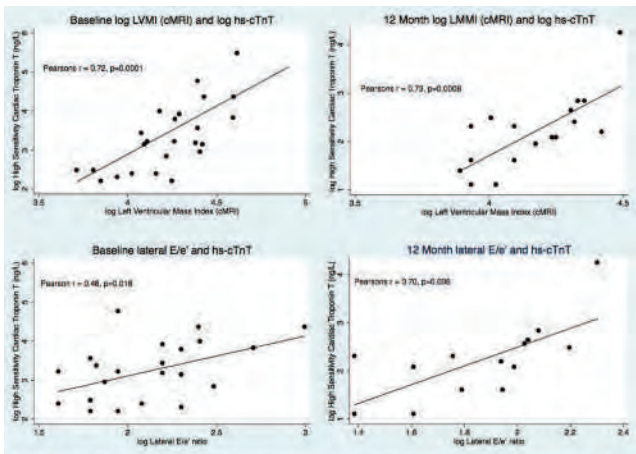


Figure 1. Correlation between high-sensitivity cardiac troponin T and baseline and 12 month (1) LVMI measured by cMRI and (2) lateral E/e' ratio measured by TTE.

Results: 22 RTR (Males 60%, age 48.1±14 years, live-donor transplant 17/22) completed follow up. No CVD clinical events were reported. After 12 months, a significant reduction in cMRI measured LVMI (69.4 g/m2 (95%CI 61-78) vs 63 g/m2 (95%CI 56-69) p=0.021), and lateral E/e' (8 (95%CI 7-9) vs 6 (95%CI 5-7), p=0.032) was demonstrated in RTR. There was a non-significant reduction in left atrial area (25cm2 (95%CI 23-27) vs 23 cm2 (95%CI 21-25), p= 0.080). Both hs-cTnT (median 27ng/L (IQR 18,40) vs 9ng/L (IQR 6,13) p<0.0001) and NT-proBNP (median 755ng/L (IQR 344,1657) vs 106ng/L (IQR 61,184) p<0.0001) were elevated at baseline and improved post transplant.

There was also continued significant decline in hs-cTnT ($p<0.0013$) and NT-proBNP ($p<0.0003$) following stabilization of GFR post transplantation. hs-cTnT demonstrated a strong correlation with LVMI (cMRI) and diastolic but not systolic dysfunction at baseline and 12 months post transplant (Figure 1). ROC analysis confirmed the ability of hs-cTnT and NT-proBNP to predict the presence of LVH (cMRI) and diastolic dysfunction (TTE) (Table 1).

Presence of Cardiac Imaging Abnormality	hs-Cardiac Troponin T		NT-proBNP	
	Baseline	12 months	Baseline	12 months
Left Ventricular Hypertrophy (ROC AUC and 95%CI)				
LVMI by cMRI	**0.85 (0.68-1.00)**	0.77 (0.29-1.00)	**0.88 (0.66-1.00)**	0.79 (0.56-1.00)
LVMI by TTE	0.46 (0.17-0.76)	0.67 (0.18-1.00)	0.64(0.34-0.94)	0.50 (0.00-1.00)
Left ventricular Systolic Dysfunction (ROC AUC and 95%CI)				
Systolic dysfunction by cMRI	0.72 (0.51-0.93)	0.65 (0.39-0.92)	0.63(0.34-0.91)	0.77 (0.48-1.00)
Systolic Dysfunction by TTE	0.55 (0.00-1.00)	0.68 (0.38-0.97)	0.76 (0.28-1.00)	**0.98(0.91-1.00)**
Diastolic Dysfunction (TTE) (ROC AUC and 95%CI)				
Diastolic Dysfunction	**0.81 (0.56-1.00)**	**0.82 (0.60-1.00)**	0.80 (0.57-1.00)	0.69 (0.09-1.00)

Table 1. AUC for Receiver Operating Characteristic (ROC) curve (95%CI) for ability of cardiac biomarkers to predict the presence of cardiac abnormalities.

Conclusion: This study demonstrates that LVMI, hs-cTnT and NT=proBNP reduce post renal transplantation. Higher hs-cTnT levels predict increased LVMI before and after transplantation. The strong association of the biomarkers with LVMI after restoring renal function suggests that increased LV mass stongly contributes to biomarker levels. The correlation of biomarkers with prognostic cardiac pathology indicates potential clinical utility in identifying RTR with early CVD.

References:

[1] Keddis, M. *American Journal of Transplantation.*2013; 13: 406–414

452.9

Integrative, multi-cohort analysis of Epstein-Barr Virus (EBV)-positive and negative tumor samples to identify gene-signatures associated with EBV oncogenesis

Eden Maloney, Erika Bongen, Francesco Vallania, Nikesh Kotecha, Purvesh Khatri, Olivia MMartinez.
Stanford University School of Medicine, Stanford, CA, United States.

Introduction: EBV is linked to a variety of lymphoid and epithelial malignancies. In transplant recipients EBV is associated with the development of B cell lymphomas in post-transplant lymphoproliferative disorder (PTLD). We performed an integrative, multi-cohort analysis of EBV-positive and negative tumor samples to identify shared gene-signatures associated with EBV oncogenesis.

Methods: For our analysis, we selected three gene expression data sets (Gastric Cancer, PTLD, and Hodgkin's Lymphoma) that compared EBV-positive to EBV-negative tumors (n =170, collected from Gene Expression Omnibus). For each data set, we plotted the geometric mean of each gene probe to check for proper normalization and batch effects. The Hedges' g effect size, a measure of magnitude, was calculated for each gene in each dataset. To study the absolute change in expression of the three data sets, a meta-effect size was created by combining the gene effect sizes from each data set. Using a False Discovery Rate of < 0.05 and an Effect Size of 0.8, we utilized two meta-analysis methods, the random-effects model and the Fischer's sum-of-logs method. Leave-one-out validation was used to prevent bias from a large dataset. We validated the EBV-positive tumor signature with an independent gene expression data set (n = 8).

Results: We identified 30 human genes that were significantly up-regulated and five human genes that were significantly down-regulated in EBV-positive tumors. Of the top 15 most significant genes, nine have proposed roles in oncogenesis; for example, PMAIP1 is up-regulated in adult T cell leukemia and SESN2 contributes to p53 signaling. However, only FGR was previously shown to be up-regulated in EBV-associated tumors; thus, these genes provide potential novel mechanisms of EBV oncogenesis. Overall, of the 35 genes, 17 have known or proposed mechanisms in oncogenesis, six have unknown functions, eight are immune regulators, and four are cellular homeostasis enzymes. Of note, CD38 and TLR7, known targets in cancer therapy, were up-regulated in EBV-positive tumors indicating that this approach can identify viable therapeutics.

Receiver operating characteristic curves, violin plots, and forest plots demonstrated that the 35 genes were able to significantly distinguish EBV-positive from EBV-negative tumors in each dataset. Importantly, the EBV-specific gene signature was able to significantly separate EBV-positive and EBV-negative samples in a validation analysis of an independent Burkitt's Lymphoma cell line dataset.

Conclusion: An integrative, multi-cohort analysis of three gene expression datasets produced a gene signature of 35 genes that robustly discerns EBV-positive from EBV-negative tumors. These results suggest common underlying mechanisms of viral transformation in EBV-positive tumors and may identify new opportunities for drug targeting or predictive diagnostics.

453.1

The relative costs and health benefits of an age-matched deceased donor allocation algorithm compared to current practice

Vaishnavi Calisa[1], Jonathan Craig[1,4], Steve Chadban[1,3], Wai Lim[1,6], Kirsten Howard[1,4], Jeremy Chapman[1,2], Stephen McDonald[1,5], Germaine Wong[1,4].
[1]Centre for Kidney Research, The Children's Hospital at Westmead, Sydney, Australia; [2]Centre for Transplant and Renal Research , The Westmead Institute for Medical Research, Sydney, Australia; [3]Royal Prince Alfred Hospital , Sydney, Australia; [4]Sydney School of Public Health, The University of Sydney, Sydney, Australia; [5]ANZDATA Registry, The University of Adelaide, Adelaide, Australia; [6]The University of Western Australia, Perth, Australia.

Introduction: Increasing demand for kidney donors in past decades has led to widespread use of expanded criteria donors (ECD), with variable outcomes for recipients. To ensure equity and utility in graft allocation, one proposed strategy is "age-matching", in which donor-recipient age mismatch is restricted. This approach may improve survival gains for younger recipients but may adversely affect survival and quality of life for older recipients due to the decreased availability of younger donor kidneys for this group. However, the extent to which health benefits occur across different age groups, under age-matching, is unknown. This study aimed to determine the incremental costs and benefits of an age-matched model of deceased donor kidney allocation, in comparison to current practice.
Materials and Methods: Probabilistic Markov models were created to determine the incremental healthcare costs and life years gained under age-matched practice, where donor-recipient age mismatch was ≤10 years, in comparison to current practice in the age groups, ≤30 years, 30 to 45 years, 45 to 60 years and >60 years. Mortality and graft survival estimates were sourced from the ANZDATA registry (1990–2012). Quality of life outcomes and direct healthcare costs were derived from published literature.
Results and Discussion: Compared to current practice, the incremental benefits and costs of age-matched allocation were 0.31 QALYs (SD: 1.95 QALYs) and -$78153 (SD: $146917) in younger recipients (≤30 years). This finding was consistent for recipients between 30 and 45 years. However, an age-matched system incurred negative benefits of 0.33 QALYs (SD: 1.38 QALYs) for those aged between 45 and 60. At a willingness-to-pay threshold of $50,000/QALY, the probability that age-matching was cost-effective compared to current practice was 77% and 63% amongst recipients aged ≤30 years and 30 to 45 years, respectively. Conversely, for those aged 45 to 60 years and over 60 years, these probabilities were reduced to 33% and 29%, respectively (Figure 1). Across all ages, an age-matching algorithm incurred a total loss of 0.16 QALYs (SD: 6.77 QALYs) in comparison to current practice.
Conclusion: At a population level, age-matched deceased donor algorithm did not incur any health benefits compared with current practice. At an individual level, the overall health benefits in the young were at best modest, and at the expense of a significant decline in survival and quality of life among the aged.

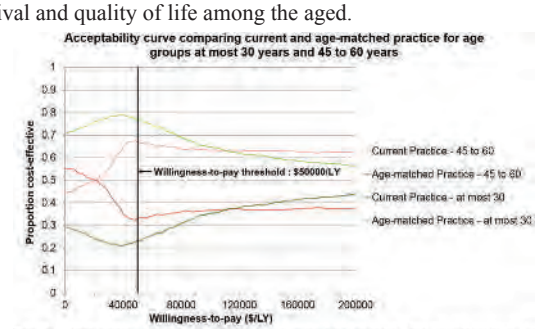


Figure 1: Cost-effectiveness acceptability curve comparing age-matched and current practice for those at most 30 years of age and those 45-60 years of age. The vertical black line indicates the standard willingness-to-pay threshold of $50,000/LY.

453.2

Donor-recipient age mismatches: the relative benefits and costs of waitlisting, transplantation and dialysis

Vaishnavi Calisa[1], Jonathan Craig[1,2], Steve Chadban[1,4], Wai Lim[1,6], Kirsten Howard[1,2], Jeremy Chapman[1,3], Stephen McDonald[1,5], Germaine Wong[1,2].
[1]Centre for Kidney Research, The Children's Hospital at Westmead, Sydney, Australia; [2]Sydney School of Public Health, The University of Sydney, Sydney, Australia; [3]Centre for Transplant and Renal Research, The Westmead Institute for Medical Research, Sydney, Australia; [4]Royal Prince Alfred Hospital , Sydney, Australia; [5]ANZDATA Registry, The University of Adelaide, Adelaide, Australia; [6]The University of Western Australia, Perth, Australia.

Introduction: Increasing demand for kidney donors in past decades has led to widespread use of expanded criteria donors (ECD). In comparison to dialysis, the survival benefits of receiving an ECD graft are age-dependent, with limited gain in life years for younger compared with older recipients. This study aimed to determine the incremental benefits of listing and transplant with various donor-recipient age differences, in comparison to being on dialysis.
Materials and Methods: Using current outcomes for patients on dialysis and graft recipients with a range of donor-recipient age mismatches, probabilistic Markov models were created to compare the health and economic benefits of waitlisting and transplantation, with dialysis. Mortality and graft survival estimates were sourced from the ANZDATA registry (1990 – 2012). Quality of life outcomes and direct healthcare costs were derived from published literature.
Results and Discussion: Compared to dialysis, listing and transplantation was cost-saving by an average of -$105,035 (SD: $326,761). Incremental benefits decreased with increasing donor-recipient age mismatch across all age groups. Amongst the 45 to 60 year-olds, gains of at least 0.70 LYs (SD: 4.54 LYs) and 1.36 QALYs (SD: 2.39 QALYs) and up to 4.58 LYs (SD: 4.90 LYs) and 4.06 QALYs (SD: 3.29 QALYs), corresponding to donor-recipient age mismatches of -25 to -20 years and +15 to +20 years, respectively, were observed. Similar trends were observed for 30-45 year-olds, with incremental benefits decreasing from 6.94 LYs (SD: 5.32 LYs), for age mismatches of -10 to -5 years, to 3.68 LYs (SD: 5.28 LYs) for +20 to +25 years mismatch. For recipients over 60, this trend was less marked, with incremental benefits ranging between 0.72 LYs (SD: 4.37 LYs) and 3.26 LYs (SD: 4.76 LYs). An age mismatch over +27 years offered no survival benefit (Figure 1). At a willingness-to-pay threshold of $50,000/LYs, the probabilities that listing and transplantation was cost-effective compared to dialysis were 90.1% and 99.3% for age mismatches of at least 20 years and -30 to -25 years, respectively (Figure 2).
Conclusion: Minimising donor-recipient age mismatch improves incremental gains in survival for all recipients, in comparison to remaining on dialysis. This strategy will incur the greatest benefits for those under 45, many of whom, currently receive much older renal allografts.

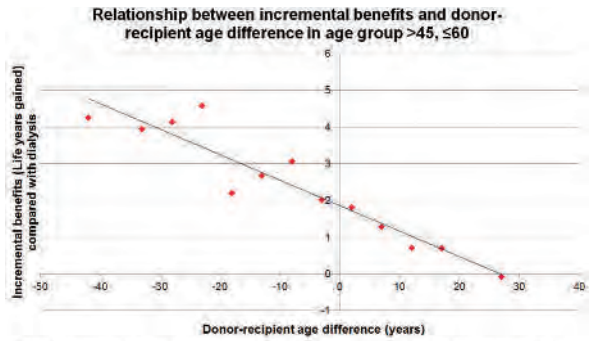


Figure 1 Association between life years gained being listed and transplanted and the donor-recipient age mismatch associated with the transplant

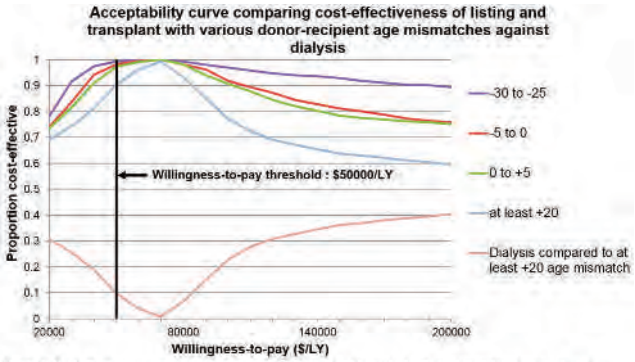


Figure 2. Cost-effectiveness acceptability curve comparing listing and transplant with a range of donor-recipient age mismatches against remaining on dialysis. The vertical black line indicates the standard willingness-to-pay threshold of $50,000/LY.

453.3

Scope and heterogeneity of outcomes reported in Cochrane systematic reviews of kidney transplantation

Benedicte Sautenet[1,2,3], Jonathan C.Craig [1,2], Nicole Evangelidis[1,2], Jeremy R.Chapman [4], John Gill [5], Germaine Wong [1,2,4], Allison Tong[1,2].
[1]Sydney School of Public Health, The University of Sydney, Sydney, Australia; [2]Centre for Kidney Research, The Children's Hospital at Westmead, Westmead, Australia; [3]University Francois-Rabelais, Tours, France; [4]Centre for Transplant and Renal Research, Westmead Hospital, Westmead, Australia; [5]Division of Nephrology, University of British Columbia, Vancouver, BC, Canada.

Aims: The heterogeneity and bias in selecting and reporting outcomes can limit the relevance and utility of systematic reviews (SRs) in informing shared decision-making. We aimed to assess the scope and consistency of outcomes reported in SRs for kidney transplant recipients.
Methods: The Cochrane Database of SRs was searched to November 2015 for published SRs of all interventions for kidney transplant recipients. All outcomes were extracted and clustered into domains, and the frequency of outcomes reported across all SRs was assessed.
Results: 30 SRs with 422 trials reported 1115 outcomes that clustered in 35 outcome domains. Only five outcome domains were reported in at least half of the SRs: mortality (29 SRs [97%]), graft function (25 [83%]), graft loss (24[80%]), graft rejection (17[57%]) and infection (15[50%]). The next three most frequently reported outcomes were cancer (14[47%]), cardiovascular diseases (13[43%]) and lipids (10[33%]). Patient-reported outcomes including mental health, health status, sleep, pain, physical function, were seldom reported (<20% of SRs). There was substantial variability in the tests, timing, and thresholds used to define and measure the outcomes.
Conclusions: Mortality and graft outcomes are frequently reported in Cochrane SRs of kidney transplantation, whereas other patient-centred outcomes including psychosocial status, mental and physical function are uncommon. These findings presumably reflect the outcomes reported in the corresponding trials. A standardised set of core outcomes based on the shared priorities of patients and health professionals in kidney transplantation may help maximise the value of SRs to inform clinical decision-making.

453.4

Timely transplantation: Improving access to early transplantation and active transplantation listing within six months of starting renal replacement therapy

Megan Brown, Kevan Polkinghorne, Orla Maney, John Kanellis. Nephrology, Monash Health, Clayton, Australia.

Introduction: Renal transplantation is the optimal therapy for end stage renal failure (ESRF). In this context, a Key Performance Indicator (KPI) was developed in Victoria measuring the proportion of new ESRF patients <65yo who either receive a transplant or are actively listed within three and six months of starting renal replacement therapy (RRT); with goals of 30% and 50% respectively. This study aims to identify the barriers to meeting this KPI within a single transplant service.

Materials and Methods: Patients <65yo commencing RRT between 01/01/2012 and 12/12/2014 were retrospectively analysed. Data was collected on demographics, dialysis factors, comorbidities, social and systemic factors. Patients were grouped according to whether they met the six month KPI or not. Those not meeting the KPI had their main barrier to listing categorised.

Table 1: Comparison of major factors between those who met the KPI and those who did not

	Listed within 6 months (N=63)		Not listed within 6 months (N=121)		P value
	Total no.	%	Total no.	%	P=
Patient Demographics					
Age at RRT	47	NA	42.8	NA	0.018
Gender (female)	22	35.4%	38	31.4%	0.578
Rural residence	11	17.7%	22	18.0%	0.769
Cause of ESRF					
Glomerulonephritis	23	37.1%	25	20.7%	<0.001
Diabetes	6	9.6%	56	46.2%	
Hypertension/Renovascular	3	4.8%	9	7.4%	
Polycystic kidney disease	12	19.4%	6	5.0%	
Other	19	29.0%	25	20.7%	
Dialysis factors					
Nephrologist review at least 3 months pre RRT	59	95.2%	97	80.2%	0.007
Education pre RRT	60	96.8%	85	70.2%	<0.001
Initial Access					
Fistula	23	37.1%	41	22.8%	<0.001
Tenckhoff	17	27.4%	19	15.7%	
Central Vein Access (Permcath or Vascath)	2	3.2%	61	50.4%	
Transplant (Pre-emptive)	20	32.3%	0	0%	
Type of RRT at 6 months					
Satellite Haemodialysis	23	37.1%	89	73.6%	<0.001
Home Haemodialysis	6	9.6%	6	5.0%	
Peritoneal Dialysis	16	25.8%	24	19.8%	
Transplant	27	43.5%	0	0%	
Other	0	0%	0	0%	
Comorbidities					
Diabetes	12	19.4%	64	52.5%	<0.001
Cardiovascular	11	17.7%	54	44.3%	<0.001
Obesity	10	16.1%	55	45.5%	<0.001
Airways disease	12	19.4%	20	16.4%	0.634
Hypertension	56	90.3%	111	91.0%	0.749
PVD	2	3.2%	26	21.3%	0.001
Infection-related risk	6	9.7%	24	19.7%	0.079
Cancer Risk	9	14.5%	17	13.9%	0.932
Psychiatric illness	10	16.1%	33	27.0%	0.092
Social Factors					
Current smoker	4	6.5%	15	12.4%	0.220
Past smoker	17	27.4%	41	33.9%	
Never Smoked	41	66.1%	65	53.7%	
Non-English Speaking Background	4	6.5%	16	13.2%	0.165
Non-Adherence	3	4.8%	47	38.8%	<0.001
Systemic issues					
Referred for transplant assessment before RRT	60	96.8%	50	40.9%	<0.001

Results:183 patients were included; 62/183 met the KPI, 121/183 did not. 27/62 (43.5%) who met the KPI were transplanted within six months, with 21/62 occurring pre-emptively. There were significant differences in the cause of ESRF and comorbidities between groups ($p<0.001$, table 1). Those not meeting the KPI were more likely to start RRT with temporary access and less likely to receive pre-dialysis education ($p<0.001$; table 1). Most delays were multifactorial, however 27/121 (22%) were limited by systemic factors alone. 96.8% who met the KPI were referred for transplantation assessment prior to RRT with a referral date of 12.8±8.8 (mean±SD) months pre-RRT.

Discussion: Multiple factors make timely transplantation and listing difficult. Living donor and pre-emptive transplants had a large role in allowing patients to meet the KPI, providing the greatest opportunity for early transplantation. Correcting systemic factors could increase the proportion of patients achieving the six month KPI to 48.6%. Other barriers are consistent across multiple units.

Conclusion: Many of the barriers identified are not easily modifiable, however systemic factors have the potential for significant improvement. This involves optimising both referral and workup processes. A state-wide evaluation may be useful to reassess the value of this KPI as an appropriate measurement tool, and to consider a shared approach to dealing with common barriers.

453.5

Roux-en-Y gastric bypass as a bridge to kidney transplantation: effective but how safe?

Ian Thomas[1], Nestor De La Cruz-Munoz[2], Tameka Joseph[3], Jeffrey Gaynor[1], Franco Cabeza[1], David Roth[1], Warren Kupin[1], Adela Mattiazzi[1], Linda Chen[1], George Burke[1], Gaetano Ciancio[1], Junichiro Sageshima[1], Rodrigo Vianna[1], Giselle Guerra[1].
[1]Kidney Transplant, Miami Transplant Institute, Miami, United States; [2]Surgery, University of Miami, Miami, United States; [3]Internal Medicine, University of Miami, Miami, United States.

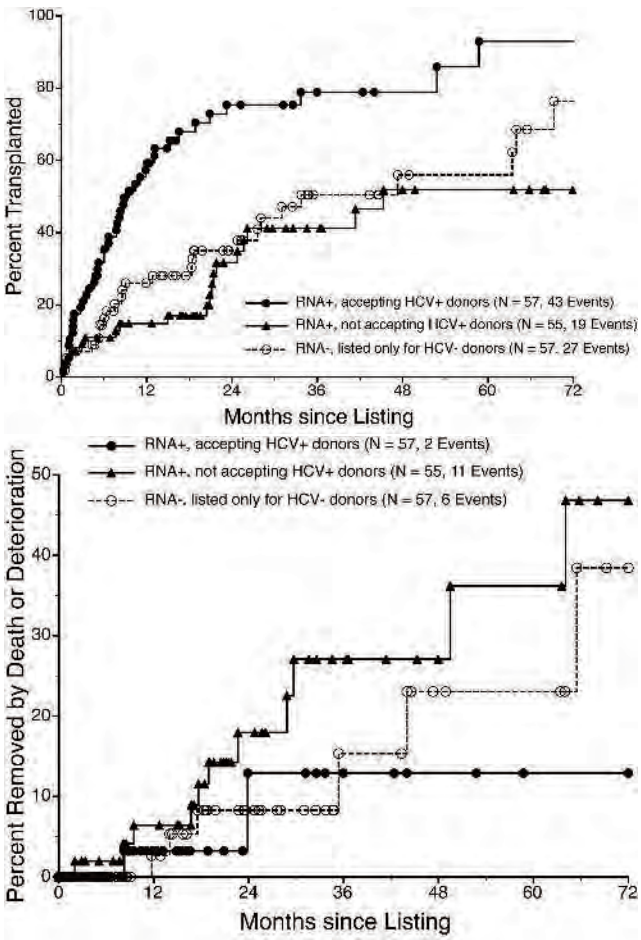
Introduction: Evaluation of body mass index (BMI) in potential kidney transplant recipients is an important component of the pre-transplant evaluation. A BMI greater than 40 kg/m2 (or in some centers more than 35 kg/m2) is considered a contraindication to transplantation due to an increased risk of wound complications, delayed graft function, venous thrombosis, new-onset diabetes after transplantation and cardiovascular complications. Roux-en-Y gastric bypass (RYGB) is frequently performed pre-transplant to help morbidly obese patients reach the target BMI. We sought to evaluate the pre-transplant outcomes for obese patients having RYGB and post-transplant outcomes in the subset of patients who were transplanted.
Materials and Methods: After reviewing the list of patients having RYGB at our institution between July 2009 and June 2014, the group of patients receiving kidney transplants after RYGB was extracted. A cohort of all patients (between March 2009 and June 2011) not having any bariatric surgery procedure with a BMI greater than 37kg/m2 at the time of kidney transplantation was used as a historical control group. The charts of both groups were reviewed to evaluate and compare pre-transplant outcomes after RYGB (where applicable) and post-transplant outcomes. These were followed until May 31, 2015.
Results: Complete anthropometric data were found for 31 of 33 kidney transplant candidates who had RYGB. 11 of these were subsequently transplanted up to the follow up period. 25 historical controls (non-RYGB obese group) were identified.
In the RYGB group, mean BMI (+ standard error) before gastric bypass was 43.5 + 0.7 kg/m2 (range: 35.4–50.5 kg/m2). A total of 87.1% (27/31) achieved a BMI <35 kg/m2. Observed mean percentage excess weight loss was 72.8% + 3.0%. The percentage having improved control of diabetes/hypertension was 29.0% (9/31); 25.8% (8/31) had complications (mostly minor) after gastric bypass.
After transplantation, no cases of new-onset diabetes or oxalate nephrolithiasis/nephropathy were observed. Biopsy-proven acute rejection was seen in 5/11 RYGB patients compared to 3/25 controls (P = 0.002). Infection requiring hospitalization was seen in 9/11 RYGB patients compared to 12/25 in the control group (P = 0.02). Death-censored graft loss occurred in the 2/11 of the RYGB group compared to 3/25 in the control group (P=0.05). No mortality was observed in the RYGB group. 4 deaths with functioning graft occurred in the non-RYGB group, p =0.2. Morbidly obese kidney transplant patients that did not have bariatric surgery before transplantation seem to have a higher mortality than the RYGB group.
Conclusion: RYGB has been successfully used as a bridge to transplantation at our center. The vast majority of patients having this procedure pre-transplant will reach the target weight allowing them to be listed for transplantation in hope of improving patient survival. Moreover, the reported complications of bariatric surgery are usually self-limited and minor. The increased rates of biopsy-proven acute rejection and borderline higher rate of death-censored graft loss may result from changes in pharmacokinetics of immunosuppressant drugs within this population. Further studies assessing this and other variables such as newer bariatric surgery procedures are warranted for short and long-term analysis.

453.6

Hepatitis C RNA-positive patients who are willing to accept hepatitis C seropositive kidneys have better transplant access and favorable waitlist outcomes

Junichiro Sageshima[1], Angelo de Mattos[2], Chandrasekar Santhanakrishnan[1], John McVicar[1], Mehul Gandhi[2], Muna Alnimri[2], Brian Gallay[2], Christoph Troppmann[1], Richard Perez[1].
[1]Surgery, University of California, Davis, Sacramento, CA, United States; [2]Internal Medicine, University of California, Davis, Sacramento, CA, United States.

Background: kidney transplantation from hepatitis C seropositive (HCV+) donors may benefit hepatitis C RNA-positive (RNA+) candidates, but it is not clear how the acceptance/listing status of such kidneys affect waitlist and transplant outcomes.
Methods: In a single-center retrospective analysis, HCV+ transplant candidates (N=169) who were placed on the deceased donor kidney waitlist from March 2004 to February 2015 were evaluated for waitlist and posttransplant outcomes. All RNA+ candidates were offered the option to be listed for HCV+ donors (in addition to HCV seronegative [HCV-] donors) in UNet after informed consent was obtained. RNA- candidates were listed only for HCV- donors.
Results: Fifty-seven patients (51% of all RNA+) were willing to accept HCV+ kidneys and listed both for HCV+ and HCV- donors. During 6-yr follow up, 43 of 57 (75%) RNA+ patients accepting HCV+ donors vs. 19 of 55 (35%) RNA+ patients not accepting HCV+ donors received a deceased donor kidney transplant with median waiting time of 9 mo vs. 45 mo, respectively (P<.0001).



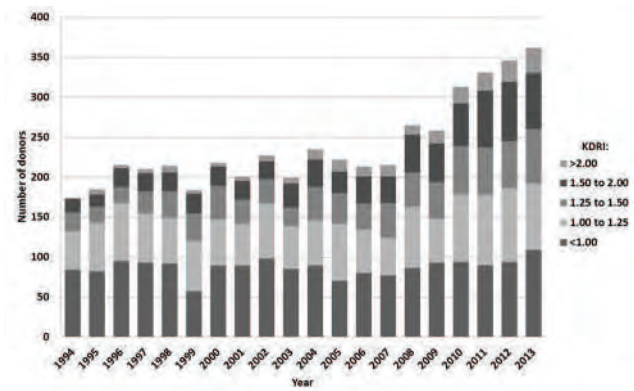
Multivariable analysis demonstrated that HCV listing status (P=.0005) and waiting time (P<.0001) were associated with receiving a transplant. More patients not accepting HCV+ donors (vs. accepting HCV+ donors) were removed from the list due to death or deteriorated medical condition (20% vs. 4%, P=.0046).
Posttransplant patient survival rates were similar for patients accepting vs. not accepting HCV+ donors (70% vs. 67% at 6 yrs, P=.55). On multivariable analysis, donor HCV status did not impact posttransplant patient or graft survival. Patient and graft survival rates for HCV+ vs. HCV- donor transplants at 6 yrs were 74% vs. 59% (P=.93) and 59% vs. 52% (P=.43), respectively.
Conclusion: HCV RNA+ patients significantly benefit if they are willing to accept HCV+ kidneys as they have superior waitlist and equivalent transplant outcomes.

453.7

Change in deceased kidney donor characteristics and kidney donor risk index (KDRI) in Australia and New Zealand over twenty years

Samuel Chan[1,2,3], Scott B. Campbell[1,2,3], Phillip A.Clayton[4,5], David W.Mudge[1,2,3], David W.Johnson[1,2,3], Ross Francis[1,2,3].
[1]Queensland Renal Transplant Service, Princess Alexandra Hospital, Brisbane, Australia; [2]School of Medicine, The University of Queensland, Brisbane, Australia; [3]Department of Nephrology, Princess Alexandra Hospital, Brisbane, Australia; [4]Central Northern Adelaide Renal and Transplantation Service, Royal Adelaide Hospital, Adelaide, Australia; [5]ANZDATA, Australia and New Zealand Dialysis and Transplant Registry, Adelaide, Australia.

Aim: To review changes in deceased kidney donor characteristics in Australia and New Zealand between 1994 and 2013
Methods: A retrospective analysis of all deceased kidney donors in Australia and New Zealand between 1 January 1994 and 31 December 2013 using data from the Australia and New Zealand Organ Donor Registry using the Kidney Donor Risk Index (KDRI) as a surrogate measure of donor quality
Results: Of 4,689 deceased donors, 57% were male and 89% were from Australia. Annual donor numbers increased from 158 in 1994 to 360 in 2013. Mean donor age increased from 35.7 ± 16 years to 46.1 ± 17.6 years over time. As the primary cause of death, motor vehicle accidents reduced from 16.5% to 3.5% and cerebral pathology increased from 52% to 64%. There was an increase in the proportion of donors with hypertension (11.4% to 24.2%) or diabetes (1.3% to 7%) between 1994 and 2013. The proportion of donors who were overweight or obese increased from 38.5% to 58.4%. These changes in donor characteristics were reflected by a rise in the median KDRI from 1.01 ± 0.29 to 1.22 ± 0.4 (slope for linear regression of log-transformed KDRI = 0.009 (95% CI 0.007 to 0.010, p<0.001). When divided into 5 categories of KDRI, the proportion of higher risk donors has increased over time (Figure 1).



Conclusions: As deceased kidney donor numbers have increased, the range of donor quality has increased, with an increase in the proportion and number of high-risk donors, and a decrease in donor quality. These data highlight the need for kidney allocation algorithms to evolve to ensure appropriate allocation of deceased donor kidneys.

453.8

99-100% cPRA deceased donor kidney transplants pre and post new kidney allocation system (KAS) - a single center perspective

Hasan Khamash, Kunam S.Reddy, Marcelo Pando Rigal, Andrew Singer, Raymond Heilman.
Transplant Center, Mayo Clinic Arizona, Phoenix, United States.

The new Kidney Allocation System (KAS) in the United States was implemented on December 4th 2014 with goals of increasing allocation fairness and providing transplant opportunities for difficult to match candidates including the highly sensitized. Prior to KAS candidates with cPRA 99-100% had dismal transplant rates of <3% that increased nationally to >14% due to regional and national priority allocation.
We wanted to assess in our center the percentage and group characteristics of deceased donor kidney alone recipients with cPRA 99-100% pre and post KAS following one complete year of implementation.
We included all kidney alone deceased donor transplants between January 1st 2010 and December 4th 2015. 871 patients were identified, and out of this cohort 30 recipients had cPRA of 99-100% prior to transplant with follow up through January 2016. Prior to KAS 13 patients were transplanted out of 634 deceased kidney transplants(2 %) compared to 17 out of 237 (7.2%) in the year following KAS implementation. The sensitized cohort was mostly female 19/30 (63%), with a history of previous transplants 18/30 (60%) and mostly on dialysis 29/30 (97%) prior to transplant. 20/30 (67%) received rATG induction and all were maintained on 3 drug immunosuppression including prednisone.
We compared the sensitized groups pre and post KAS. They had similar age, demographics, retransplant rates and Kidney Donor Profile Index. They significantly differed in the number of HLA mismatches and the wait time on the list. Table. The increase in wait post KAS is likely due to the roll back of wait time to dialysis start date. Number of HLA mismatches (ABDR) was significantly lower pre KAS with higher percentage of 0 mismatch transplants compared to post KAS.There were 4 cases of rejection in the pre KAS group with one antibody mediated. Only one cellular rejection case post KAS and no graft loss in either group.

	Pre-KAS(13)	Post-KAS(17)	p
Age(yr)	48±12	51±14	.6
Retransplants	8(62%)	10(59%)	.9
Wait Time(days)	1434±1235	2418±1169	.03
HLA mismatches	0.9±1.5	3±1.5	.001
0 mismatch	9(69%)	2(12%)	.001

Conclusion: We demonstrate at the center level the increased transplant percentage for candidates with cPRA 99-100% through regional and national priority allocation of deceased donor kidneys.

453.9

A retrospective cohort study on the association between donor kidney KDRI score and re-hospitalization in transplant recipients

Wilson Lin, Yuliana Noah, Colin Dunn, Stuart Greenstein.
Albert Einstein College of Medicine, Bronx, NY, United States.

Introduction: Although kidney transplantation is considered the best treatment for end stage renal disease, the number of kidneys available for transplantation each year is insufficient to reduce the number of patients on the waiting list. One strategy to expand the kidney donor pool has been to incorporate the use of non-ideal donor kidneys, such as those from hypertensive or cardiac death donors. Several scoring systems exist to assess the quality of these 'marginal' kidneys. While Expanded Criteria Donor (ECD) status has been mainly used in the past, the Kidney Donor Risk Index (KDRI) has recently come to be regarded as a better measurement, since it incorporates more donor factors and is a continuous score rather than a binary indicator. However, studies evaluating its capability to predict recipient outcomes are scarce. This study seeks to determine the degree of correlation between KDRI values of donor kidneys and incidence of re-hospitalization in transplant recipients.
Materials and Methods: A cohort of 526 deceased donor kidneys transplanted between 2003 and 2013 was retrospectively examined. Cox proportional hazards modeling was used to determine the association between KDRI and the occurrence of at least one recipient re-hospitalization within the first two years post-transplant. Poisson regression analysis was used to assess the relationship between KDRI and total number of re-hospitalizations within the first two years post-transplant, as well individually for the first year and the second year. Both analyses were adjusted for the covariates that significantly affected re-hospitalization: time on dialysis, presence of psychiatric illness, and use of mycophenolate mofetil. The Poisson regression analysis only incorporated 471 recipients due to insufficient follow-up duration (< 2 years).
Results and Discussion: The recipient cohort was 42.1% Black, 36.6% Hispanic, and 21.3% White/Other; the mean age was 52.7, mean time on dialysis was 4.61 years, and mean BMI was 27.4. The mean donor kidney age was 43.9; KDRI values ranged from 0.639 to 2.80, with a mean of 1.29. Cox proportional hazards modeling revealed a hazard ratio of 1.180 ($p=0.1609$), meaning the hazard rate increased by ~18% with each unit increase in KDRI. Poisson regression analysis indicated that the expected log count increase in total number of re-hospitalizations per unit increase in KDRI is 0.3006 ($p<0.001$). The increase was 0.3253 ($p<0.001$) during the first year post-transplantation, and 0.2654 ($p=0.002$) during the second year.
Conclusion: KDRI did not correlate significantly with the occurrence of recipients being re-hospitalized at least once ($p=0.1609$). However, it was a significant predictor of total number of re-hospitalizations ($p<0.001$), with a greater impact during the first year post-transplant than the second year (0.3253 versus 0.2654).

454.1

Assessment of immune mediators in plasma for detection of chronic rejection after heart transplantation

Christoph Bara[1], Bianca Przybylek[1], Anneke Neumann[1], Axel Haverich[1], Christine Falk[2].
[1]Department of Cardiothoracic, Transplantation and Vascular Surgery, Hannover Medical School (MHH), Hannover, Germany; [2]Institute of Transplant Immunology, IFB-Tx, Hannover Medical School (MHH), Hannover, Germany.

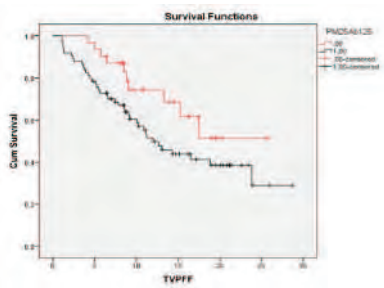
Purpose: Typical manifestation of chronic rejection in transplanted heart is onset of so called cardiac allograft vasculopathy (CAV) in the coronary arteries. It remains a common cause of death after heart transplantation (HTx) and one of the most limiting aspects for long-term graft survival. The inflammatory process is supposed to be one of the central factors in the pathogenesis of CAV. Immune mediators, which are part of an inflammatory immune response, are serologically detectable in various cardiovascular diseases, including native coronary artery disease and myocardial infarction. Therefore, we hypothesized that certain mediators, being present in blood in higher amounts than others, may serve as non-invasive biomarkers for CAV.
Methods: In order to investigate the cytokine microenvironment in these patients, we performed profiling of plasma samples of patients after HTx (n=31) with angiographic confirmed moderate or severe CAV (ISHLT 2 or 3) and a control group of patients without CAV (ISHLT 0, n=23) at least 5 years after HTx. We focused on IL-4, IL-6, IL-10, IL-17, IL-21, IL-23, IL-31, IL-33, IFN-g, TNF-α and sCD40L in order to uncover potential changes in the Th1/2/17 balance. Furthermore, we compared the plasma levels among themselves and correlated the increase or decrease of each of them with another one.
Results: There were no significant differences between the groups for IL-6, IL-23, IL-33, IFN-g and sCD40L concentrations. Although IL-4, IL-21 and IL-31 concentrations were higher in CAV sera, they did not show any statistical significance (p=0,058 – 0,067). However, IL-10 and TNF-α concentrations were significantly elevated in plasma of CAV patients (p<0,05). Eleven combinations of mediator levels correlated with each other in both - the CAV and non-CAV group. There were 15 combinations that correlated with each other in the non-CAV patients but only two of them (IL-21 vs. IL-33 and IL-6 vs. sCD40L) could be found solely in the CAV patients.
Conclusion: The detection of higher IL-10 and TNF-α concentrations in plasma of CAV patients indicates a systemic pro-inflammatory process associated with chronic rejection and the development of CAV. The simultaneous increase of IL-21 and IL-33 or IL-6 and sCD40L may support an acceleration of this process. Thus, cytokine quantification could be helpful to identify the patients with increased risk of developing CAV after HTx.

454.2

Air pollution can support a chronic rejection after heart transplantation

Christoph Bara, D. Böthig, Y. Scheibner, B. Przybylek, A. Haverich.
Department of Cardiothoracic, Transplantation and Vascular Surgery, Hannover Medical School (MHH), Hannover, Germany.

Purpose: The main clinical form of chronic rejection after heart transplantation (HTx) is the development of cardiac allograft vasculopathy (CAV). It is the most limiting factor leading to reduced graft function and one of the most common causes of death in long-term follow-up after HTx. Despite the development of novel immunosuppressive regimes, the prevalence of CAV has not significantly been reduced. A number of studies have shown the association between exposure to air pollution and the development of cardiovascular disease. The impact of air pollution on chronic rejection after lung transplantation has been demonstrated. Therefore, we hypothesized that air pollution could also affect the development of CAV after HTx.
Methods: In order to assess the potential impact of air pollution on CAV, we selected 50 consecutive patients with angiographic-confirmed moderate or severe CAV (ISHLT 2 or 3) that visited our out-patient clinic and 55 consecutive patients without CAV (ISHLT 0). All patients were at least 5 years post-HTx. Based on air pollution data published by the German Environment Office, we estimated the exposure to particulate matter (PM) of different sizes (eg. PM2.5 and PM10). Various dichotomization values for both available PM concentrations were entered into series of Kaplan Meier freedom from CAV estimations to maximize the contrast between the resulting groups. Statistical differences between the curves were assessed with log rank, Breslow-, and Tarone-Ware tests.
Subsequently, a Cox regression model was formed including the potential risk factors i.e. body mass index, CMV-infection, postoperative need for dialysis, diabetes, and hypertension. In order to take into account potential factors with non-proportional hazards, the product of event times and potential factors were also included in the model.
Results: There were no statistically significant differences between patients with or without CAV concerning age, body mass index, donor and recipients' CMV-antigen status, previous CMV-infection, number of previous organ rejections, diabetes, or hypertension. However, the most contrasting air pollution indicator threshold was more than 15 µg/m³ of PM sized 2.5 µm: According to the log rank test, the error probability was 6.5%, Breslow estimated 3.3% and Tarone Ware 4.3%. The stepwise backwards Cox regression resulted in considering the same pollution indicator as the only remaining significant risk factor (p=1.9%, HR = 2.44). Including PM2.5 as a continuous variable, PM2.5 concentration was also a significant independent risk factor for CAV development (p=0.039, HR = 1.155).
Conclusion: Air pollution should be considered as an additional risk factor in the development of CAV after heart transplantation. Further investigations are necessary to confirm these observations in a larger study population and to identify the underlying pathological mechanisms.



454.3

Pre-sensitized immune condition of host exaggerates prolonged cold ischemia-mediated injury of cardiac graft involving in regulatory T cells

Weihua Gong[1], Fangmin Ge[1], Baoqing Liu[2].
[1]Surgery and Medicine, Second Affiliated Hospital of Zhejiang University, Hangzhou , People's Republic of China; [2]Zhejiang University, Hong Kong, Hong Kong.

Background: The detrimental effect of prolonged cold ischemia time (PCI) on pre-sensitized transplanted graft is conceivable, but impact of pre-sensitization status of recipient on PCI-mediated graft injury and inflammation is not well-defined.
Methods: Allogeneic skin grafts from Balb/c donors were transplanted into C57BL/6 recipients for pre-sensitization. Syngeneic or allogeneic heterotopic heart transplantations with PCI were performed using C57BL/6 or BALB/c donors for these recipients through different treatments.
Results: We revealed that PCI could not affect isograft survival but significantly shortened allograft survival in the pre-sensitized recipients. Depletion of regulatory T cells (Tregs) starting 1 day before and after heart transplantation with anti-CD25 mAb remarkably induced intragraft Foxp3 gene expression, worsened architecture damage and subepicardial and intramuscle inflammatory cellular infiltration, and caused a dramatic fall of intragraft CD4+/CD8+ ratio, while adoptive transfer of exogenous wild-type Tregs or endogenous Tregs promoted by Rapamycin had a beneficial effect on preventing the infiltration of T lymphocytes and Gr-1+ neutrophils, reversed intragraft CD4+/CD8+ ratio, preserving cardiac graft architecture. However, their distinct protective mechanisms showed that Rapamycin treatment mainly diminished CD4+ T cells infiltration. Nevertheless, CD4+ still outnumbered CD8+ T cells in the graft, while adoptive transfer of Tregs expanded both CD4+ and CD8+ T cells, particularly CD8+ T cells.
Conclusion: Allogeneic immunoresponses synergistically enhanced PCI effect under pre-sensitized condition. PCI could affect subsequent immunoresponses. Tregs were closely involved in this pathophysiological process. Our data may pave the way to utilize Tregs as a novel therapeutic approach to prevent PCI-mediated injury in the pre-sensitized transplant recipients.
The work was supported by the Department of Education of Zhejiang Province (Grant No. Y201226017), the National Natural Science Foundation of China (No. 81270323), the Qianjiang Talents Project of Zhejiang Province (Grant No.2012R10022), and Zhejiang Provincial Outstanding Youth Foundation (Grant No. LR13H020001)

454.4

Altered immunogenicity of porcine hearts via ex vivo perfusion

William Critchley[1,2], John P.Stone[1,2], Parminder Chaggar[1,2], Qiuming Liao[4], Guangqi Qin[4], Rajamiyer Venkateswaran[3], Trygve Sjöberg[4], Stig Steen[4], James Fildes[1,2].
[1]Manchester Collaborative Centre for Inflammation Research, University of Manchester, Manchester, United Kingdom; [2]The Transplant Centre, University Hospital of South Manchester, Manchester, United Kingdom; [3]Department of Cardiothoracic Surgery, University Hospital of South Manchester, Manchester, United Kingdom; [4]Department of Cardiothoracic Surgery, Lund University, Lund, Sweden.

Introduction: Graft rejection is a major barrier to heart transplantation success and fundamentally requires the activation of recipient immunity. Many donor organs a contain significant leukocyte reservoirs, which, upon transplantation, are able to migrate to recipient lymph nodes and prime recipient allospecific T cells, initiating a rejection response. We aimed to profile the donor myocardial leukocyte content and to determine whether ex vivo perfusion of the heart promotes immune migration into the circuit and thus immunodepletion of the donor organ prior to transplantation.
Methods: Six isolated porcine hearts were attached to an ex vivo circuit and underwent hypothermic, cardioplegic perfusion for 8 hours. Biopsy samples were taken both pre- and post- perfusion. Perfusate samples were taken before organ attachment, and at 2, 4, 6 and 8 hours. Leukocyte populations were quantified in biopsy and perfusate samples by flow cytometry. In perfusate samples, cell-free DNA and 13 cytokines were quantified via PCR and Luminex respectively. A targeted proteomic approach was used to assess the state of the tissue on a molecular level.
Results: Ex vivo perfusion results in the significant depletion of leukocytes from myocardial tissue ($p=0.003$, $p=0.023$, $p=0.024$, $p=0.043$, $p=0.003$ and $p=0.042$ for neutrophils, basophils/eosinophils, classical monocytes, macrophages, NK cells and B cells respectively). The perfusate displayed a selective, pro-inflammatory pattern during perfusion with steadily increasing concentrations of IFN-γ ($p=0.003$, peaking at >8000pg/ml), GM-CSF ($p=0.021$), IL-8 ($p=0.001$), IL-18 ($p=0.001$) and TNF-α ($p=0.001$) detected. Within the perfusate, an increasing concentration of cell-free genomic DNA ($p=0.037$) and mitochondrial DNA ($p=0.063$) was detected. Diminished expression of 9 pro-apoptotic proteins (heme oxygenase 1, death receptor 5, Bad, Bcl-x, pro-caspase-3, claspin, clusterin, serine 46 phosphorylated p53 and TNF receptor 1) was demonstrated in the tissue following perfusion. Stable expression was observed for a further 26 cell death and apoptosis related proteins.
Discussion: We demonstrate for the first time that the donor myocardium contains a significant immune population with the potential to elicit a strong recipient alloresponse. Furthermore, ex vivo perfusion significantly reduces the immunogenicity of the donor heart via the profound loss of resident donor leukocytes, which is likely to reduce direct allorecognition. This novel process may be magnified by active migration in response to inflammatory cytokines in the circuit, particularly IFN-γ, which reaches an extremely high concentration. Despite this pro-inflammatory environment, we also demonstrate a pro-survival profile in the tissue at 8 hours, indicating that myocardial status is significantly improved by perfusion. Preservation of donor hearts via hypothermic, cardioplegic perfusion may therefore provide significant benefit for both organ viability and immunogenicity.

454.5

C4d positive staining is a poor prognostic indicator in cardiac transplant recipients

Carmela D. Tan, E. Rene Rodriguez.
Anatomic Pathology, Cleveland Clinic, Cleveland, OH, United States.

Introduction: The current diagnostic criteria for antibody-mediated rejection (AMR) in heart transplant recipients are based on a combination of histologic and immunopathologic features as recommended in the International Society for Heart and Lung Transplantation (ISHLT) working formulation. Despite published criteria, there are still considerable variations in the technical aspects of diagnosing AMR amongst transplant centers. In this study, we sought to evaluate the clinical significance of C4d staining by immunofluorescence technique in the clinical outcome of heart transplant patients.
Materials and Methods: The study population included 296 adults patients transplanted between January 2006 through March 2012 and who survived more than a year posttransplant. Patient demographics and clinical data including recipient age at the time of transplantation, cytomegalovirus (CMV) status, pre-transplant panel-reactive antibody (PRA), history of cardiovascular surgery or mechanical circulatory support, and immunosuppression at the time of discharge were obtained from the electronic medical record. All endomyocardial biopsies were routinely processed as frozen sections for evaluation of acute cellular rejection (ACR) and AMR by staining for C4d and C3d.
Results: Complement staining was positive in 70 of 296 patients. Fifty-one (17%) of patients were C4d+ on the index biopsy while 19 (6%) of patients were C4d+C3d+. Eleven of 51 (22%) C4d+ patients converted to C4d+C3d+ over an average of 10 month follow-up. C4d+ biopsies occurred mostly as asymptomatic single events that resolved without therapy. At the end of an average length of 73 month follow-up (range: 34-110 months), 38% (15/40) of patients from the C4d+ group died. Mortality in the C4d+C3d+ group was 37% (1/19), in the C4d+ converters 36% (4/11) and only 11% (25/226) in the control group. In a multivariable model, the significant variables that were associated with decreased survival were the presence of complement deposition on biopsy and the use of cyclosporine as maintenance immunosuppression. The results of a multivariable backward Cox proportional hazards regression indicated that the hazard ratios for all three patient groups relative to the control group were significant.
Discussion: Our results show an inferior survival for all patients with evidence of any complement staining in their biopsies. While a high short-term mortality in patients with overt clinical AMR having C4d+C3d+ biopsies and allograft dysfunction is known, this study shows that the poor survival rate of patients with subclinical AMR having C4d+ biopsies without allograft dysfunction was comparable to clinical AMR patients on longer follow-up.
Conclusion: Positive C4d staining in surveillance endomyocardial biopsies is a strong prognostic indicator of worse survival. The optimal management of subclinical AMR however is not yet established.

454.6

Calcineurin inhibitor (CNI) delay with ATG induction: Teaching an old dog, new tricks

Arezu Z. Aliabadi[1], Johannes Goekler[1], Olivia Salameh[1], Thomas Haberl[1], Barbara Steinlechner[2], Phillip Opfermann[2], Alexandra Kaider[1], Keziban Uyanik-Uenal[1], Guenther Laufer[1], Andreas O.Zuckermann[1].
[1]Cardiac Surgery, Medical University of Vienna, Vienna, Austria; [2]Cardiothoracic and Vascular Anesthesiology, Medical University of Vienna, Vienna, Austria.

CNI delay early after heart transplantation is a recommended therapeutic option according to ISHLT guidelines. However, there exist only few published data on feasibility of CNI delay. The aim of this single center retrospective study was to analyze safety and efficacy of CNI delay with ATG induction therapy.
A total of 706 patients were transplanted at our center from 1/96-9/14. All patients received thymoglobuline induction therapy for 1-10 days. CNI therapy was started after the end of ATG-induction. Patients were grouped according to CNI start day (0-1, 2-3, 4-5, 6-7, >7). Freedom from rejection at 1 and 12 months was analyzed in each group by Kaplan-Meier analysis with log-rank test. Changes of creatinine levels (Crea) pre-transplant (preTX) vs. 7 days post-transplant (7d PostTX) were compared in all groups and in sub-groups with crea preTX levels >1.5mg/dl. Differences were analyzed with paired t-test. P-values <0.05 were defined as significant.
Group 0-1 consisted of 20 patients (Crea>1.5mg/ml: n=8; 40%), 2-3: n=173 (n=30, 17%), 4-5: n=219 (n=53; 24%), 6-7: n=224 (n=64; 29%), >7: n=70 (n=25; 36%). Freedom from acute rejection at 1 and 12 months post transplant were: 0-1: 100%, 95%; 2-3: 92.4%, 88.4%; 4-5: 95%, 91.3%; 6-7: 93.3%, 87.5% and >7: 88.6%, 82.9%; p=0.282). Whereas there was no significant change between preTX Crea and 7d postTX in the 0-1 (1.68±1.11 vs. 1.69±1.18) and >7 groups (1.59±1.02 vs. 1.32±0.8), there was significant improvement in groups 2-3 (1.35±0.82 vs. 1.18±0.61; p<0.001), 4-5 (1.43±0.85 vs. 1.21±0.46; p<0.001) and 6-7 (1.42±0.63 vs. 1.18±0.56; p<0.001). In patients with preTX crea >1.5 there was also significant improvement in crea 7d postTX in most groups. 2-3 (2.15±1.52 vs. 1.71±1.00; p<0.01), 4-5 (2.13±1.32 vs. 1.41±0.48; p<0.001), 6-7 (2.02±0.78 vs. 1.48±0.50; p<0.001), >7 (1.78±0.51 vs. 1.42±0.50; p<0.01). Only group 0-1 showed no improvement (2.55±1.35 vs. 2.38±1.54; p=n.s.).
CNI delay of 2 to even >7 days in combination with ATG induction seems to be associated with low rejection rates and improvement of early renal function. Prospective studies are needed.

454.7

Cellular rejection beyond 2 years after cardiac transplantation

Christian Heim, Johannes Roesch, Michael Weyand, Rene Tandler.
Cardiac Surgery, University of Erlangen, Erlangen, Germany.

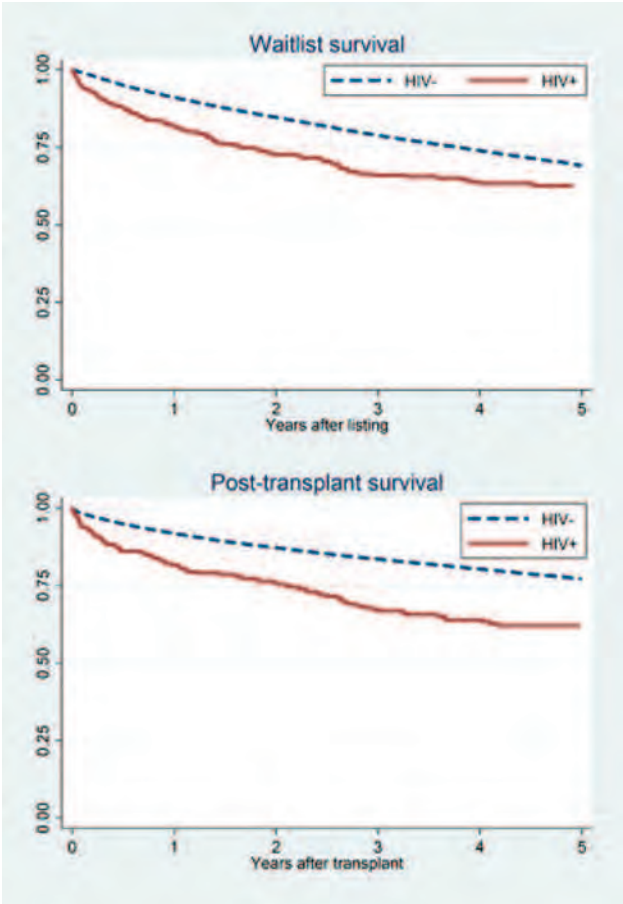
Introduction: Endomyocardial biopsy (EMB) is widely used for routine surveillance of cardiac allograft rejection. The need for continued EMB beyond the first year after cardiac transplantation is controversial. EMB is performed through the jugular or femoral veins and is associated with a complication rate of less than 1%. The aim of this study was to investigate the use of EMB in monitoring long term surviving heart transplant recipients.
Methods: We conducted a retrospective chart review of all patients at our center 2 years or more after heart transplantation. 154 HTx patients between 2000-2012 were included in this study. Significant cellular rejection was defined as grade 2R or 3R using ISHLT nomenclature. Patients were analyzed assessing immunosuppressive regimen and procedural related complications.
Results: Of 154 cardiac transplant patients, 110 (71.4%) had a follow-up of more than 2 years. 17 of these long-term survivors of cardiac transplantation developed at least 1 episode of significant late (>2 years after Tx) cellular rejection (15.5%). Analyzing the respective immunosuppressive regimen showed increased number of calcineurin inhibitor (CNI)-free regimen (64.7%) in patients rejecting late after heart transplantation. Only 35.3% of late cellular rejections occurred in patients treated with Ciclosporin A or Tacrolimus. The overall incidence of procedural related complications was low (1.0%) and none was life threatening.
Conclusion: The above data demonstrates that endomyocardial biopsies continue to detect clinically significant rejection beyond 2 years after cardiac transplantation. Late rejection was not depending on previous episodes of early cellular rejections. Therefore, we recommend routine endomyocardial biopsies in cardiac transplant recipients even though late after transplantation.

455.1

Survival benefit of liver transplantation for HIV+ candidates

Allan B. Massie[1], Christine M. Durand[1], Jayme E. Locke[2], Rhiannon D. Reed[2], Brittany A. Shelton[2], Andrew M. Cameron[1], Dorry L. Segev[1].
[1]Surgery, Johns Hopkins, Baltimore, United States; [2]Surgery, University of Alabama at Birmingham, Birmingham, United States.

In the modern era of antriretroviral therapies, HIV-positive patients are living longer and end-stage renal disease is increasing in this population. Many seek liver transplantation. However, the survival benefit of liver transplantation in HIV+ patients is unknown.
Materials and Methods: We linked SRTR data (comprehensive national registry for transplant patients in the United States) on liver transplant candidates March 2002-Dec 2014 to pharmaceutical claims from IMS Health (a major commercial dataset covering 65% of liver candidates during the study period). HIV status was ascertained by prescription fills of medications specific to HIV. We modeled relative risk of waitlist mortality and post-transplant mortality in HIV+ patients vs HIV- patients using Cox regression, adjusting for MELD score, age, and sex. We modeled benefit of transplant in HIV+ patients in various MELD strata using Cox regression, with transplant date as time of origin for patients who received a transplant, and the date of the earliest MELD score in the stratum for patients who did not receive a transplant in that MELD stratum.
Results and Discussion: Among 71,561 waitlist candidates, 607 (0.8%) were HIV+ at time of listing. In the first 30 days after listing, HIV infection was associated with 6.7-fold risk of waitlist mortality (aHR=$_{4.6}$ 6.7 $_{10.0}$, p<0.001), an added mortality risk equivalent to 9.3 MELD points (Figure, upper panel). Thereafter, HIV was associated with 60% higher risk of waitlist mortality (aHR=$_{1.4}$ 1.6 $_{1.9}$, p<0.001, HIV/time interaction p<0.001), an added mortality risk equivalent to 2.4 MELD points. Waitlist mortality risk associated with HIV was consistent across the range of MELD (MELD/HIV interaction p=0.6). One, three, and five-year post-transplant patient survival rates were 82%, 67%, and 62% for HIV+ recipients vs 92%, 84% and 77% for HIV- recipients (Figure, lower panel). Among transplant recipients, HIV was associated with threefold risk of mortality in the first 6 months post-transplant (aHR=$_{2.1}$ 3.1 $_{4.4}$, p<0.001) and 40% increased risk thereafter (aHR=$_{1.1}$ 1.4 $_{1.9}$, p<0.01, HIV/time interaction p<0.001). Nevertheless, liver transplantation was associated with substantial survival benefit for recipients with MELD of 20 and above (aHR of transplantation = $_{0.5}$ 0.8 $_{1.4}$, p=0.4 for MELD 6-12; $_{0.4}$ 0.7 $_{1.1}$, p=0.1 for MELD 13-19; $_{0.3}$ 0.5 $_{0.8}$, p=<0.01 for MELD 20-29; and $_{0.1}$ 0.2 $_{0.3}$, p=0.<0.001 for MELD 30-40; aHR less than 1 indicates survival benefit from liver transplantation).
Conclusion: Despite increased risk of post-transplant mortality (particularly in the short-term post-operative period), HIV+ transplant recipients in this US-based cohort had high rates of five-year survival and received substantial survival benefit from liver transplantation, suggesting that liver transplantation is a viable treatment for HIV+ patients with end-stage liver disease.



Sally Gustafson, SRTR

455.2

Treatment of HCV-recurrence after liver transplantation with sofosbuvir/ledipasvir: the role of ribavirin

Dennis Eurich[1], Brigitta Globke[1], Raschzok Nathanael[1], Eva Teegen[1], Iman Dimrah[1], Eckart Schott[3], Johann Pratschke[1,2].
[1]General, Visceral and Transplant Surgery, Charité Campus Virchow, Berlin, Germany; [2]General, Visceral, Thoracic and Vascular Surgery, Charité Campus Mitte, Berlin, Germany; [3]Gastroenterology and Hepatology, Charité Campus Virchow, Berlin, Germany.

Introduction: HCV-recurrence after liver transplantation used to be a hard and sad issue in the era of interferon-based treatment. Since the introduction of modern direct acting antivirals, treatment became easier and shorter. According to published data, antiviral treatment duration with sofosbuvir (SOF) und ledipasvir (LDV) may be shortened to 12 instead of 24 weeks using ribavirin (RBV) additionally in the natural course of hepatitis-C-infection. Furthermore, the question, if ribavirin is really necessary in a 12-week SOF/LDV-treatment in transplant setting, is still unanswered.
Patients and Methods: 98 liver transplant patients with HCV-recurrence underwent interferon-free sofosbuvir-based treatment at our institution. 51 genotype 1 and 4 patients were included in the analysis of antiviral efficacy of SOF/LDV with or without RBV. Group A with the shortest and simplest treatment (SOF/LDV for 12 weeks) comprised 29 patients with histologically proven low stage fibrosis (F0-2) and was compared to 22 individuals in the group B (SOF/LDV with RBV or 24 weeks treatment), which comprised 15 patients treated with SOF/LDV with RBV for 12 weeks, 5 patients treated with SOF/LDV and RBV for 24 weeks and 2 patients treated with SOF/LDV for 24 weeks predominantly in case of advanced fibrosis stages (F3-4).
Results: All patients demonstrated a rapid decline of HCV-rna that was measured once a week. By the end of week 4, all patients had HCV-rna-concentration below the limit of detection. All 51 patients achieved ETR and SVR 12 disregarding the treatment mode, previous treatment history and transplant fibrosis stage. No patient in any treatment group had virological breakthrough during the treatment and relapse after treatment. The highest prevalence of adverse events that was significantly different among the groups A and B was observed in the group B with RBV or prolonged treatment regimen (27.6% vs. 86.4%; $p<0.001$). In 55% (n=11) of 20 patients with RBV-comedication RBV-dose had to be reduced and in 40% (n=8) of 20 patients RBV-therapy had to be stopped due to adverse events predominantly due to significant anemia with hemoglobin concentrations lower than 10g/dl in 11 (21.6%) patients. No acute cellular rejection episodes occurred during the treatment and the observation period.
Conclusion: SOF/LDV-combination is a reliable therapy of recurrent HCV-infection after liver transplantation. It is easy to administer and to achieve SVR in immunocompromised patients without interactions with the immunosuppressive medication. Regarding the high rate of adverse events, frequent RBV-treatment discontinuation and 100%-SVR in patients with advanced fibrosis stages, there is no need for RBV in the 12 week SOF/LDV-regimen after liver transplantation.

455.3

Interferon-free antiviral treatment of HCV-recurrence with sofosbuvir containing regimen: evaluation of kidney function

Dennis Eurich[1], Eva Teegen[1], Brigitta Globke[1], Nathanael Raschzok[1], Iman Dimrah[1], Eckart Schott[3], Johann Pratschke[1,2].
[1]General, Visceral and Transplant Surgery, Charité Campus Virchow, Berlin, Germany; [2]General, Visceral, Thoracic and Vascular Surgery, Charité Campus Mitte, Berlin, Germany; [3]Gastroenterology and Hepatology, Charité Campus Mitte, Berlin, Germany.

Background: Since the introduction of directly acting antiviral drugs (DAAs), treatment of hepatitis-c before and after liver transplantation has finally become a highly effective procedure with a low risk profile. The use of sofosbuvir (SOF) is limited to patients with all stages of hepatic and transplant fibrosis and is not recommended for patients with glomerular filtration rate (GFR) lower than 30ml/min, thus denying this HCV-positive subpopulation with renal insufficiency stage IV and V an effective antiviral therapy. Therefore, we performed a retrospective analysis of SOF-based antiviral treatment after liver transplantation especially focusing on kidney function in order to deliver some additional data on this topic.
Patients and Methods: 98 patients were identified who underwent a virologically successful interferon-free, SOF-based antiviral treatment of HCV-recurrence after liver transplantation at our institution. The indication for antiviral treatment was based on viral genotype, transplant fibrosis stage and urgency. Apart from virological and biochemical data renal function (creatinine, GFR) were assessed before, during and after antiviral treatment.
Results: SOF-based antiviral treatment in full dose (400mg/d) was performed with ledipasvir in 52 (53.1%), daclatasvir in 21 (21.4%), simeprevir in 22 (22.4%) and ribavirin (RBV) in 3 (3.1%) genotype 2 patients. There were 3 patients on dialysis (stage V), 3 patients in stage IV, 34 patients in stage III of renal insufficiency and 2 patients after liver and kidney transplantation. The antiviral therapy was well tolerated although no dose reduction was performed and no SOF-related adverse events occurred during and after the treatment. Mean GFR did not show any significant differences at the moment of treatment initiation 64.3ml/min at the end of treatment 63.8ml/min and 12 weeks after treatment 63.1ml/min. Stages of renal insufficiency were equally distributed before and after the antiviral treatment.
Conclusion: Although SOF-based treatment is not recommended in patients with severely impaired kidney function (stages IV-V), no further deterioration of kidney function could be observed in the present analysis suggesting that SOF may be used as a therapy backbone without dose reduction in this specific, difficult to treat, liver transplant population.

455.4

A closing chapter – therapy of HCV genotype III recurrence in liver transplants

Eva Teegen[1], Brigitta Globke[1], Eckart Schott[2], Johann Pratschke[1], Dennis Eurich[1].
[1]Department of General, Visceral and Transplantation Surgery, Charité, Berlin, Germany; [2]Medical Department, Division of Hepatology and Gastroenterology, Charité, Berlin, Germany.

Introduction: Historically, HCV-genotype (GT) 3 was not as hard to treat as genotype-1 HCV-infection using interferon-based therapy. Nowadays, GT-3-HCV-infection can easily be assessed using interferon-free regimes such as the combination of sofosbuvir (SOF) and daclatasvir (DCV) as a highly successful and reliable therapeutical option.
We report a successful antiviral treatment of our last 10 GT-3 patients suffering from HCV-recurrence after liver transplantation with SOF/DCV.
Patients and Methods: This report presents a descriptive analysis of 10 patients who were transplanted due to a GT-3 cirrhosis in our center. Only two of them were naïve for any antiviral therapy. All of them received antiviral treatment with SOF/DCV for 12 weeks after liver transplantation; in one case ribavirin was additionally applied. The endpoint was HCV-RNA free survival after 12 weeks of therapy. Secondary endpoints were preservation of renal and liver function and incidence of adverse events.
Results: All patients were free of HCV-RNA at the latest from 8 weeks of therapy. Elevated transaminases and gamma-glutamyl transferase at the beginning of therapy normalized during the therapy. Bilirubin and alkaline phosphatase were stable at all dates. There were no severe side effects especially on renal function or blood count. Sustained virological response rates at week 12 were achieved in all 10 patients.
Conclusion: HCV could be eliminated in all patients after liver transplantation by antiviral treatment of SOF/DCV over 12 weeks. SOF/DCV is a safe and reliable antiviral therapy of recurrent GT-3 HCV-infection that allowed us to close the chapter of HCV-recurrence after liver transplantation in our outpatient clinic.

455.5

The use of hepatitis B core antibody positive donors for liver transplantation does not lead to inferior survival

Tiffany C.L. Wong[1], James Y.Y. Fung[2], See Ching Chan[1], Chung Mau Lo[1].
[1]Surgery, The University of Hong Kong, Hong Kong, Hong Kong;
[2]Medicine, The University of Hong Kong, Hong Kong, Hong Kong.

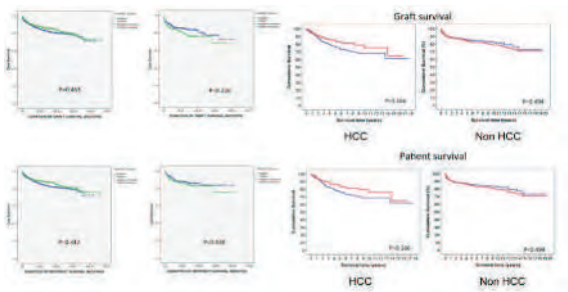
Introduction: It is still controversial whether the use of hepatitis B core antibody (HBcAb) positive graft would lead to an inferior survival after liver transplantation (LT). Consensus is lacking regarding the optimal regimen of anti-viral prophylaxis in these patients and the reported risk of de novo hepatitis B (HBV) infection ranged from 0-14%. It was suggested that such risk was higher especially when HBcAb positive grafts were transplanted to non-HBV recipients. Our study aims to evaluate the long-term outcomes and risk of de novo HBV in using HBcAb positive grafts.
Method: We evaluated all patients who were transplanted in our center from 1996 to 2015. All data was retrieved from a prospectively collected database.
Results: There were 1004 adult LT and 438 grafts (43/6%) were from HBcAb positive donors and 326 (74.4%)of these grafts were transplanted to HBV recipients while 112 (25.6%) were transplanted to non-HBV recipients. All recipients received oral antiviral prophylaxis only and had no hepatitis B immunoglobin (HBIG). Table 1 showed the clinical characteristics of donors and recipients as related to HBcAb status.
Figure 1 showed the use of HBcAb positive grafts to HBV and non-HBV recipients. In fact the long-term patient and graft survival at 10 years are >80% for both group. The use of HBcAb positive graft in patients with and without hepatocellular carcinoma did not influence long-term graft and patient survival either.

Table 1. Clinical characteristics of donor and recipient as related to HBcAb status

Donors	HBcAb+ graft (n=438)	HBcAb- graft (n=566)	P value
Age	47 (12 – 77)	35 (2 – 84)	0.000*
% Male	202 (46.1%)	270 (47.7)	0.618
BMI	22.5 (12.9 – 35.2)	22.2 (12.8 – 35.6)	0.079
Cold ischemic time	160.5 (60 – 924)	133 (53 – 742)	0.001*
Warm ischemic time	52 (25 – 146)	51 (24 – 151)	0.043*

Recipients	HBcAb+ graft (n=438)	HBcAb- graft (n=566)	P value
Age	51 (17 – 69)	52 (16 – 73)	0.210
% Male	325 (74.2%)	407 (71.9%)	0.418
HCC vs. non HCC	330 : 308	161 : 405	0.669
BMI	23.8 (15.1 – 47.7)	24.2 (15.4 – 42.9)	0.627
MELD	22 (6 – 59)	21 (6 – 53)	0.702
LDLT vs. DDLT	218 : 220	339 : 227	0.001*

In fact, there was no difference in outcomes when HBcAb positive grafts were used in living donor vs. deceased donor LT; recipient MELD <30 vs. ≥30 and MELD <45 vs. ≥45.
Among the 112 non-HBV patients who received a liver graft from HBcAb positive donors, only 1/112 (0.9%) developed de novo HBV. HBV DNA was 179.3 copies/ml at time of HBV recurrence and liver biopsy showed graft fibrosis and had graft failure.
Conclusion: Our study has demonstrated the outcomes of HBcAb positive grafts in LT were excellent with minimal risk of de novo HBV. The use of oral antiviral therapy alone is adequate to prevent de novo HBV. The use of HBcAb positive grafts in non-HBV recipients is also safe.



455.6

Hepatitis B vaccination in patients receiving antiviral monotherapy after liver transplantation for chronic hepatitis B

Tiffany Wong[1], James Y.Y.Fung[2], See Ching Chan[1], Chung Mau Lo[1].
[1]Surgery, The University of Hong Kong, Hong Kong, Hong Kong;
[2]Medicine, The University of Hong Kong, Hong Kong, Hong Kong.

Introduction: Life long antiviral prophylaxis is required for patients who are transplanted for hepatitis B (HBV). Our study aimed to determine if a double-dose pre-S containing HBV vaccination (Sci-B-VacTM) could elicit an adequate and sustainable immune response in HBV patients who developed previous hepatitis B surface antibody (Anti-HBs) response after liver transplantation (LT).
Method: All patients who were transplanted for HBV related disease for >1 year with normal graft function and hepatitis B surface antigen (HBsAg) seronegativity were evaluated. They were recruited to receive a 40mcg pre-S containing HBV vaccine if they were responders in our previous vaccine trial, or anti-HBs was positive for >1 year after LT or a peak anti-HBs at any time point after LT was >100mIU/ml. All patients received oral antiviral therapy as prophylaxis, none of them had hepatitis B immunoglobulin (HBIG) at any time point before or after LT. Primary endpoint was the development of anti-HBs ≥10mIU/ml from previous negative value or a 1-log increase from baseline. Anti-HBs up to 32 months after vaccination was monitored.
Results: Eighty-six patients were recruited; 5 were responders from previous trial; 45 patients had detectable anti-HBs >1 year after LT and 36 patients had an anti-HBs >100mIU/ml. Figure 1 showed the anti-HBs response of all responders. All (5/5,100%) previous responders responded to booster vaccination and all demonstrated a positive response 2 weeks after vaccination with median anti-HBs of 638 (range 146-1000)mIU/ml. Anti-HBs remained detectable in all patients at 32 months followup. For the remaining 81 patients, 10/81 (12.3%) responded. Table 1 showed the characteristics between responders and nonresponders in the 81 patients. Majority (8/10, 80%) of them developed a positive response at 2 weeks after vaccination; the remaining 2 patients responded at 1 month and 3 months after vaccination. At 32 months after vaccination, anti-HBs remained detectable in 3/10 (30%) patients.
Conclusion: All previous responders responded to booster vaccination, implying durability and memory of HBV immune response, which is an important prerequisite for definitive host immunity for HBV. In patients who had spontaneous and significant anti-HBs production after LT, a single HBV vaccination can induce response in 12.3% of patients which was higher than that reported in literature. The fact that none of our recipient received HBIG, and anti-HBs persisted up to 32 months in 8 patients after vaccination, demonstrating a spontaneous production of anti-HBs which is independent of adoptive immunity transfer from donor and passive immunity. This group of patients might be potential target for anti-viral prophylaxis withdrawal.

Figure 1. Anti-HBs response in responders. a) Previous responders b) Patients who had persistent anti-HBs >1 year after LT or peak anti-HBs >100mIU/ml

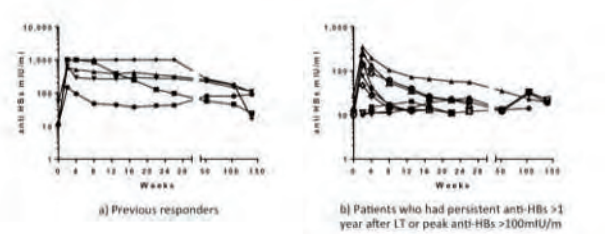


Table 1. Baseline characteristics of patients

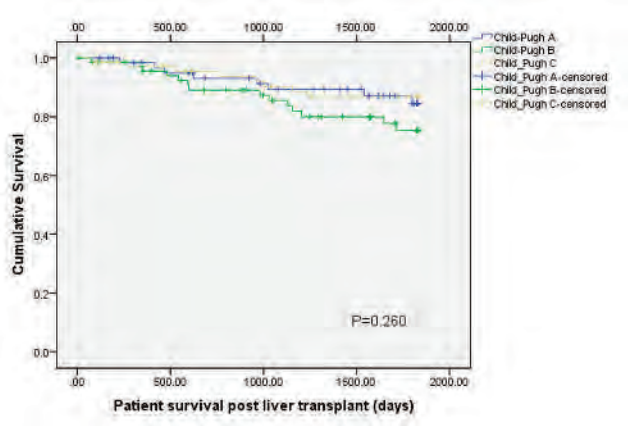
	Responders n=10	Non-responders n=71	P value
Age	48.5 (27-63)	52 (24-67)	0.31
Sex (n, % male)	8 (80)	60 (84.5)	1.00
Disease indication			
Acute/acute on chronic	3 (30)	9(12.7)	
Cirrhosis	3 (30)	35(49.3)	
Hepatocellular carcinoma	4 (40)	27 (38)	0.46
Graft type			
LDLT vs. DDLT (n,%)	8 (80): 2 (20)	41(57.7): 30 (42.3)	0.32
Time from LT to current study (months)	131.5 (62.5-155.6)	105.1 (57.4-163.2)	0.11
Antiviral therapy			
Lamivudine	8 (80)	29 (40.8)	
Lamivudine + adefovir	0 (0)	11 (15.5)	
Lamivudine + tenofovir	1 (10)	4 (5.6)	
Entecavir	1 (10)	26 (36.6)	
Tenofovir	0 (0)	1 (1.4)	0.15
Duration of antiviral therapy before LT (months)	0.1 (0-82.2)	1.3 (0-131.5)	0.06
HBeAg seropositive before LT	1 (10)	24 (33.8)	0.25
HBV DNA at LT (copies/ml)	15x 10^3 (35-7.2x 10^6)	12x 10^3 (0-4.2x 10^6)	0.84
Presence of HBV mutant (n,%)	0 (0)	14 (19.7)	0.27
Time for HBsAg seroconversion after LT (months)	0.7 (0-1.2)	0.8 (0-14.8)	0.16
Peak anti-HBs after LT	61 (11-342)	165 (20-1000)	0.09
Donor anti-HBc (n,%)	5 (50)	42 (59.2)	0.70
Donor anti-HBs (n,%)	7 (70)	63 (88.7)	0.04

455.7

Child-Pugh classification cannot predict survival of hepatocellular carcinoma patients after liver transplantation

Walid Elmoghazy[1,2], Norman Kneteman[1].
[1]Department of Surgery, Hepatobiliary, Pancreatic Surgery, and Transplantation, University of Alberta Hospital, Edmonton, AB, Canada; [2]Department of Surgery, Sohag University, Sohag, Egypt.

Background: According to Barcelona clinic algorithm for management of hepatocellular carcinoma (HCC) patients, patients who are classified Child-Pugh class (CPC) C, should be offered only palliative care. So, we aimed to study patients who were transplanted at our institution due to HCC and to assess impact of CPC on their prognosis after transplant.
Patients and Methods: A retrospective analysis of 202 patients with non-incidental HCC transplanted between October 1990 and October 2013 was done. The end point was 5-year survival after liver transplantation. Comparison of survival according to CPC was done using log-rank test.
Results: Over a period of 20 years, 202 patients with non-incidental HCC were transplanted in our center with a mean follow-up of 4.5 years. Mean age at time of transplant was 55.5 ± 8.3 years and 164 (81.2%) patients were males. HCV-related cirrhosis represented the underlying pathology in 107 patients (53%). Patients with CPC classes A, B, and C were 63 (31.2%), 70 (34.6%), and 69 (34.2%), respectively. The 5-year survival was 85.1% while the overall patient survival was 70.6% during the follow-up period. The 5-year survivals for patients with Child-Pugh classes A, B, and C were 87.3%, 80%, and 88.4%, respectively with no significant statistical difference (P = 0.26) (figure 1).
Conclusions: Child-Pugh classification could not stratify the outcome of patients undergoing liver transplantation for hepatocellular carcinoma and should not be used in selection of patients.



455.8

Hepatic epithelioid haemangio-endothelioma and adult liver transplantation: Extra-hepatic disease is not a contraindication to liver transplantation. Report of the European liver transplant registry including 149 patients

Quirino Lai[1], Estelle Feys[1], Vincent Karam[2], Rene Adam[2], Jurgen Klempnauer[3], Martin Oliverius[4], Vincenzo Mazzaferro[5], Andreas Pascher[6], Piotr Remiszewski[7], Helena Iesoniemi[8], Jacques Pirenne[9], Aksel Foss[10], Bo GEriczon[11], Sasa Markovic[12], Jan Lerut[1].
[1]UCL, S. Luc Hospital, Brussels, Belgium; [2]Liver Transplant Unit, Centre Hépatobiliaire Paul Brousse, Paris, France; [3]Liver Transplant Unit, Medizinsche Hochschule Hannover, Hannover, Germany; [4]Liver Transplant Unit, Transplancenter IKEM, Prague, Czech Republic; [5]Liver Transplant Unit, Istituto Nazionale Tumori, Milan, Italy; [6]Liver Transplant Unit, Charité Campus Virchow Klinikum, Berlin, Germany; [7]Department of General Transplant and Liver Surgery, Medical University of Warsaw, Warsaw, Poland; [8]Liver Transplant Unit, UCJ Helsingfors, Helsinki, Finland; [9]Liver Transplant Unit, Universitaire Ziekenhuizen Gasthuisberg KULeuven, Louvain, Belgium; [10]Liver Transplant Unit, Oslo Universitetssykehus, Oslo, Norway; [11]Liver Transplant Unit, Karolinska University Hospital, Stockholm, Sweden; [12]Liver Transplant Unit, University Medical Centre Ljubljana, Ljubljana, Slovenia.

Background: Hepatic epithelioid haemangio-endothelioma (HEHE) is a rare vascular tumour with an intermediate aggressiveness. Due to its rarity, the surgical management is still not yet standardized despite the fact that liver transplantation (LT) has been valued in its treatment even in the presence of extrahepatic disease (EHD). The aim of the present study is to further define the value of LT in the current management of HEHE and to identify risk factors for recurrence after LT.
Methods: outcome of one hundred forty-nine patients collected in the European Liver Transplant Registry (ELTR) during the period November 1984-May 2014 has been analysed. Median follow-up after LT is 7.6 years (IQR; 2.8-14.4).
Findings: At Cox regression analysis, only macro-vascular invasion (hazard ratio 4.8; p-value < 0.001) and waiting time before LT ≤ 120 days (HR: 2.6; p-value: 0.01) were significant risk factors for recurrence. Hilar lymph node invasion and LT performed during the period 1984-2003 almost reached significance (p-values 0.07 and 0.12, respectively). Importantly, pre-LT EHD was not a risk factor for post-transplant recurrence.
Five-year disease-free survival rates were 84.7 vs. 44.0% in patients without or with macro-vascular invasion (p-value < 0.001) and 86.7 vs. 73.3% in patients without or with WT ≤ 120 days (p 0.02).
Interpretation: The detailed analysis of this worldwide largest cohort of adult liver recipients transplanted because of HEHE clearly confirms the value of LT in the treatment of this rare vascular hepatic disease. EHD is not a contraindication to transplantation. Tissue examination of lymph nodes and tissues, including Factor VIII antigen immunohistochemistry staining, are needed in order to stage the real disease extension. This information is of importance as it will allow to better appreciate the risks for tumour recurrence after (partial as well as total) hepatectomy.

455.9

Liver transplantation and liver resection for treatment of hepatoblastoma: 10-years single-center experience

Oleg Kotenko, Marat Grigiryan, Denis Fedorov, Alexander Grinenko, Alexander Korshak, Alexey Popov, Andrey Gusev, Alexander Ostapishen, Artem Minich.
Liver Surgery and Transplantation, National Institute of Surgery and Transplantology, Kiev, Ukraine.

Introduction: Hepatoblastoma is the most common malignant liver tumor in childhood. Achievement of complete surgical resection is the mainstay of multimodal treatment for hepatoblastomas. Overall, outcomes have greatly improved because of advances in chemotherapy and innovations of surgical technologies, including the liver transplantation.
Materials and Methods: The aim of this study was to retrospectively review our results of hepatoblastoma surgical treatment. The medical records of patients with hepatoblastoma, treated in our institution from 2005 to 2015, were reviewed. 60 children with a hepatoblastoma have entered the study. The age range at surgery was 8 months to 5.6 years (median 28 months), with a male to female ratio of 3:1. The patients were classified according to the pretreatment extent of disease (PRETEXT) grouping system. 3 patients (5%) were PRETEXT group I, 19 (31,6%) group II, 30 (50%) group III and 8 (13,4%) group IV. 12 patients PRETEXT I-II underwent primary surgery. 48 patients were treated with preoperative chemotherapy. 51 patients received postoperative chemotherapy. The standard perioperative treatment is 4 cycles of preoperative chemotherapy followed by surgical treatment and 2 postoperative cycles of chemotherapy («PLADO» or «superPLADO»). Patients with PRETEXT IV tumors, multifocal tumors and tumors invading major vessels of the liver underwent liver transplantation. Surgical procedures performed included: right trisectionectomy (N.23), left trisectionectomy (N.7), right hemihepatectomy (N.9), left hemihepatectomy (N.4), left lateral sectionectomy (N.3), mesohepatectomy (N.1) , central and anterior bisegmentectomy (N.5), living donor liver transplantation (N.7), living donor liver transplantation with total pancreatectomy, splenectomy, gastroduodenectomy, mesenterico-caval shunting, cavoportal transposition (N.1) in one case hepatoblastoma with portal vein and splenic vein invasion and tumor thrombosis.
Results: The overall 1-,3- and 5-year survival rate for 52 patients after liver resection and for 8 patients after LDLT in the study was 90,4%, 82,7%, 71,1% and 100%, 87,5%, 75%, respectively. Recurrence-free 1-,3- and 5-year survival rate for 52 patients after liver resection and for 8 patients after LDLT in the study was 86,5%, 76,9%, 69,2% and 100%, 87,5%, 75%, respectively. Operative morbidity and mortality after liver resection and after LDLT were 17,3% and 12,5%, 9,6%, and 0%, respectively. 5 patients died after extended hepatectomies. After a median follow-up of 3.8 years (range 1.5-5.3 years), 42 (70%) of the patients were tumor-free in remission and 18 (30%) had died.
Conclusion: Favorable long-term outcome could be expected for hepatoblastoma with multidisciplinary treatment involving neoadjuvant chemotherapy, surgical treatment with complete tumor excision followed by adjuvant chemotherapy. Liver transplantation is a successful treatment option for children with unresectable hepatoblastoma.

456.1

Characterization of hematopoietically-derived CNS macrophages following bone marrow transplantation

Kurt Pruner, Zachary Heroux, Matthew DeFazio, Marina Rocha, Todd Williams, Josef Kurtz.
Department of Biology, Emmanuel College, Boston, MA, United States.

Microglia/macrophages of the central nervous system (CNS) consist of a heterogeneous population, although unlike other tissue-specific macrophages, post-fetal microglial populations have been observed to undergo self-renewal in the brain parenchyma, and limited contribution from hematopoietically-derived monocytes in the absence of an inflammatory state has been described. We have previously described the appearance of a small, but readily observable population of hematopoietically-derived CNS macrophages that demonstrate similar characteristics to resident microglia (both by flow cytometry within the CD11b+/CD45mid population and by immunofluorescence microscopy of Iba1^{+} cells with ramified morphology) following syngeneic bone marrow transplantation.

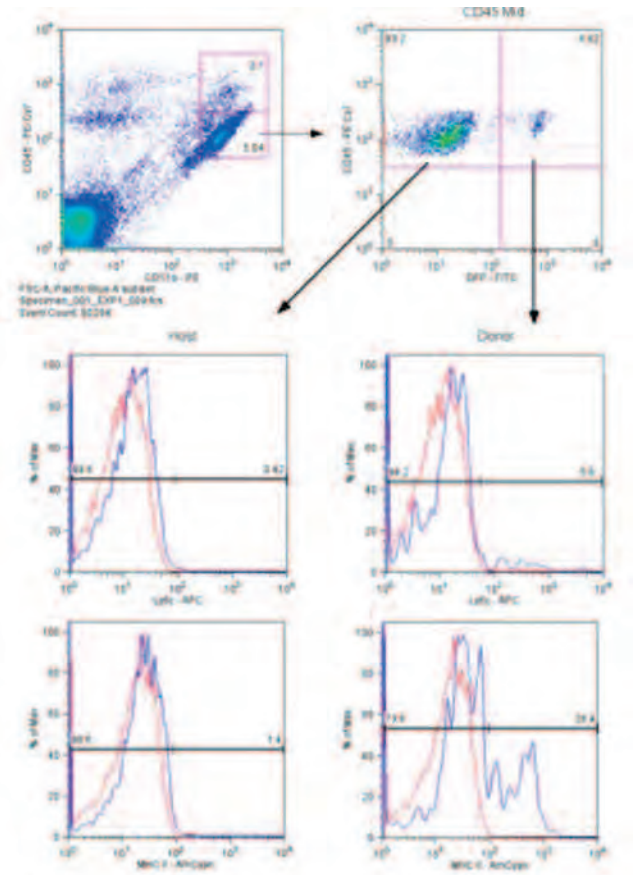


Figure 1. Pseudo-dot plots of data obtained by flow cytometry isolate cell populations characterized by positive expression of CD11b and mid expression of CD45 in the brains of mice 29 weeks post-BMT. These cells are further characterized by donor specific expression of GFP. Histograms compare the expression of Ly6c and MHC II among these CD11b^{+} CD45^{mid} GFP^{+} (donor) and GFP^{-} (host) populations.

To further investigate the phenotype of these CNS infiltrating macrophages, recipient C57BL/6 mice were treated with a (previously described) non-inflammatory myeloablative busulfan regimen followed by a bone marrow transplantation from syngeneic green fluorescent protein (GFP)-expressing C57BL/6 donors. Analysis by flow cytometry of a CNS macrophage phenotype population (CD11b^{+}/CD45^{mid}) in chimeric mice demonstrated that 1-5% of these cells were GFP^{+} at 7 post-BMT, although no significant further increase in percentages were observed at later time points (up to 52 weeks post-BMT). Interestingly, no GFP^{+} cells were detected before 7 weeks post-BMT, suggesting that the recruitment/migration of circulating blood monocytes into the CNS was not due to an acute inflammation process in response to busulfan conditioning. Analysis of Ly6C expression found that Ly6c^{low} cells were observed in the GFP^{-}/CD11b^{+}/CD45^{mid} resident population, but Ly6c^{hi} cells were observed in the peripheral circulation as well as in the GFP^{+}/CD11b^{+}/CD45^{mid} CNS population, suggesting recent migration from blood into the CNS. Interestingly, analysis of MHC class II expression revealed that only the hematopoietically-derived GFP^{+}CD11b^{+}/CD45^{mid} population expressed surface MHC class II, in contrast to no expression by resident (GFP^{-}) populations, suggesting that these cells may have an immune function upon migration into the CNS.

Additionally, immunofluorescence (IF) microscopy identified Iba-1^{+}/GFP^{+} CNS macrophages at 8 weeks post-BMT, however they did not demonstrate a ramified morphology until ~ 13 weeks post-BMT, suggesting that these cells (as detected by flow cytometry) are not merely in transit through the blood. Finally, IF microscopy confirmed the presence to GFP^{+}/Iba1^{+}/MHC class II^{+} cells.

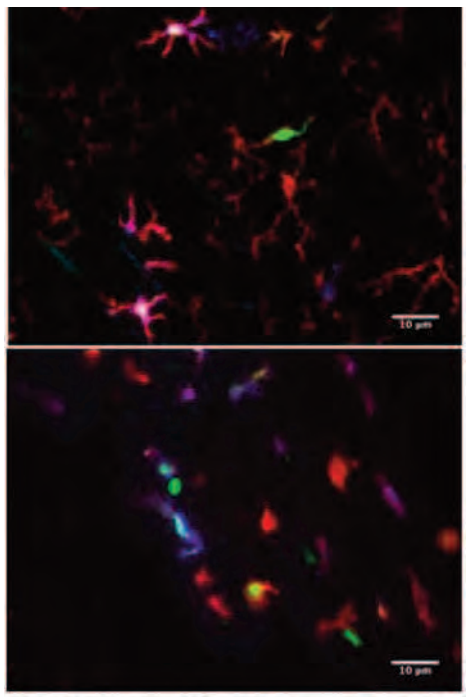


Figure 2. IF imaging of 13µm thick sections of the brains of mice 29 weeks post-BMT assess MHC II (blue) expression of Iba1^{+}(red) macrophages from GFP^{+} donor (green) and GFP^{-} host populations. 40x imaging of the lateral ventricle depicts amoeboid shaped macrophages of donor origin with MHC II expression located in the choroid plexus. 40x imaging of cerebral cortex reveal ramified 'microglia-like' macrophages of donor origin expressing MHC II in the parenchyma.

Together, these data suggest a possible continuum of cells that originate as hematopoietically-derived monocytes into CNS macrophages, with potential immune surveillance function, and further investigations into the mechanisms of recruitment will be important in understanding the interplay between the traditional CNS and immune systems.

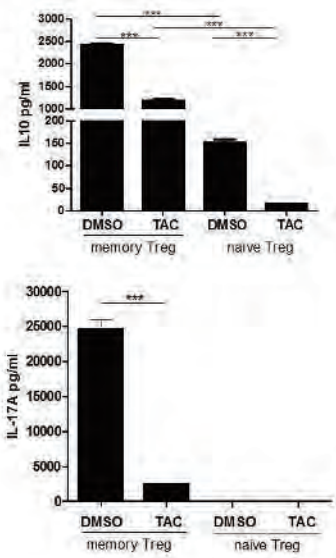
456.2

CD45RA identifies TSDR demethylated regulatory T cells with a stable phenotype and suppressive cytokine profile

Rebeca Arroyo Hornero[1], Gareth J.Betts[1], Birgit Sawitzki[2], Katrin Vogt[2], Paul N.Harden[3], Kathryn J.Wood[1].
[1]Transplant Research Immunology Group, Nuffield Department of Surgical Sciences, University of Oxford, Oxford, United Kingdom; [2]Institute for Medical Immunology, Charité University of Medicine, Berlin, Germany; [3]Oxford Kidney Unit, Churchill Hospital, Oxford, United Kingdom.

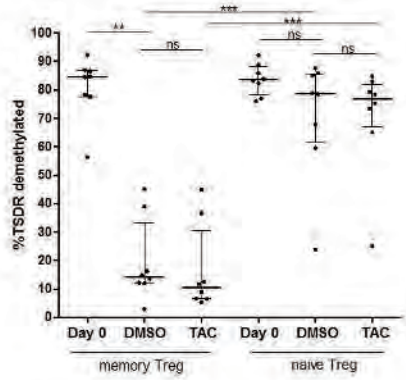
Introduction: Regulatory T cell (Treg) therapy is a promising strategy to induce transplant tolerance and may permit a reduction of IS chemotherapy and its toxic side effects[1]. The ONE study clinical trial is currently examining Treg cell therapy in combination with low tacrolimus (TAC) in living donor kidney recipients. Therefore, Treg populations that are able to survive, expand and remain suppressive in the presence of TAC may be critical to the therapy's success. Stable *bona fide* Tregs may be accurately identified through measurement of the methylation status of their Treg-specific demethylated region (TSDR) on the FOXP3 promoter[2]. While useful as a research technique, TSDR cannot assist in identification of cells for therapy. The identification of cell surface markers sufficient to isolate Treg that remain stable after expansion is therefore key. We focussed on distinct Treg subpopulations using CD25, CD127 and CD45RA cell surface markers[3],[4].

Materials and Methods: Total Tregs ($CD4^+CD127^{lo/-}CD25^+$); $CD127^{lo/-}CD25^{hi}CD45RA^-$($CD25^{hi}$memoryTreg); $CD127^{lo/-}CD25^{int}CD45RA^-$($CD25^{int}$memoryTreg); and $CD127^{lo/-}CD25^{int}CD45RA^+$(naiveTreg) were sorted from PBMC and expanded *in vitro* for 14 days using αCD3/αCD28 coated beads. Cells were expanded in the presence of a therapeutic concentration of TAC (8ng/ml)[5],[6] or control DMSO for the final 7 days of culture. FOXP3 expression, TSDR demethylation, suppressive function and IL-10, IL-17 and IFN-γ production were analysed after expansion.



IL-10 and IL-17A production by expanded Treg subsets.
IL-10 and IL-17A production by memory Tregs and naive Tregs after *in vitro* expansion in the presence of TAC or DMSO was determined in triplicate wells. 1 representative donor of 2 different donors IL-17 production by naive Tregs was under the detection limit. Bars represent means with SEM (***p<0.001).

Results: TAC reduced $CD25^{int}$memoryTreg and naiveTreg expansion equally, whilst $CD25^{hi}$memoryTreg were highly anergic and did not proliferate, even in the absence of TAC. FOXP3 expression after expansion was higher in naiveTregs than in memoryTregs, and was reduced by TAC. Importantly, naiveTregs had a stable demethylated TSDR following *in vitro* expansion whilst memoryTregs lost their demethylated phenotype.
This effect was independent of their exposure to TAC. Suppressive capacity of memoryTreg and naiveTreg was similar in the absence of TAC; however, naiveTregs had partially reduced suppressive function following exposure to TAC in 4/5 donors, compared to 1/6 donors for memoryTregs. MemoryTregs produced substantially more IL-10, IL-17 and IFN-γ than naiveTregs, however this production was reduced by TAC.



Demethylation status of the TSDR region of expanded Treg cell subsets.
Percentages of TSDR demethylation (n=8 donors) before (day 0) and after expansion in the presence of DMSO control or TAC. For statistical analysis, Wilcoxon-matched pair test was performed comparing TSDR demethylation between the same Treg population and the Mann-Whitney test was used to compare between cell populations (*p<0.05, **p<0.01, ***p<0.001). Median with interquartile range is represented.

Discussion: Suppressive mechanisms of naiveTregs and memoryTreg may differ given their differential susceptibility to TAC and levels of cytokine production.

Conclusion: Although memoryTregs retained a greater suppressive capacity when expanded with TAC, the marked loss of TSDR demethylation highlights the potential for loss of stability of these cells after infusion into transplant recipients. By contrast, expanded naiveTregs were still highly suppressive, retained TSDR demethylation and did not produce IL-17, suggesting that this $CD4^+CD127^{-/lo}CD45RA^+$Treg population is a promising candidate for Treg therapy and should be studied further.

Joanna Hester ; Fadi Issa

References:

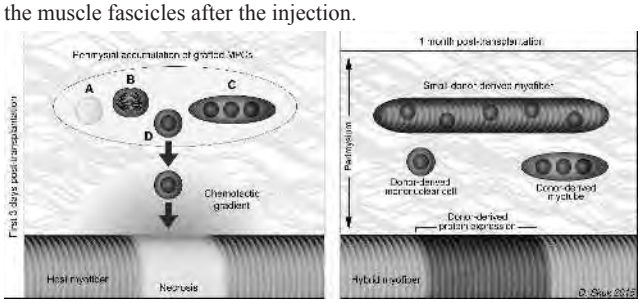
[1] van der Net J. Regulatory T cells: first steps of clinical application in solid organ transplantation. *Transplant International*. 2016;29(1):3-11
[2] Baron, U. DNA demethylation in the human FOXP3 locus discriminates regulatory T cells from activated FOXP3(+) conventional T cells. *European Journal of Immunology*. 2007;37(9): 2378-2389.
[3] Hoffmann, P. Only the CD45RA+ subpopulation of CD4+CD25high T cells gives rise to homogeneous regulatory T-cell lines upon in vitro expansion. *Blood*. 2006;108(13): 4260-4267.
[4] Miyara, M. Functional delineation and differentiation dynamics of human CD4+ T cells expressing the FoxP3 transcription factor. *Immunity*. 2009;30(6): 899-911.
[5] The 3C Study Collaborative Group. Alemtuzumab-based induction treatment versus basiliximabbased induction treatment in kidney transplantation (the 3C Study): a randomised trial. *Lancet*. 2014;384(9955):1684 - 1690
[6] Ekberg H. Calcineurin Inhibitor Minimization in the Symphony Study: Observational Results 3 Years after Transplantation. *American Journal of Transplantation*. 2009;9:1876–1885

456.3

The early behavior of muscle-precursor cell transplantation in skeletal muscles and how this conditions the outcome of the graft: A study in non-human primates

Daniel Skuk, Jacques P.Tremblay.
Axe Neurosciences, Research Center of the CHUQ, Québec, QC, Canada.

Introduction: Transplantation (Tx) of muscle-precursor cells (MPCs) has potential applications in the treatment of skeletal muscle disorders. Clinical results must be improved[1],[2], but this must be based on adequate knowledge of what happens to the MPCs after Tx, which is not sufficiently known. To clarify this issue in a model extrapolable to human, we conducted a study in nonhuman primates.
Material and Methods: MPCs proliferated in vitro and labeled with ß-galactosidase (ß-Gal) were injected in muscle regions of 1 cm^3 in macaques immunosuppressed with tacrolimus. The MPC-grafted regions were biopsied at post-Tx periods of 1 hour, 1, 3, 7 days and 3-4 weeks and analyzed by histology.
Results and Discussion: One hour post-Tx, grafted cells formed compact accumulations randomly in the perimysium. Few cells remained in the trajectories of injection in the muscle fascicles. On days 1, 3 and 7, there was a progressive depletion of the grafted cells in the perimysium, some of which fused between them to form myotubes. Paralelly, there was necrosis (day 1), phagocytosis (day 3) and regeneration (day 7) of the myofibers damaged by the injections. At day 7, regenerating myofibers began to express ß-Gal. At 3-4 weeks, regeneration was completed and ß-Gal was mostly in the myofibers of the muscle fascicles, reproducing the injection trajectories. Few ß-Gal+ elements remained in the perimysium as myotubes, small myofibers or mononuclear cells. Necrosis was observed in several grafted cells at day 1 by immunodetection of intracellular complement deposition, while immunodetection of active caspase 3 suggests that some grafted cells undergo apoptosis. Proliferating cell nuclear antigen immunodetection evidenced proliferation in the grafted-cell collections during the first week.
We interpret that the cell suspension leaks from the muscle fascicles and dissects the perimysium to accumulate there. In these cell accumulations, MPCs have different fates (figure). Some die by necrosis and probably by apoptosis (A). Some survive and proliferate (B), fuse between them to form myotubes (C) or migrate towards damaged myofibers to participate in regeneration (D). After 1 month (figure), the process is complete: the accumulations of grafted cells disappeared leading to few graft-derived elements in the perimysium: mononuclear cells, myotubes and small myofibers. ß-Gal+ myofibers in the fascicles occur more likely by MPCs that migrated from the perimysial accumulations than by the few MPCs that remained within the muscle fascicles after the injection.



Conclusion: The primary distribution of the grafted cells depends on the physical properties of the host tissue components and the cell suspension, while the graft outcome depends on different interacting biological factors: cell death vs proliferation, and local fusion vs migration to participate in myofiber regeneration.
This work was supported by the Jesse's Journey Foundation for Gene and Cell Therapy of Canada

References:
[1] Skuk D, et al. Dystrophin expression in muscles of Duchenne muscular dystrophy patients after high-density injections of normal myogenic cells. *J. Neuropathol. Exp. Neurol.* 65: 371-386; 2006.
[2] Skuk D, et al. First test of a "high-density injection" protocol for myogenic cell transplantation throughout large volumes of muscles in a Duchenne muscular dystrophy patient: eighteen months follow-up. *Neuromuscular Disorders* 17: 38-46; 2007.

456.4

Mobilized human peripheral blood mononuclear cells (PBMCs) contain higher proportion of IL-10 producing B cells in CD8+TCR- facilitating cell population than non-mobilized PBMCs

Yujie Wen, Esma S.Yolcu, Yiming Huang, Larry E.Kahn, Suzanne T.Ildstad.
Institute for Cellular Therapeutics, University of Louisville, Louisville, KY, United States.

Introduction: We recently reported that facilitating cell (FC) enriched hematopoietic progenitor stem cell (HSPC) allografts (FCRx) induced tolerance without graft-versus-host disease (GVHD) or engraftment syndrome in HLA-mismatched living donor renal transplant recipients. Recent studies suggest that dysfunction of B cells that maintain homeostasis may play a role in the pathogenesis of chronic GVHD after HSPC transplantation and alterations in B cells may be a biomarker for transplantation tolerance in kidney transplant recipients.
Materials and Methods: We analyzed B cell progenitors and interleukin 10 (IL-10)-producing B cell subsets in FC obtained from mobilized peripheral blood mononuclear cell (mPBMC) for clinical transplantation, control un-mobilized PBMC and G-CSF mPBMC from healthy volunteer donors. Clinical donors received 9 doses of 10 µg/Kg G-CSF plus 1 dose of Plerixafor while volunteer donors received 4 doses of 10 µg/Kg G-CSF (q.d. for 4 days).
Results and Discussion: FC obtained from mPBMC for FCRx contained significantly more $CD19^+CD24^{hi}CD27^+$ activated B cells and memory B cells ($p<0.01$; n=7-15) and $CD19^+CD24^{hi}CD27^+CD38^{-/lo}IgM^+$ memory IL-10-producing B cell subsets ($p<0.05$; n=7-15), but contained a similar proportion of B cell progenitor subsets (Pro-B $CD34^+CD19^+CD38^+CD10^+IgM^-$, Pre-B $CD34^-CD19^+CD38^+CD10^+IgM^-$ and Immature B $CD34^-CD19^+CD38^+CD10^+IgM^+$) compared to the two control groups. The percentage of $CD8^+TCR^-$ FC in the lymphoid gate was 9 fold less than that of the Non-FC cell population (reverse gate of $CD8^+TCR^-$) in mPBMC for FCRx. However, the FC population contained a significantly higher proportion of activated and memory B cells ($p<0.05$, n=7) and IgM^+ memory IL-10-producing B cell subsets ($p<0.01$, n=7) compared to the Non-FC cell population. We then evaluated the impact of depleting $CD19^+$ FC on in vivo engraftment of mPBMC in a non-obese diabetic / severe combined immune deficiency mutation and IL2 receptor gamma chain deficiency (NSG) mouse model. NSG mice were conditioned with 325 cGy TBI and transplanted with 50-70 x 106 mPBMC for FCRx with or without $CD19^+$ FC. Depletion of $CD19^+$ FC resulted in a significant impairment in engraftment (6 % vs. 14 %; p = 0.03, n = 6) compared to mPBMC for FCRx containing $CD19^+$ FC.
Conclusion: In summary, our results suggest that $CD8^+TCR^-$ facilitating cells in mPBMC from clinical donors for FCRx are enriched with IL-10 producing B cell subsets, which may contribute to the prevention of GVHD and the induction of transplantation tolerance.
References:
[1] de Masson A. *Blood*. 2015; 125(11):1830-1839.
[2] Khoder A. *Blood*. 2014;124(13):2034-45
[3] Newell KA. *Am J Transplant*. 2015; 15(11):2908-2920
[4] Newell KA. *J Clin Invest*. 2010; 120(6):1836-1847.

456.5

Therapeutic benefit of adipose-derived stem cell transplantation in rats of acute-on-chronic liver failure

Cheng-Maw Ho[1,2], Ya-Hui Chen[3], Chin-Sung Chien[2], Shu-Li Ho[1,3], Chih-Chiang Hsiao[4], Jung-Cheu Chen[4], Hui-Ling Chen[2,3], Daniel Tzu-bi Shih[4], Po-Huang Lee[1,2].
[1]Department of Surgery, National Taiwan University Hospital, Taipei, Taiwan; [2]Graduate Institute of Clinical Medicine, National Taiwan Univerisity, Taipei, Taiwan; [3]Hepatitis Research Center, National Taiwan University Hospital, Taipei, Taiwan; [4]Department of Pediatrics, Taipei Medical University, Taipei, Taiwan.

Background: Acute-on-chronic liver failure resulted in high mortality. Adipose-derived stem cells (ADSCs) hold promise to complement cell therapy for this dread syndrome. The aim of this study is to demonstrate the therapeutic benefit of CD34 expressed cells derived from adipose tissue in complementing hepatocyte transplantation for acute-on-chronic liver failure in rats.
Methods: Acute-on-chronic liver failure was induced by temporary bile duct obstruction (for 2 weeks) and D-galactosamine (350mg/kg, one day before transplantation) in Sprague-Dawley (SD) rats. Donor hepatocytes were freshly isolated from green fluorescent SD rats by in situ liver perfusion and collagenase digestion before transplantation. ADSCs of stromal vascular fraction were freshly isolated from human adipose tissue and cryopreserved for future transplantation. Three groups, CD34+, CD34-, and unsorted ADSCs were classified based on cell sorting. Rat hepatocytes (1 x 10^7/1ml) were transplanted intraportally into rats of acute-on-chronic liver failure, followed by ADSCs (1 x 10^6/0.5 ml, PKH 26 Fluorescent-labeled) 1 hour later. Animals were sacrificed in 1 and 2 weeks after transplantation, if survived. Liver fibrosis, donor cell identification, serologic tests, immunohistopathological examination, were analyzed.
Results: Acute-on-chronic liver failure was evidenced by the development of acute coagulopathy and hepatocyte necrosis in fibrotic livers. Transplanted ADSCs were found universally in the periportal connective tissue. Rats of CD34+ group exhibited better resolution of obstructive jaundice and biliary fibrosis, followed by those of unsorted and CD34- groups. Ductular reaction, marked by CK19+ progenitor cells, was prominent in CD34- group, and less so in unsorted and CD34+ groups. The CK19+ progenitor cells were not co-localized with transplanted ADSCs. Rats of unsorted group had the trend of superior survival benefit than the other two groups (unsorted vs. sorted, p=0.087).
Conclusions: Co-transplantation of CD34+ ADSCs and hepatocytes resulted in significantly better amelioration of liver fibrosis and ductular reaction in rats of acute-on-chronic liver failure, compared to CD34- ADSCs, although co-transplantation with non-sorted ADSCs instead showed potential survival benefit.

456.6

Tracking of Iron nano-particle labelled Mesenchymal stem cells in Ovine heterotopic kidney transplantation

Bron Lett[1,4], Kisha NSivanathan[1,4], Dyane Auclair[3], Julie Johnston[1,4], Christine HRussell[2], Santosh Olakkengil[2], Raj Perumal[3], Chris Drogemuller[1,2,4], Patrick TCoates[1,2,4].
[1]School of Medicine, University of Adelaide, Adelaide, Australia; [2]Renal Department, Royal Adelaide Hospital, Adelaide, Australia; [3]Large Animal Research and Imaging Facility, Adelaide, Australia; [4]Center for Clinical and Experimental Transplantation, Royal Adelaide Hospital, Adelaide, Australia.

Mesenchymal stem cells (MSCs) are a potent group of non haematopoietic progenitor cells with the hallmark qualities of differentiation into bone, cartilage and fat cells. They also possess immunomodulatory and immunosuppressive properties. Given these traits they are a promising candidate for cellular therapy with potential use in solid organ transplantation.
Applying these potential therapeutics to large animal models is essential for understanding their mechanism of action and improving their eventual clinical application. To this end we have a developed an Ovine model of kidney transplantation and tracked the in vivo movement of these cells using MRI.
Methods: Bone marrow aspirate was isolated from the iliac crest and MSCs were selected for by washing off the non adherent cells 7 days after plating. The MSCs were characterised by their ability to differentiate into bone, cartilage, and fat cells as well as flow cytomertry staining. The immunosuppressive abilities of these cells were assessed via mixed lymphocyte reaction.
In order to track the cells in vivo we use an iron nano particle, resovist. To ensure that this did not interfere with the function of the cells, MSCs were labelled and characterised again.
The transplant model can be either an autograft or an allograft and involves the heterotopic transplantation of the donor kidney into the neck of the recipient sheep. The renal artery undergoes end to end anastamosis with the left carotid artery and the renal vein undergoes end to side anastamosis with the jugular vein. The ureter is then passed through the skin flap that covers the kidney and catheterised to allow urine flow and collection.
Heparinised and labelled mesenchymal stem cell suspension (1x106 cells/kg in volume) was infused directly into the renal graft artery immediately prior to completion of the anastomosis, with additional vascular clamps used to prevent loss of solution at the anastomosis. Alternatively the cells were given at the time of the arterial anastamosis via a central venous line. The animals then underwent MRI scans to try and locate the iron labelled cells in the graft.
Results: Differentiation potential, cell surface markers, and immunosuppressive abilities of the MSCs were comparable between unlabelled and resovist labelled cells with no significant difference being observed in any of the assays.
MRI imaging of the transplanted kidney allowed for the detection of iron labelled MSCs up to 7 days post infusion, the distribution of which was confirmed histologically using Prussian blue staining.
Conclusion: In conclusion, these translational studies provide techniques to label large animal MSCs and follow the distribution of these cells within a living kidney using non-invasive MRI scanning. The heterotopic transplant model should allow testing of the effectiveness of MSCs as a cell therapy and provide a tool for refinement of their application.

456.7

Ex-vivo expansion of human alloantigen-reactive regulatory T cells using monocyte-derived dendritic cells versus CD40L-stimulated B cells

Hong Zhang[1], Yifei Zhao[1], Qizhi Tang[2], Mohammed Ezzelarab[1], Angus Thomson[1].
[1]Surgery, University of Pittsburgh, Pittsburgh, PA, United States; [2]Surgery, UCSF, San Francisco, CA, United States.

Introduction: Alloantigen-reactive murine and human CD4+CD25+FOXP3+ regulatory T cells (arTreg) are more potent in promoting long-term allograft survival than polyclonal Treg in experimental models. Dendritic cells (DCs) are well known professional antigen-presenting cells (APC) specialized to initiate and regulate immunity. CD40L-stimulated B cells (CD40L-sBc) have been reported to expand human arTreg. However, no direct comparison of the ability of these different APCs to expand arTreg has been reported. In order to establish a protocol to generate large numbers of human arTreg with potent allospecific suppressive capacity for therapeutic application, we compared immature monocyte-derived DC (mono-DC), LPS-matured DC, and CD40L-sBc for their ability to expand arTreg.
Materials and Methods: To generate mono-DCs, immunobead-selected peripheral blood CD14+ cells were cultured with GM-CSF and IL-4 for 7 days. To mature the mono-DCs, LPS was added for the last day of culture; to make CD40L-sBc, flow-sorted CD20+ cells were cultured with irradiated 3T3-CD40L cells, IL-4 and cyclosporine A for 7 days before restimulation with CD40L-3T3 and IL-4 for an additional 3 days. The APCs were irradiated and co-cultured with flow-sorted peripheral blood CD4+CD25+CD127- Tregs and IL-2 for 12 days. To test their function, the expanded arTreg were co-cultured with Teff from same Treg donor at different ratios under stimulation of allo-sBc or anti-CD3/CD28 beads. % of suppression of proliferation correlates with suppressive capacity of the arTreg.

Figure 1. (A) Phenotype of CD40L-stimulated B cells (sBc), monocyte-derived DC (untreated DC), and LPS-treated DC (LPS-DC). (B) Expansion kinetics of arTreg stimulated by sBc, DC, or LPS-DC

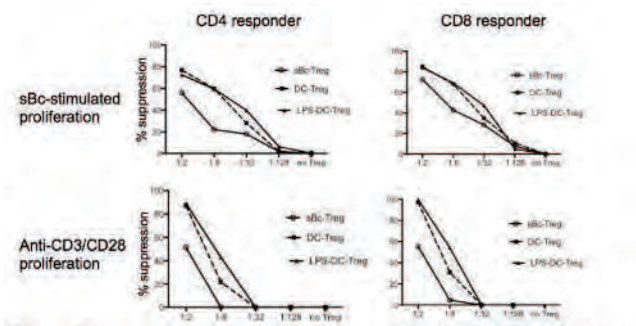


Figure 2. Human arTreg flow-sorted and expanded with allo-sBc, unstimulated DC, or LPS-DC were co-cultured with VPD450-labeled CD4+CD25- Teff from the Treg donor at the indicated ratios under stimulation of allo-sBc (top) or anti CD3/CD28 beads (bottom) for 4 days. % of suppression of proliferation is shown..

Results and Discussion: We observed that LPS-matured DC expressed higher levels of co-stimulatory CD80 and CD86 than immature DC and CD40L-sBc (Figure 1A), suggesting a stronger stimulatory capacity. Treg cultured with LPS-matured DCs were expanded ~40 fold, while those cultured with immature DC or CD40L-sBc were expanded 10- to 20- fold (Figure 1B). Expression of FOXP3 and CTLA-4 was higher

in Treg expanded by either DCs or LPS-matured DCs. In addition, Treg expanded by DCs were superior suppressors of effector T cell proliferation stimulated by either anti-CD3/CD28 beads or allo-sBc (Figure 2). T cell receptor diversity analysis of expanded arTreg is currently being performed to ascertain and compare the clonality of arTreg expanded by the different types of allo-APCs.
Conclusion: LPS-matured human mono-DCs are superior to immature DC and CD40L-sBc in their ability to expand functionally suppressive FOXP3+ Treg. Given the importance of Treg specificity in their in vivo efficacy, our data and ongoing studies will allow determination of which APC is optimal for expansion of human arTreg for in vivo therapy.

456.8

De- and recellularization of porcine pancreas

Erik Elebring[1], Bengt Gustafsson[2], Niclas Kvarnström[2], Suchitra Sumitran-Holgersson[1].
[1]Laboratory for Transplantation and Regenerative Medicine, Department of Surgery, University of Gothenburg/Sahlgrenska Academy, Gothenburg, Sweden; [2]Sahlgrenska University Hospital, Gothenburg, Sweden.

Introduction: Diabetes mellitus is a global metabolic disease affecting endocrine pancreas with extremely high prevalence worldwide[1]. The currently available treatments for diabetes mellitus are not adequate[2],[3]. A technique with the potential to improve therapies that has evolved over the last decade is whole organ perfusion decellularization and recellularization. The ultimate goal of decellularization is to, with the help of different agents, remove cell material from the organ without negatively affecting the extracellular matrix (ECM) scaffold[4]. An innate ECM scaffold has the basic biomolecules, preservation of scaffold-bound growth factors and cytokines, an intact vascular structure and the ability to direct cell differentiation during recellularization.
Apart from endocrine tissue, pancreas also consists of exocrine tissue[5]. The release of digestive enzymes from exocrine tissue during decellularization results in a damaged ECM structure. Therefore an alternative decellularization method is presented, where cold temperature is used to keep activity of digestive enzymes low for preservation of ECM structure.
Materials and Methods: Porcine pancreata were dissected with duodenum and aorta intact. The aorta was cannulated and the portal vein was kept open, while all other vessels were ligated. Organs were washed with cold PBS with heparin for 2 hours and then frozen until use. When ready for decellularization the organ was placed at +4°C and perfused with 4% sodium deoxycholate and 6% Triton X-100 in distilled water for 8 hours. Lastly, the organ was washed by perfusion with distilled water for a minimum 120 hours. The outcome of decellularization was characterized using histological and, immunohistochemical stainings for ECM components, quantification of ECM components and quantification of DNA. 0.5 cm3 pieces of decellularized scaffolds were recellularized by seeding cells isolated from human fetal small intestine.
Results and Discussion: After decellularization, pancreata look pale in comparison to normal (Figure 1). Histology results showed that decellularization protocol at +4°C can effectively remove nuclei and other cellular remnants while affecting the ECM minimally (Figure 2). Pancreata decellularized at cold temperature had a much more conserved ECM structure compared to pancreata decellularized at room temperature. DNA quantification confirmed removal of nuclei material during decellularization. Results of recellularization with human fetal small intestine cells will be presented.
Conclusion: Porcine pancreas can be effectively decellularized in a cold surrounding using detergents, while preserving the ECM structure.

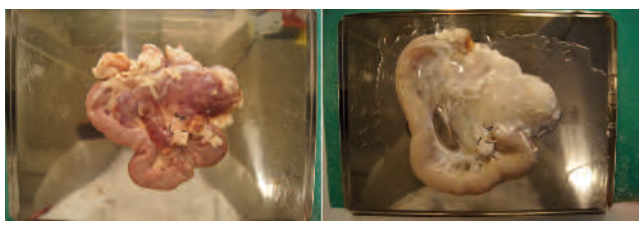


Figure 1. Gross anatomy of normal and decellularized porcine pancreas.

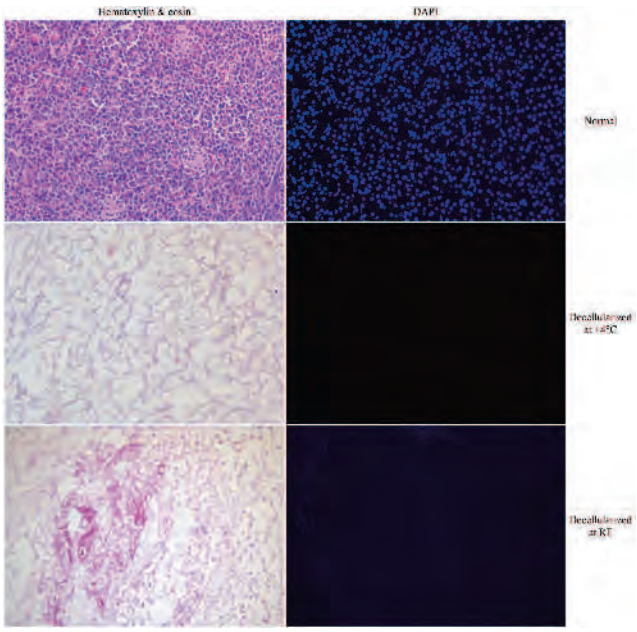


Figure 2. Histological characterization of normal and decellularized pancreata. H&E is used to characterize the ECM while DAPI is used to characterize nuclei.

References:

[1] J. E. Shaw, et al., Global Estimates of the Prevalence of Diabetes for 2010 and 2030, *Diabetes Research and Clinical Practice*, 87 (1) (2010) 4-14

[2] R. T. Wagner, et al., Stem Cell Approaches for the Treatment of Type 1 Diabetes Mellitus, *Translational Research*, 156 (3) (2010)169-179

[3] G. Orlando, et al., Pancreas Transplantation for Type 2 Diabetes Mellitus, *Current Opinion in Organ Transplantation*, 16 (1) (2011) 110-115

[4] S. F. Badylak, et al., Decellularization and Recellularization of Three-Dimensional Matrix Scaffolds, *Annual Review of Biomedical Engineering*, 13 (2011) 27-53

[5] G. K. Gittes, Developmental Biology of the Pancreas: a Comprehensive Review, *Developmental Biology*, 326 (1) (2009) 4-35

456.9

Stem cell therapy as an emerging modality for post-traumatic paraplegia and for renal parenchymal diseases

Umang G. Thakkar[1], Hargovind L. Trivedi[1,3], Aruna V. Vanikar[1,2]. [1]Department of Regenerative Medicine and Stem Cell Therapy, Institute of Kidney Diseases & Research Centre (IKDRC) - Institute of Transplantation Sciences (ITS), Ahmedabad, India; [2]Department of Pathology, Laboratory Medicine, Transfusion Services and Immunohematology, Institute of Kidney Diseases & Research Centre (IKDRC) - Institute of Transplantation Sciences (ITS), Ahmedabad, India; [3]Department of Nephrology and Transplantation Medicine, Institute of Kidney Diseases & Research Centre (IKDRC) - Institute of Transplantation Sciences (ITS), Ahmedabad, India.

Background: Stem cell therapy (SCT) holds promise for spinal cord injury (SCI) and renal parenchymal diseases (RPD).[1][2] We present experience of co-infusion of autologous adipose-derived neuronal differentiated mesenchymal stem cells (N-AD-MSC)+ hematopoietic stem cells (HSCs) in post-traumatic paraplegia (PTP), and allogenic AD-MSC+ HSC in RPD.

Materials and Methods: Informed consent forms of these prospective, non-randomized, open-labeled studies were approved by Institutional Review Board. Study-1 was carried out in 10 PTP patients (8 males, 2 females) with mean age of 28 years and mean injury duration, 3.42 years, from January,'09 to March,'12. Seven patients had SCI at dorsal level and 3, at lumbar level. Spine-fixation was performed in 7 patients. All patients had bowel-bladder incontinence, sexual dysfunction, 8 had neurogenic pain and pressure sores, 6 had kyphosis/scoliosis, 4 had recurrent urinary tract infections, 1 had bladder calculi, and 5 were depressed. Nerve-conduction studies showed motor axonopathy in 4, upper motor neuron lesion in 2 and radiculoneuropathy in 4 patients. Mean Hauser Ambulation index (HAI) was 9. American Spinal Injury Association (ASIA) score was "A" in all.

Seven steroid-resistant RPD patients (5 males, 2 females) in study-2 underwent SCT from May,'13 to March,'14. Mean patient age was 29.85 years with disease duration, 5.43 years, 24-hours proteinuria, 1.45 gm, serum Creatinine (SCr), 1.17 mg/dL and serum albumin (S.alb), 3.08 gm/dL. Three patients had primary focal segmental glomerulosclerosis, 2 had lupus nephritis, and 1 each had primary IgA nephropathy and mesangio-proliferative glomerulonephritis.

MSCs were generated in vitro from abdominal fat and differentiated into neuronal cells. HSCs were generated from bone marrow. N-AD-MSCs, 2.08 ml (expressing beta-3 tubulin and glial fibrillary acid protein, Figure-1) and HSCs, 1.96 ml, with mean nucleated cell counts of $2.0\times10^2/\mu L$, and $4.5\times10^4/\mu L$ respectively were infused into cerebrospinal fluid below injury site.

In study-2, AD-MSCs + HSCs were infused by femoral catheterization into bilateral renal arteries, portal circulation and thymus. Mean counts of MSC and HSC were 33.79 $x10^4$ and 4.86x 10^8/Kg BW respectively with mean $CD34^+/CD90^+/CD73^+$ of 0.81%/ 37.95% /20.6%.

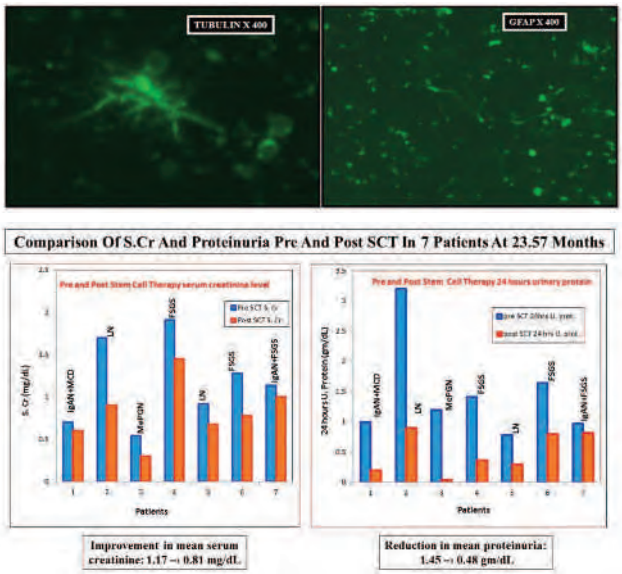
Results and Discussion: SCT was safe and uneventful.

In study-1, over mean 4.95 years follow-up, ASIA score improved to B in 6, C in 3, D in 1; HAI improved to 7.4, with subjective improvement. Muscle power improved to grade-2 in 6 patients. Touch and pressure sensation regained in 4 and 3 patients respectively. The required natural recovery period is ≈1-year, suggesting positive role of SCs here.

In study-2, over mean 23.57 months follow-up, there was sustained reduction in proteinuria to 0.48 gm/day, decreased SCr to 0.81 mg/dL (Figure-2) and increased S.alb to 3.54 gm/dL. Remission was found in all with absence of relapse in 5 patients.

MSCs promote recovery via angio/neuro/synaptogenesis and axonal remodeling. Demyelinated axons are re-myelinated by SCs releasing various trophic factors and induce endogenous mechanisms of tissue repair.[3] HSCs help in angiogenesis and remodeling. MSCs facilitate immunomodulation and regeneration.

Conclusion: Co-infusion of N-AD-MSC+ HSC in CSF for SCIs, and AD-MSC+ HSC infusion in portal, thymic and renal circulation for remission of RPD is safe and viable.



References:
[1] Mehta T, Feroz A, Thakkar U, Vanikar A, Shah V, Trivedi H. Subarachnoid placement of stem cells in neurological disorders. Transplant Proc 2008;40:1145-7.
[2] Perin L, Da Sacco S, De Filippo RE. Regenerative medicine of the kidney. Adv Drug Deliv Rev. 2011; 63(4-5):379-87
[3] Hsu YC, Chen SL, Wang DY, Chiu IM Stem cell-based therapy in neural repair. Biomed J 2013;36:98-105

457.1

Macrophage-specific deletion of mTOR inhibits chronic allograft rejection and induces long-term heart allograft survival

Yue Zhao, Wenhao Chen, Wentao Liu, Malgosia Kloc, Rafik Ghobrial, Peixiang Lan, Xian C. Li.
Immunobiology and Transplant Research Center, Houston Methodist Hospital, Houston, United States.

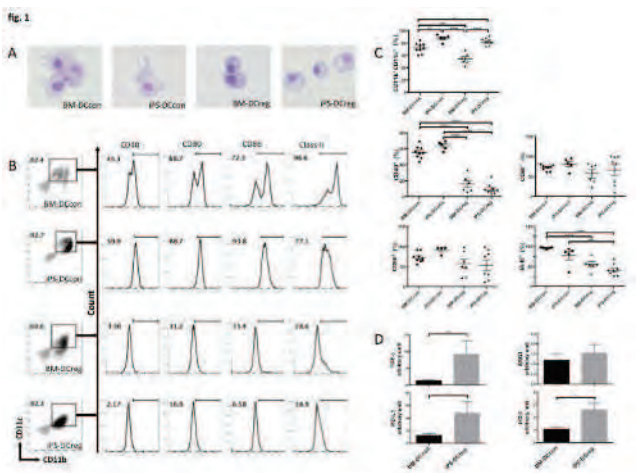
Most transplants are eventually lost to chronic rejection under broad immunosuppression therapies where activation of innate immune cells is suspected to play a major role in graft loss. Indeed, monocytes and macrophages are major cell types infiltrating the chronically rejected allografts, but the molecular mechanisms governing macrophage activities in the grafts are poorly understood. In the present study, we generated mTOR-conditional knockout mice in which deletion of mTOR in macrophages was driven by LyzM-Cre and used this model to examine the role of mTOR in regulating innate immunity in chronic allograft rejection. We observed in a heart transplant model that treatment of wild type C57BL/6 mice with CTLA-4Ig prevented acute rejection of Balb/c heart allograft, but allowed chronic rejection to develop. There was an extensive macrophage infiltration in chronically rejected heart allografts, and the graft infiltrating macrophages preferentially expressed markers associated with M2 phenotype. Interestingly, treatment of macrophage specific mTOR deleted mice with CTLA4-Ig showed that neointima formation and M2 infiltration were marked ameliorated in the grafts, and as such, the graft survival was marked prolonged (>100 days). Mechanistically, we found that deletion of mTOR in macrophages resulted in an impaired induction of M2 cells, but M1 polarization was intact, and this impaired M2 induction was related to reduced STAT6 activation. These findings highlight the importance of mTOR in regulation of macrophages in chronic rejection and provide potential new therapeutic targets for treatment of chronic graft loss.

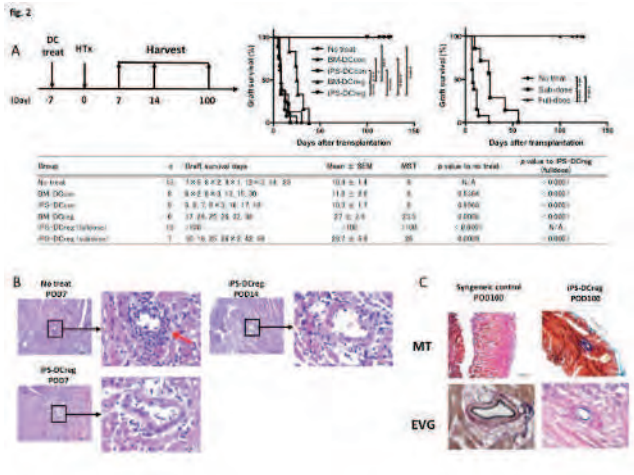
457.2

Systemic therapy of regulatory dendritic cells derived form induced pluripotent stem cells allows allogeneic cardiac grafts acceptance

Songjie Cai[1,2], Jiangang Hou[1], Masayuki Fujino[1,3], Naotsugu Ichimaru[2], Yoshiaki Nishio[1], Lina Lu[4], Shiro Takahara[2], Xiao-Kang Li[1].
[1]Division of Transplantation Immunology, National Research Institute for Child Health and Development, Tokyo, Japan; [2]Department of Advanced Technology for Transplantation, Osaka University Graduate School of Medicine, Osaka, Japan; [3]AIDS Research Center, National Institute of Infectious Diseases, Tokyo, Japan; [4]Department of Immunology, Lerner Research Institute, Cleveland Clinic, Cleveland, United States.

Backgroud: Regulatory dendritic cells (DCreg) play an essential role in inducing and maintaining immune tolerance, representing a potential therapeutic tool for organ transplantation.
Methods: In the present study, we attempted weather systemic administration of donor-type iPSCs derived DCregs (iPS-DCregs) could lead to permanent acceptance of fully MHC-mismatched murine allogeneic cardiac grafts[1]. We used the mouse cardiac transplantation model (C57BL/6, H-2Kb into CBA/N, H-2Kk) to test the hypothesis.
Result: Recipients (CBA/N) pretreated with donor-type (C57BL/6, B6) iPS-DCregs accepted B6 cardiac grafts but rejected third-party (BALB/c, H-2Kd) grafts. The number of graft infiltrating CD8+ T cells in iPS-DCregs treated recipients was significantly decreased compared with no-treat group, and the Foxp3+ T cells were significantly increased. Further, the naïve secondary recipients (CBA/N) treated with the adoptive transfer of splenocytes from iPS-DCregs treated primary recipients on POD100 accepted B6 cardiac grafts but rejected BALB/c grafts. Dramatically, the above effects were reversed by infusion of anti-TGFβ or anti-CD25.
Conclusion: Our data indicated that preconditioning of donor-type iPS-DCregs to recipients generated antigen-specific Tregs in association with TGFβ. These findings highlight the iPS-DCregs will be a key cell therapy in clinic transplantation.





Naoto Ishii; Shiguang Qian; Hiroshi Hirano; Shizue Iwasaki; Chengcheng Huang; Qi Zhang

References:

[1] Zhang Q., Cai S., et al. Generation and characterization of regulatory dendritic cells derived from murine induced pluripotent stem cells.*Scientific Reports*. 2014. 4: p. 3979.

457.3

Human regulatory macrophages are capable of suppressing the allogeneic response in vitro via soluble molecules

Yuanfei Zhao[1,2], Yixiong Tan[1,2], Fei Guo[1,2], Dandan Huang[1,2], Min Hu[1,2], Wayne J.Hawthorne[1,2], Shounan Yi[1,2], Philip J.O'Connell[1,2]. [1]Centre for Transplant and Renal Research, The Westmead Institute for Medical Research, Sydney, Australia; [2]The University of Sydney, Sydney, Australia.

Introduction: Human regulatory macrophages (Mreg) have been shown to suppress mitogen-driven T cell proliferation in vitro and are under evaluation as a cellular therapy in renal transplantation in THE ONE STUDY clinical trial. In this study we investigated whether human Mreg were capable of suppressing the allogenic response in vitro and the mechanisms involved.
Methods: Human CD14+ cells were isolated from healthy donor peripheral blood monocytes (PBMC) using CD14 magnetic beads. Mreg induction was performed by culturing CD14+ cells with macrophage colony-stimulating factor (M-CSF) for 6 days. On day 6 cells were stimulated with IFN-γ for a further 24 hr. The phenotype of Mreg were characterized by flow cytometry, and their suppressive activity was tested by mixed-lymphocyte reaction (MLR) using human PBMC as responder cells, autologous Mreg as suppressor cells and irradiated allogeneic PBMC as stimulator cells. In a separate transwell MLR setting, responder and allogeneic stimulator cells were cultured in the upper chamber and autologous Mreg were seeded in the lower chamber. Supernatants collected from MLR cultures were used for cytokine detection using cytokine beads array and ELISA kits and IDO activity assay by HPLC, respectively.
Results: The resulting induced human Mreg demonstrated a typical Mreg phenotype, CD14low CD80low CD86+ HLA-DR+ CD83low CD16-, and as a result, potent suppression of proliferating alloreactive PBMC even at Mreg: responder cells ratios >1:16. Separation of Mreg from responder and allogeneic stimulators in a transwell MLR system had mild impact on their suppressive potency in the allogeneic response at all ratios of Mreg:responder cells tested. Upregulated levels of Mreg function related molecules TGF-β and IDO were detected in the supernatants collected from both non-transwell and transwell MLR cultures containing Mreg.
Discussion: In vitro induced human Mreg showed capacity to suppress the allogeneic response in vitro and Mreg-mediated suppression was not cell-cell contact dependent.
Conclusions: This study suggested soluble molecules TGF-β and IDO involved mechanisms used by human Mreg to suppress the allogeneic response in vitro. The requirement of these soluble molecules for Mreg-mediated immunomodulation of the allogeneic response is being under further investigation by blockading TGF-β and IDO activity in MLR assays.

457.4

Anti-A immune response in mice to ABO-blood carbohydrates antigen is T cell dependent

Ibrahim Adam[1,4], Bruce Motyka[2,4], KeSheng Tao[2,4], Lori West[1,2,3,4]. [1]Medical Microbiology & Immunology, University of Alberta, Edmonton, AB, Canada; [2]Pediatrics, University of Alberta, Edmonton, AB, Canada; [3]Surgery, University of Alberta, Edmonton, AB, Canada; [4]Alberta Transplant Institute, University of Alberta, Edmonton, AB, Canada.

Background: ABO-incompatible heart transplantation (ABOi HTx) is safe during infancy and allows increased access to donors. B-cell tolerance develops to donor A/B antigen(s) (Ag) following ABOi HTx, but mechanisms of tolerance are not well-defined. Using a recently developed A-transgenic (A-Tg) mouse on B6 background (express A Ag on vascular endothelium and erythrocyte (RBC)), we investigated the role of CD4+ T-cells in anti-A antibody (Ab) production.
Methods: Wild-type C57BL/6 mice (WT) were injected i.p. 3x, 1 week apart with human blood group A (n=3) or A-Tg (n=12) RBCs and adjuvant. Four weeks later, mice injected with A-Tg RBCs were injected i.p. with human A-RBCs. Anti-A Ab in serum was measured by hemagglutination and ELISA (both IgG and IgM). To deplete CD4+ T-cells, rat anti-mouse Ab (GK1.5) was used, 3x 1 week apart, pre and post human A-RBC injection (n=5). To study the effect of antigens on human RBCs, human group-O and A-Tg RBCs were mixed, injected i.p. 3x, 1 week apart in WT (n=5), then anti-A IgM titer was measured.
Results: Despite comparable A-Ag expression, A-Tg RBCs did not induce anti-A Ab in WT mice (titer ≤1:2), in contrast to human A-RBCs (titer=512). Four weeks after A-Tg RBC injection, human A-RBC injection induced anti-A Ab (median titer=256). Human A-RBC injection following CD4+ T-cell depletion did not produce anti-A Ab to human A-RBCs (titer <4). Moreover, co-injection of A-Tg and human O-RBC into WT mice did not induce anti-A Ab (titer ≤1:2).
Conclusions: Administration of A-Ag alone (A-Tg RBC) to WT mice was not sufficient to induce an anti-A Ab response. In contrast, human A RBC (A antigen plus foreign glycoproteins/glycolipids) induced a strong anti-A Ab response which was CD4+ T cell-dependent. The lack of an anti-A Ab response following co-injection of A-Tg RBCs and human O RBC is consistent with a requirement for a chemical linkage of foreign protein/lipid with A-antigen. Administration of A-antigen alone (A-Tg RBC) did not induce tolerance inasmuch as subsequent administration of human A RBC elicited anti-A Ab.

457.5

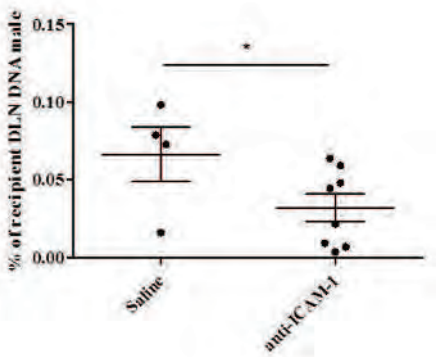
Anti-ICAM-1 treatment hinders lymphatic trafficking of donor passenger leukocytes and prolongs murine kidney allograft survival

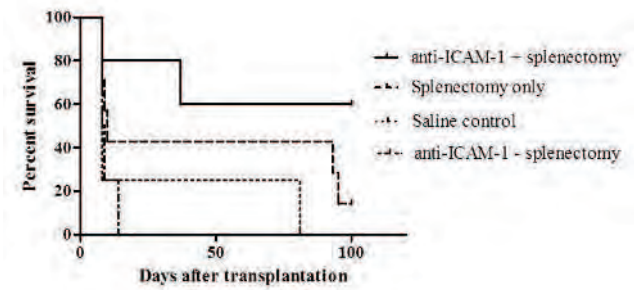
Lucy Meader, Lindsey A.Edwards, Anna Nowocin, Kathryn Brown, Wilson Wong.
Medical Research Council (MRC) Centre for Transplantation, King's College London, London, United Kingdom.

The immune response to an allograft is initiated when donor-derived foreign antigen is recognised by the host immune system, leading to the priming of graft-destructive T cells. This process relies on efficient trafficking of donor passenger leukocytes (DPL) out of the graft to secondary lymphoid organs, i.e. the local draining lymph nodes and spleen, via lymph and blood, respectively. This provides a suitable niche for interactions between antigen presenting cells and alloreactive T cells. The lymphatic vasculature permeates tissue and provides a conduit for immune surveillance. It is now known that lymphatic endothelial cells (LEC) play an active role in the adaptive immune response as they can; present antigens to T cells, affect dendritic cell function and alter the inflammatory milieu by secreting cytokines and chemokines. In addition to this; during inflammation LEC upregulate adhesion molecules important for DC transmigration, such as ICAM-1. We hypothesised that blocking ICAM-1 in a transplant model would hinder DPL trafficking to draining lymph nodes, and thus dampen the rejection process.

Fully allogeneic male BALB/c mouse kidneys were transplanted into female C57BL/6 recipients. Recipient mice were treated with either saline control, or a single dose of 200µg anti-ICAM-1 on the day of transplantation to inhibit transmigration of DPL across the lymphatic endothelium, and thus their passage to the local draining lymph nodes. Recipient spleens were removed to exclude this alternative route of T cell priming. Real-time quantitative PCR, for a donor male specific HY gene, was used to quantify donor passenger leukocytes in recipient draining lymph nodes.

Trafficking of DPL to recipient draining lymph nodes, in the initial post-transplant period, was reduced in anti-ICAM-1 treated recipients compared to saline treated controls. Figure 1 shows that the proportion of recipient draining lymph node DNA that was donor derived was significantly higher in saline treated recipients (0.06%) than anti-ICAM-1 treated recipients (0.03%) (p=0.0375), 24 hours after transplantation. In addition, graft survival was prolonged in anti-ICAM-1 treated splenectomised recipients, with 60% of recipients surviving indefinitely, compared to 14% in the splenectomy only group, and zero in both the saline control and anti-ICAM-1 only groups (figure 2).





These results demonstrate the effect of anti-ICAM-1 treatment on lymphatic trafficking of DPL in the immediate post-transplant period, and highlight the importance of interactions between antigen presenting cells and recipient T cells within secondary lymphoid organs to the rejection process.

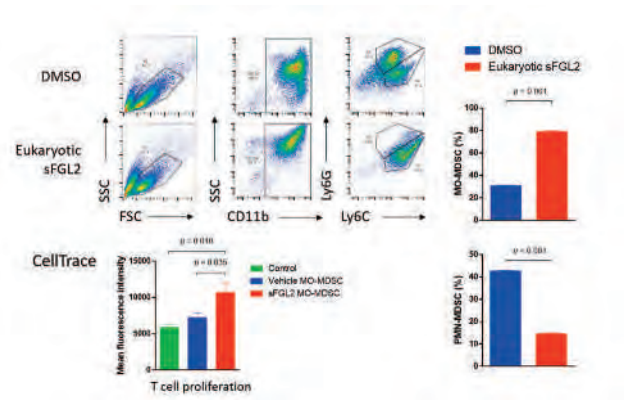
This work was funded by an MRC Centre grant (MR/J006742/1)

457.6

Soluble fibrinogen-like protein 2 regulates differentiation and enhances immunosuppressive function of myeloid-derived suppressor cells in allograft immunity

Cheng Yang[1], Lingyan Wang[2], Ming Xu[3], Tongyu Zhu[3].
[1]Plastic Surgery, Zhongshan Hospital, Fudan University, Shanghai, People's Republic of China; [2]Biomed Research Center, Zhongshan Hospital, Fudan University, Shanghai, People's Republic of China; [3]Urology, Zhongshan Hospital, Fudan University, Shanghai, People's Republic of China.

Soluble fibrinogen-like protein 2 (sFGL2) is a novel immunoregulatory molecule, secreted mainly by regulatory T cells. CD11b+ Gr1+ myeloid-derived suppressor cells (MDSCs) are an important regulatory innate cell population and have significant inhibitory effect on T cell-mediated responses. Here, we synthesized murine full length sFGL2 by eukaryotic expression system, and investigated the impact on differentiation and function of MDSCs. Bone marrow cells from BABL/c mice were cultured with or without 10 μg/ml sFGL2 for 3 days and 5 days under 10 ng/ml GM-CSF stimulation. Compared with PBS, sFGL2 significantly induced CD11b+Ly6G-Ly6Chigh MDSC (MO-MDSC) differentiation but inhibited CD11b+Ly6G+Ly6Clow MDSC (PMN-MDSC) differentiation. The sFGL2-induced MO-MDSCs significantly inhibited T cells proliferation compared with those induced by PBS. Besides, sFGL2-induced MO-MDSCs demonstrated higher expression of arginase-1 and iNOS at both mRNA and protein level. Furthermore, adoptive transfer sFGL2-induced MO-MDSCs prolonged the skin allograft survival in mice. In the sFGL2-induced MO-MDSCs infusion group, the transplanted skin allograft showed mild inflammatory immune cell infiltration, less apoptosis and necrosis, and lower pro-inflammatory cytokines expression. T cells in the recipient mouse displayed a lower autoimmune phenotype (lower TCR+ CD44high CD62low cells). Taken together, our results indicate sFGL2 prompts MO-MDSCs differentiation and enhances their immunosuppressive function.



References:
[1] Wang L, Yang C, Xu M, Hu M, Wang X, Zhu T. *Transplantation*. 2014, 97:1201-1206.

457.7

Reduction in CXCL9–CXCR3 signals diminishes TRAIL-expressing liver NK cells in partially hepatectomized mice under depressed immune status

Takuya Yano, Masahiro Ohira, Ryosuke Nakano, Hideki Ohdan.
Department of Gastroenterological and Transplant Surgery, Hiroshima University, Hiroshima, Japan.

Introduction: Liver resident natural killer (NK) cells express the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), a critical molecule for NK cell-mediated anti-tumor cell killing. However, dynamics of the TRAIL-expressing NK cells after partial hepatectomy (PH) or liver transplantation (LT) is unknown. In the present study, we used a mouse model to evaluate the effect of PH on the number and function of TRAIL-expressing NK cells in the liver.
Methods: To quantify the number and function of NK cells after PH, the liver was perfused and lymphocytes were purified using a density gradient. Flow cytometry revealed the changes in surface phenotype and intracellular protein production.
To assess the anti-tumor activity in vivo, Hepa1-6 cells were injected intra-portally into C57BL/6 mice following a 70% hepatectomy.
To investigate the liver environment during hepatectomy, cytokines and chemokines in the liver and their corresponding receptors on liver NK cells were measured.
Results: The expression of TRAIL in liver NK cells was temporarily down-regulated after a 70% PH (7 mice experiments; $p < 0.01$ for day 3 vs control). Although PH allowed tumor growth in the liver, adoptive transfer of TRAIL-expressing liver NK cells prevented the spread of metastases.
After PH, mRNA and protein levels of CXCL9 and interferon (IFN)-gamma significantly decreased in the liver (Fig 1A). Comprehensive analysis of chemokine receptors revealed that CXCR3, which is the receptor of CXCL9, was strongly expressed by TRAIL-expressing NK cells (98.8% ± 0.1 %) but not by TRAIL-negative NK cells (20.4% ± 2.3 %), (n = 5, p = 0.008) (Fig1B 1C 1D). Augmentation of CXCL9 in the liver by injecting poly I:C into mice maintained the TRAIL-expressing NK cells in the liver even after a 70% PH (Fig2A 2B 2C). Analysis of intracellular flow cytometry results showed that Kupffer cells extensively produced CXCL9 in response to IFN-gamma in various liver component cells.

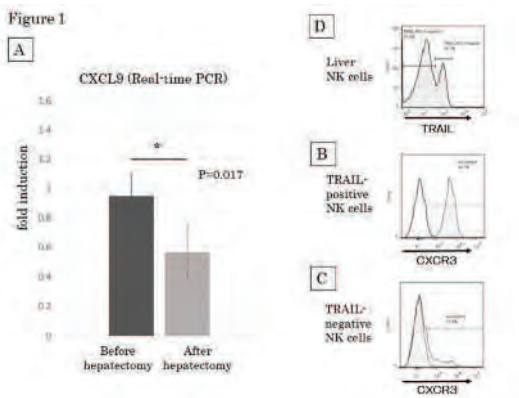
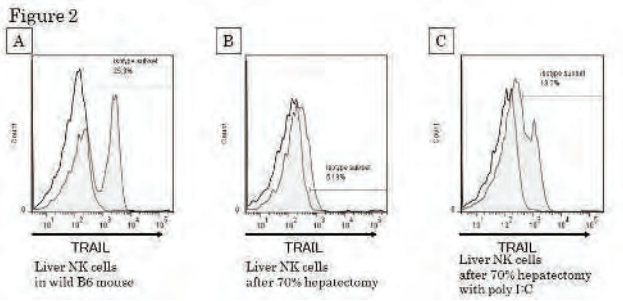


Figure 2



Discussion: In this study, we have shown that the number of TRAIL-expressing NK cells and anti-tumor activity in the liver decreased after 70% PH. The depressed immune status including activity of liver resident NK cells caused hepatocellular carcinoma (HCC) recurrence after PH or LT. The CXCL9-CXCR3 pathway in the liver is the possible molecular mechanism causing the recurrence. Since CXCL9 decreased after PH, TRAIL-expressing NK cells that had CXCR3 could not be maintained in the liver. Furthermore, intrahepatic IFN-gamma regulated Kupffer cells to produce CXCL9.
Conclusion: Significant reduction in TRAIL-expressing NK cells in the liver after 70% PH is associated with the impaired intrahepatic CXCL9–CXCR3 pathway. Our results suggest the possibility of developing strategies to augment CXCL9 levels in the liver to prevent HCC recurrence after PH or LT, i.e., the combination of immunotherapy using TRAIL-expressing NK cells and chemokine CXCL9.

457.8

NK cells promote kidney graft rejection through evasion of cyclosporine a therapy

Muhammad Imtiaz Ashraf[1], Thomas Resch[2], Cornelia Fabritius[2], Susanne Ebner[2], Paul Ritschl[1], Vanessa Mellitzer[2], Julia Günther[2], Felix Aigner[1], Johann Pratschke[1], Katja Kotsch[1].
[1]Department of General, Visceral, and Transplantation Surgery, Experimental Surgery and Regenerative, Charité-Universitätsmedizin Berlin, Berlin, Germany; [2]2Department of General, Visceral, and Transplantation Surgery, Innsbruck Medical University, Innsbruck, Austria.

Background: Despite advances in immunosuppressive regimens having significantly increased short-term graft outcome, overall long-term graft survival has not dramatically changed. Frequently used immunosuppressive drugs e.g. calcineurin inhibitors (CNI), either cyclosporine A (CsA) or tacrolimus (Tac) are primarily targeting T cells, whereas their efficacy in suppressing other immune subsets such as NK cells might be limited, which may explain the incomplete long-term benefits. As NK cells have recently been recognized as key players in chronic allograft failure comprehensive studies are required to address whether NK cells can escape conventional immunosuppressive regimens and play a role in recurrence of allograft rejection.
Methods: We characterized the effects of cyclosporine A (CsA) in vitro and ex vivo on human and murine NK cells and further assessed its functional influence on NK cells during transplantation using a murine model of allogenic KTX (Balb/C to C57Bl/6). Immunophenotyping of NK cells and other graft infiltrating lymphocytes was performed by flow cytometry. Allograft function was assessed by measuring serum creatinine and urea levels. For histology tissue sections were stained with HE and PAS and evaluated according to the Banff criteria. Expression of cytokines and inflammatory markers was performed by RT-qPCR.
Results: In vitro, human NK cells treated with CsA concentrations (5-1000 ng/ml over 1-3 days) were insensitive concerning viability, expression of activation markers (CD16, NKp30, NKp44, NKp46), pattern of differentiation (CD65dim/CD56bright) and IFNγ production. Concordantly, ex vivo, CD3-NKp46+ NK cells isolated from CsA treated C57BL/6 mice (10 mg/kg for 7 days) revealed normal function regarding degranulation and IFNγ production, whereas CD8+ T cells were functionally impaired. In vivo, application of CsA to C57BL/6 recipients of fully allogeneic Balb/C kidneys resulted in a significant reduction of creatinine levels at day 7 (KTX: 35.6±4 vs. KTX+CsA: 19.8±4 mmol/L; $p<0.05$). Flow cytometric analysis revealed a CsA mediated reduction of intragraft CD4+ and CD8+ T cells by halve, whereas intragraft NK cell frequencies significantly increased (KTX: 10±0.4 vs. 17.5±1.5; $p<0.01$) and remained unaffected within the spleen or liver. Importantly, the additional depletion of NK cells (anti-NK1.1 Ab) resulted in a further improvement of kidney function (12±0.5 mmol/L; $p<0.01$) associated with reduced intragraft and splenic IFNγ expression levels ($p<0.05$, respectively).
Conclusion: CsA insufficiently targets human and murine NK cell function ex vivo and fails to affect NK cells in vivo. We show for the first time that NK cell depletion combined with CsA synergistically improves graft function in an acute transplantation setting, suggesting that selective NK cell targeting might constitute a novel approach to ameliorate outcomes after KTX in the long-term.

457.9

Klotho deficiency aggravates tacrolimus-induced renal injury via the PI3K-Akt-FoxO pathway

Jian Jin, Longt Jin, Chul Woo Yang, Kang Luo, Sun Woo Lim.
Seoul St. Mary's Hospital, The Catholic University of Korea 641 Banpo-ro, Seocho-Ku, Seoul 137-040, Korea, Seoul, Korea.

Background: Klotho is highly expressed in the kidney, is present in the circulation and urine, and has protective effects against various renal injuries. We examined whether reduced Klotho expression affects tacrolimus (Tac)-induced renal injury in an experimental model of chronic Tac nephropathy.
Methods: First, we evaluated the association between the Tac dose and Klotho expression by giving different doses of Tac (0.25, 0.5, and 1 mg/kg) to wild-type (WT) mice for 4 weeks. Second, we compared Klotho levels, renal function, fibrosis, and apoptosis between WT mice and Klotho heterozygous (HT) mice in an experimental model of chronic Tac nephropathy. Third, we examined whether the oxidative stress and signaling pathway is involved in the protection by Klotho against Tac-induced renal injury.
Results: Klotho levels in renal tissue and urine were reduced in a dose-dependent manner in Tac-treated WT mice. Tac-treated HT mice showed lower levels of Klotho in the renal cortex and urine, and higher serum creatinine level, fibrosis, and apoptosis compared with WT mice. Treatment of Tac to WT mice increased markers of oxidative stress such as PI3K–Akt and FoxO3a phosphorylation but decreased FoxO1 dephosphorylation. These effects were greater in HT mice. HT mice exhibited a much lower level of manganese superoxide dismutase level and higher level of Bim, target genes of FoxOs, compared with the levels in WT mice.
Conclusion: Reduced Klotho expression aggravates Tac-induced renal injury via the PI3K–Akt–FoxO pathway.

458.1

VDJ immune repertoire sequencing predicts patients at risk of alloimmune injury

Marina Sirota[1], Tara Sigdel[1], S. Boyd[2], A. Fire[2], Minnie Sarwal[1].
[1]University of California San Francisco, San Francisco, CA, United States; [2]Stanford University, Stanford, CA, United States.

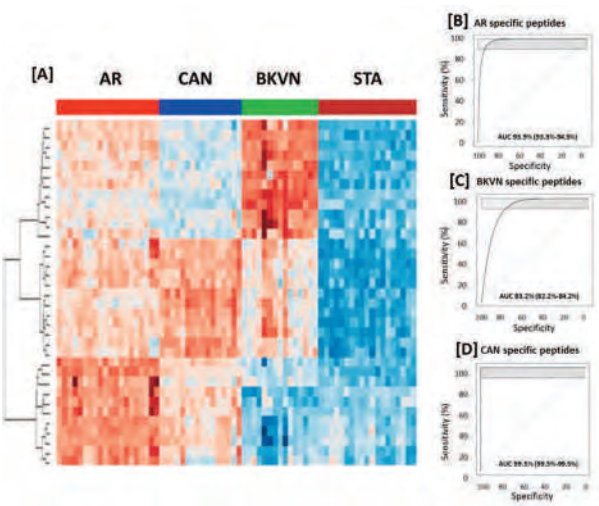
Background: The complex repertoire of immune receptors generated by B cells enables allo-recognition in the context of transplantation across HLA barriers, and is a major contributor for the persistence of unrecognized, sub-clinical lymphocyte immune activation, resulting in acute and chronic immune mediated graft injury. High-throughput DNA sequencing technologies have been advancing at an extremely rapid pace and are transforming our understanding of humoral immune responses.
Methods: DNA sequencing of rearranged immune receptor loci was performed in 99 serial blood samples, collected at 0, 6, 12 and 24 months after transplantation in 36 unsensitized kidney transplant recipients with and without acute rejection and progressive chronic injury. Matched, centrally scored, histopathology compartment scores and graft function were also obtained on all patients at all time points. We used a series of redundant primers to amplify diverse DNA rearrangements, and the resulting mixtures of barcoded amplicons were sequenced using long-read ultra-deep sequencing. Individual DNA molecules were then characterized on the basis of DNA segments that were joined to make functional (or nonfunctional) immune effectors.
Results: Nearly 600,000 sequences were generated across 99 samples resulting in roughly 200,000 unique clones, defined by the VDJ recombination patterns and the CDR3 region sequence. We observed significantly more B-cell sequences and clones present in the acute rejection group in comparison to the other sub-groups (ANOVA p= 0.0006545), with greater expansion also seen in the patients with greater biopsy chronicity at 24 months (ANOVA p=5.016e-05). Importantly, in the acute rejection group B cell sequences and clones were expanded across all time points, inclusive of the pre-transplant sample. Longitudinal analysis tracking clones across the various time points observed similar and related clones across different people.
Conclusions: VDJ sequencing demonstrates expansion of specific B-cell clones prior to transplantation, even in the absence of allo-sensitization. This B cell clonal expansion is highly predictive of acute rejection and accelerated chronic rejection. B cell immune priming is currently unrecognized, and maybe harnessed for immunosuppressive drug selection at engraftment.

458.2

Mining the human proteome for monitoring renal transplant injury

Tara Sigdel[1], Y. Gao[2], J. He[2], A. Wang[1], C. Nicora[2], D. Smith[2], W. Qian[2], D. Camp[2], Minnie Sarwal[1].
[1]Surgery, University of California San Francisco, San Francisco, CA, United States; [2]Pacific Northwest National Laboratory, Richland, CA, United States.

The human urinary proteome reflects systemic and inherent renal injury perturbations and can be harnessed to define biomarkers for different kidney transplant injury states. Through a NIH-funded study, we sought to identify and validate panels of urine proteins that could serve as non-invasive surrogate biomarkers for kidney transplant injuries.
396 unique urine samples were collected contemporaneously with an allograft biopsy from 396 unique kidney transplant recipients. Centralized, blinded histology on the graft phenotyped matched urine samples into categories of acute rejection (AR), chronic allograft nephropathy (CAN), BK virus nephritis (BKVN), and stable (STA). A strategy involving liquid chromatography–mass spectrometry (LC-MS) based shotgun proteomics using iTRAQ based discovery (n=108) to quantify pooled samples and global label-free LC-MS analyses of individual samples (n=137) and targeted validation with selected reaction monitoring (SRM; n=151) was applied to identify and validate minimal urine protein/peptide biomarkers to accurately segregate organ injury causation and pathology. The panel of peptides was selected with an algorithm called LASSO and its extension elastic net.
A total of 958 proteins were initially quantified by iTRAQ, 87% of which were also identified among 1574 urine proteins detected in label-free LC-MS validation. 103 urine proteins were significantly ($p<0.05$) perturbed in graft injury and enriched for humoral immunity, complement activation and lymphocyte trafficking. A set of 131 peptides corresponding to 78/103 proteins were measured by SRM and validated as significant in an independent sample cohort. A rigorous A minimal set of 35 peptides mapping to 33 proteins, were modeled to segregate different injury groups (AUC =93% for AR, 99% for CAN, 83% for BKVN) [Fig1].



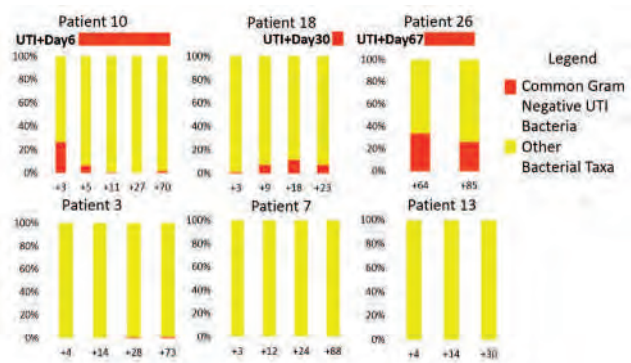
This translational research used biomarker discovery and validation used mass spectrometry based urinary proteomics and has identified urine protein fingerprints for non-invasive differentiation of kidney transplant injuries, thus opening the door for personalized immune risk assessment and therapy.

458.3

Gut microbial composition predictive of gram-negative urinary tract infections in kidney transplant recipients

John Lee[1], Muthukumar Thangamani[1], Darshana Dadhania[1], Lilan Ling[2], Matthew Magruder[1], Eric Pamer[2], Manikkam Suthanthiran[1].
[1]Medicine/Nephrology and Hypertension, Weill Cornell Medicine, New York, United States; [2]Medicine/Infectious Diseases, Memorial Sloan Kettering Cancer Center, New York, United States.

Introduction: Disturbance in the gut flora (i.e. antibiotic administration) is a known risk factor for development of *Clostridium difficile*, but its relationship to other infectious bacterial complications is not well defined. We reported that fecal *Enterococcus* abundance precedes and/or is associated with development of *Enterococcus* urinary tract infections (UTI) in kidney transplant recipients[1]. In this cohort of transplant recipients, we examine the relationship between the gut microbiota and common gram-negative UTIs in kidney transplantation.
Methods: We prospectively enrolled 26 kidney allograft recipients from 2011 to 2012 and collected serial fecal specimens during the first three months after kidney transplantation (N=85 specimens). We characterized the gut microbiota in all 85 fecal specimens using 16S rRNA deep sequencing of the V4-V5 hypervariable region. We evaluated whether the fecal abundance of common gram-negative UTI bacteria (defined by the following genera: *Escherichia*, *Klebsiella*, *Pseudomonas*, *Enterobacter*, *Citrobacter*, and *Proteus*) was associated with common gram-negative UTIs.
Results: Three of the 26 recipients developed common gram-negative UTIs (*Escherichia coli*, *Klebsiella oxytoca*, and *Proteus mirabilis*) and had available fecal specimens during the time frame of the UTI. Figure 1 shows the 3 recipients with UTI and 3 representative recipients who never developed common gram negative UTIs. In this figure, the gut microbial composition as a percentage of common gram-negative UTI bacteria is shown on the y axis and post transplantation days when the fecal specimens were collected for microbial composition analyses are shown on the x axis. The 3 transplant recipients who developed UTIs had a higher fecal abundance of common gram-negative UTI bacteria at the time of UTI (median, 7.3% [range: 6.5% to 33.6%]) than the 23 transplant recipients who did not develop common gram negative UTIs (time-matched specimens, median 0.4% [interquartile range: 0% to 1.5%]) (P=0.005, Wilcoxon rank sum test).



Conclusions: Fecal abundance of common gram-negative UTI bacteria is associated with common gram-negative UTIs, highlighting a novel interplay between the gut microbiota and urinary tract infections, and importantly the potential for personalized infection prophylaxis.
References:
[1] Lee JR, Muthukumar T, Dadhania D, Toussaint NC, Ling L, Pamer E, Suthanthiran M. Gut microbial community structure and complications after kidney transplantation: a pilot study. Transplantation 2014; 98(7):697-705. PMCID: PMC4189837.

458.4

Host microbiota of cardiac allograft recipients is durably altered by fecal microbiota transplant and these changes correlate with allograft outcome

C. Colin Brinkman[1], Lauren Hittle[2], W. Florian Fricke[2], Emmanuel F.Mongodin[2], Jonathan Bromberg[1].
[1]Surgery, Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD, United States; [2]Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD, United States.

Hypothesis: We previously presented highly significant differences in the bacterial community structures of human renal allograft recipients as a result of transplantation and attendant medications that persisted over time. These results suggest that longitudinal changes in each individual's microbiota have the potential to be indicative of health or disease in the context of transplantation. We also found that murine normal, colitic, and pregnant fecal samples influenced murine cardiac allograft survival and were markedly distinct from each other in bacterial composition and community structure, notably, in the abundance of Bifidobacterium. We hypothesized that these differences persist after fecal microbiota transplant (FMT) into mice with cardiac allografts and that they correlate with allograft outcome.
Methods: Mice were fed antibiotics (kanamycin, gentamicin, colistin, metronidazole, vancomycin) ad libitum in drinking water on days -6 to -1 before transplant. On d0 C57BL/6 mice received BALB/c hearts. Fecal samples from healthy C57BL/6, female mice on d11-12 of pregnancy, colitic T cell receptor transgenic mice that spontaneously develop colitis, or cultured Bifidobacterium pseudolongum (ATCC25526) were also transferred on d0 by gavage. Mice received daily immunosuppression: tacrolimus (2 mg/kg/d sc d0-40 or 3 mg/kg/d sc d0-60) starting on day 0. Fecal pellets and intestinal tissue were collected from transplanted mice, and analyzed via 16S rRNA gene sequencing and RNA-Seq. Cardiac allografts were assessed for survival, harvested at d40, 60, or rejection, and stained with H&E and Masson's Trichrome.
Results: 16S rRNA gene analysis of transplant recipient stool samples revealed highly significant differences in the bacterial community structures of recipients of normal, colitic, and pregnant FMT, and cultured B. pseudolongum, as determined by bacterial composition and relative abundance, principle component analysis and hierarchical clustering. Bacteria from the genus Bifidobacterium were absent in colitic, yet present in normal and pregnant source samples and remained fairly abundant in pregnant transplanted samples for at least 40 days. In general the microbiota of recipients of normal, colitic, and pregnant fecal samples converged over time, but remained distinct for at least 40 days. Mice receiving pregnant FMT or B. pseudolongum had the highest graft survival rates, and least histological evidence of graft inflammation and fibrosis indicating a possible anti-inflammatory effect of Bifidobacterium bacteria.
Conclusion: FMT of pro- and anti-inflammatory fecal microbiota and specific components of the microbiota result in durable changes to host microbiota. These changes correlate with alterations in systemic immunity with consequences for graft survival, rejection, and inflammation.

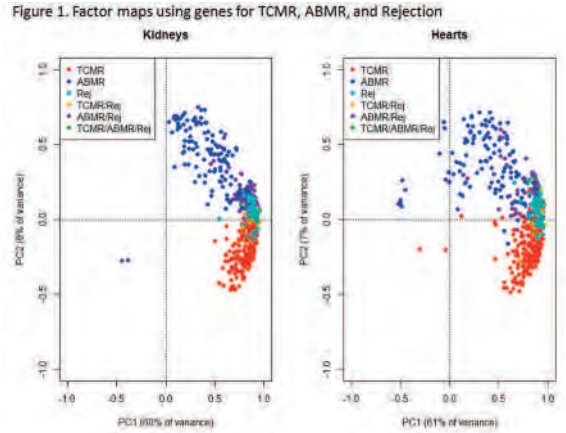
458.5

A molecular microscope diagnostic system (MMDx) for heart transplant biopsies developed from the molecular phenotypes of rejection in kidney transplants.

Philip Halloran[4], Daniel Kim[4], Alexandre Loupy[2], Jean-Paul Duong Van-Huyen[2], Patrick Bruneval[3], Luciano Potena[1], Ornella Leone[1], Jeff Reeve[4].
[1]University of Bologna, Bologna, Italy; [2]Necker Hospital, Paris, France; [3]Hôpitaux de Paris, Paris, France; [4]University of Alberta, Edmonton, AB, Canada.

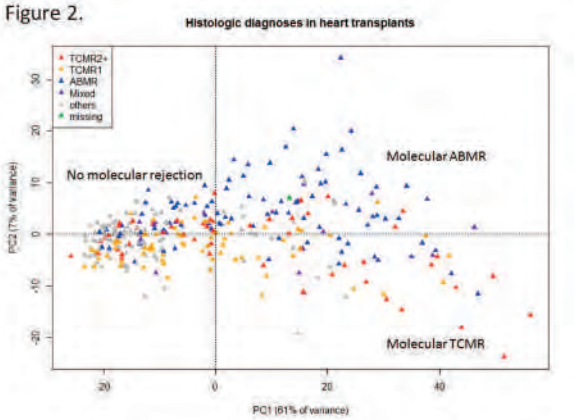
The diagnosis of heart transplant rejection by histology in endomyocardial biopsies (EMB) is challenging. A molecular system (MMDx) has been developed to assess both T cell-mediated (TCMR) and antibody-mediated (ABMR) rejection in kidney transplant biopsies. The present project adapted the MMDx derived in kidney transplant biopsies to heart transplant EMBs. We collected a single bite from 331 standard-of-care EMBs from three centers and processed them on Affymetrix microarrays. EMB diagnoses were assessed by histology using ISHLT guidelines. To develop diagnostic tests for EMBs, the genes most highly associated with Rejection, ABMR, and TCMR in kidney transplants were used to perform semi-supervised clustering on the EMBs. Figure 1 shows that the multivariate distribution of these genes was highly conserved between kidney biopsies (left panel) and heart biopsies (right panel).

Figure 1. Factor maps using genes for TCMR, ABMR, and Rejection



The biopsies segregated into three overlapping molecular clusters in the discovery set, roughly corresponding to histologic diagnoses of TCMR, ABMR, and No Rejection (figure 2). Some histology ABMR and TCMR (particularly TCMR1) biopsies were molecularly No Rejection. Two-thirds of the biopsies were used as a discovery set, and the remaining third as a validation set. A predictive model for probability of ABMR, TCMR, or no rejection, based on the molecular distribution of biopsies in the discovery set, was used to assign scores to the biopsies in the validation set. These were distributed similarly to those in the discovery set. Biopsies designated as ABMR by the molecular test showed reasonably good agreement with histology, and were highly associated with HLA antibody. There was considerable less agreement between molecular and histologic TCMR, supporting concerns that the current histologic ISHLT assessment of EMBs is poorly predictive of true TCMR. We conclude that the MMDx can interpret rejection in EMBs, and the results show strong molecular similarity to rejection in kidney biopsies. The results also raise concerns about the accuracy of histologic assessment, particularly of TCMR, but open the possibility of using the molecular classification as a reference standard to improve conventional assessments of EMBs.
(Clinicaltrials.govNCT#02670408).

Figure 2.

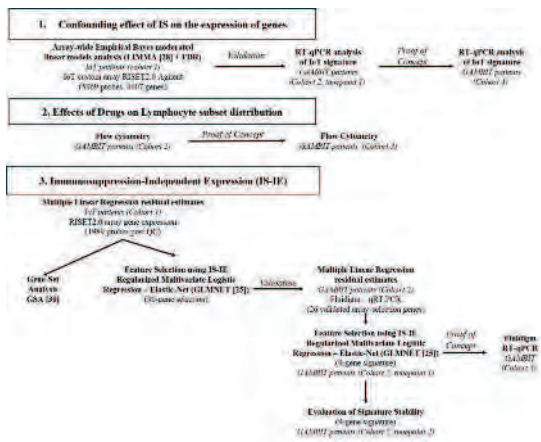


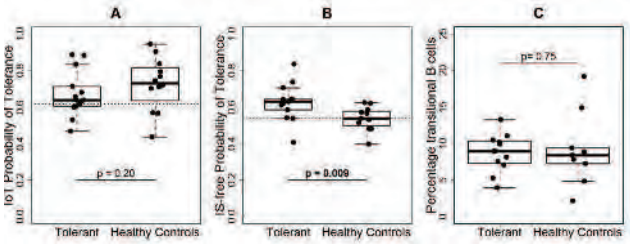
458.6

Biomarkers of tolerance in kidney transplantation: Immunosuppression-effects on gene-expression. Can we really detect tolerance?

Maria Hernandez-Fuentes[2,5], Irene Rebollo-Mesa[1,5], Christakoudi Sofia [1,5], Estefania Nova-Lamperti[5], Paula Mobillo[5], Manohursingh Runglall[2], Sonia Norris[5], Yogesh Kamra[2], Rachel Hilton[4], David Game[4], GAMBIT-Consortium www.GAMBITstudy.org.uk[5], Robert I.Lechler[3,5], Graham M.Lord[2,5].
[1]Biostatistics; IoPPN, King's College London, London, United Kingdom; [2]NIHR Biomedical Research Centre at Guy's & St Thomas' NHS Foundation Trust and King's College London, London, United Kingdom; [3]King's Health Partners, London, United Kingdom; [4]Renal Unit, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom; [5]MRC Centre for Transplantation, King's College London, London, United Kingdom.

We and others have previously analysed rare kidney transplant recipients (KTR) that are operationally tolerant in that they have retained their transplant despite stopping all immunosuppression. These studies described a signature that displayed over-expression of B-cell-related genes and a relative expansion of the transitional-Bcell subset size. A key question confounding these studies was whether the features seen in the tolerant patients merely reflected the fact that they were not taking immunosuppression (IS), unlike all the comparator patient groups.
We re-analysed microarray data from our previous study, and validated on an independent cohort of 250 KTR on IS and 14 newly identified tolerant recipients.
We demonstrate that many of the genes with altered expression between tolerant subjects and the other KTR are influenced by IS. Additionally, flow-cytometry analyses show that IS drugs affect transitional B-cells in a way that mirrors their effects on gene-expression, but not other lymphocyte subsets as much. To address this problem, we have used a regression approach to obtain IS-independent expression and define a new unbiased signature of tolerance, using elastic-net regularized regression tuned via cross-validation. The new IS-independent signature was validated with AUC=0.93, and also differentiated tolerant patients from healthy controls. The statistical results are validated by a proof of concept prospective study that demonstrates that the IS-independent signature is robust to actual changes in IS, and remains stable when comparing samples before and after steroid withdrawal.
Conclusion: We report on a validated and highly accurate gene-expression signature that could be used reliably to identify patients suitable for IS reduction, irrespective of the IS drugs they are receiving. Only this approach will make the conduct of pilot clinical trials for IS-minimization safe, and hence allow critical improvements in kidney post-transplant management.





MRC in UK: G0801537/ID:88245 grant and MRC Centre for Transplantation, – MRC grant no. MR/J006742/1; Guy's and St Thomas' Charity (grants R080530 and R090782); EU-FP7 HEALTH-F5–2010–260687: The ONE Study; EU-FP7 HEALTH-2012-INNOVATION-1 project number 305147: BIO-DrIM; National Institute for Health Research (NIHR) Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London; Clinical Research Networks in UK, study portfolio number 7521

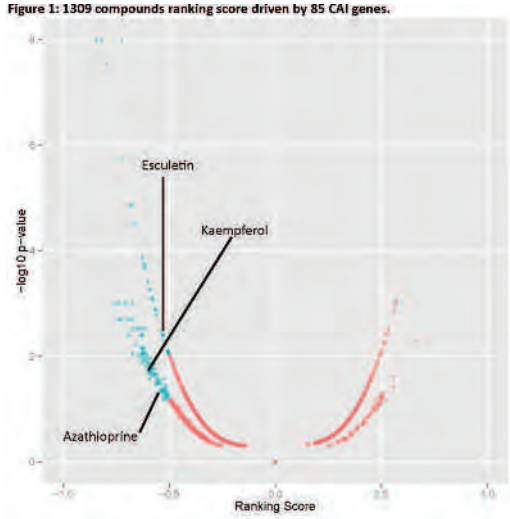
458.7

Identification of novel therapeutics and targets for chronic allograft injury using integrative informatics approach

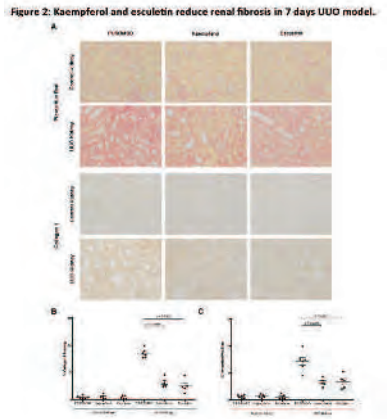
Li Li[1], Ilana Greene[2], Benjamin Readhead[1], Brian Kidd[1], Andrew Uzilov[1], Madhav Menon[2], Bernd Schroppel[2], John Cijiang He[2], Rong Chen[1], Joel Dudley[1,3], Barbara Murphy[2].
[1]Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, United States; [2]Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, United States; [3]Health Policy and Research, Icahn School of Medicine at Mount Sinai, New York, NY, United States.

Background and Hypothesis: Chronic allograft injury (CAI) is a leading cause of allograft failure and significantly impacts with long-term allograft survival[1]-[3]. Few effective therapeutic options are available to prevent or revert the progression of CAI. In this study, we aimed to identify novel therapeutic targets to potentially ameliorate CAI through an integrative computational framework. We hypothesized that a robust molecular signature from a meta-analysis of multi-center independent gene expression data sets integrated with a computational drug repurposing approach would suggest novel therapeutic targets for rational drug discovery and design.
Methods: We developed a computational framework to identify novel therapeutic targets with potential to ameliorate CAI. Our analysis utilized six independent microarray datasets comprising 275 samples generated by ourselves and others from Gene Expression Omnibus (GEO) as well as chemogenomic profiles of 1,309 compounds from the Connectivity Map[4]. We applied a meta-analysis approach to identify the gene signature specific to CAI, which was used as input for computational drug repurposing analysis. We hypothesized that if a CAI state is signified by a specific set of genome-wide transcriptional expression changes, and if exposure to a particular drug causes the reverse set of changes in a model cell line, then that drug has the potential to have a therapeutic effect on CAI. We used human kidney 2 (HK2) cells and mouse unilateral ureteric obstruction (UUO) model to validate the cellular pathways and study the development of renal interstitial fibrosis that were perturbed by the predicted drugs.
Results: We identified 85 genes that were associated with CAI. We thus generated a ranked list of potential treatments for CAI from compounds predicted to induce an anti-correlated state to CAI ($P \leq 0.05$)[5],[6]. Among the top ranked compounds predicted to be therapeutic for CAI were azathioprine (score=-0.53, p=0.04), a drug to prevent acute rejection in renal transplantation[7] and treat pulmonary fibrosis[8], and kaempferol (score=-0.57, p=0.02) and esculetin (score=-0.52, p=0.01), two drugs not previously described to have efficacy for CAI (Figure 1).

Figure 1: 1309 compounds ranking score driven by 85 CAI genes.



In UUO model, kaempferol reduced the expression of *Snai1* (p=0.04); esculetin reduced the expression and protein levels of *cyclin D1* (p=0.004). In HK2 cells, kaempferol attenuated TGF-b1 induced fibrotic and inflammatory mechanisms, as measured by reduction in the phosphorylation of *SMAD3* (p=0.01), NFkB *P65* (p=0.02), and reduction in expression of *SNAI1* (p=0.01) and *CDH1* (p=0.045). Esculetin attenuated Wnt-induced fibrotic mechanism, as measured by reduction in protein levels of *CCND1* (p=0.005) and reduction in expression of *CCND1* (p<0.0001) and *MYC* (p=0.01). Kaempferol and esculetin reduced fibrosis as compared with PBS/DMSO vehicle in UUO model as measured by collagen I immunohistochemistry and picrosirius red staining. Histology confirmed significantly abrogated fibrosis by kaempferol (p<0.0001 and p=0.0009) and esculetin (p<0.0001 and p=0.001) (Figure 2).



Conclusion: We developed and applied an integrative computational approach to identify kaempferol and esculetin as putatively novel therapies for CAI and provided experimental evidence for their therapeutic activity in vitro and in vivo using preclinical models. The findings suggest that both drugs might serve as therapeutic options for CAI.

References:

[1] Meier-Kriesche HU, Schold JD, Kaplan B. Long-term renal allograft survival: have we made significant progress or is it time to rethink our analytic and therapeutic strategies? American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2004;4:1289-1295.
[2] Pascual M, Theruvath T, Kawai T, Tolkoff-Rubin N, Cosimi AB. Strategies to improve long-term outcomes after renal transplantation. The New England journal of medicine. 2002;346:580-590.
[3] Hariharan S, Alexander JW, Schroeder TJ, First MR. Impact of first acute rejection episode and severity of rejection on cadaveric renal allograft survival. Clinical transplantation. 1996;10:538-541.
[4] Lamb J, Crawford ED, Peck D, et al. The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. Science. 2006;313:1929-1935.
[5] Sirota M, Dudley JT, Kim J, et al. Discovery and preclinical validation of drug indications using compendia of public gene expression data. Science translational medicine. 2011;3:96ra77.
[6] Dudley JT, Sirota M, Shenoy M, et al. Computational repositioning of the anticonvulsant topiramate for inflammatory bowel disease. Science translational medicine. 2011;3:96ra76.
[7] Bergan S, Rugstad HE, Bentdal O, et al. Monitored high-dose azathioprine treatment reduces acute rejection episodes after renal transplantation. Transplantation. 1998;66:334-339.
[8] Izumi S, Iikura M, Hirano S. Prednisone, azathioprine, and N-acetylcysteine for pulmonary fibrosis. The New England journal of medicine. 2012;367:870; author reply 870-871.

458.8

First clinical results of a saliva testing for monitoring after renal transplantation

Dietmar Abendroth[1], Jürgen Kaden[2], Michael Marzinzig[1], Manfred J.Stangl[3].
[1]Center of Surgery, University of Ulm, Ulm, Germany; [2]Clinical Chemistry, Vivantes-Klinikum Friedrichshain, Berlin, Germany; [3]Transplant Center Munich-Großhadern, Ludwig-Maximilians-University, Munich, Germany.

Introduction: There remains a need for biomarker in transplantation for monitoring and early identification of rejection episodes. We developed a test using a colorimetric assay to detect kynurenine (Kyn) changes in serum and saliva. Kynurenine is generated downstream in the tryptophan metabolism and one of the key players concerning inflammatory response and known for immune-modulation not only on T-regulatory and NK cells. Furthermore, we developed a monoclonal antibody against kynurenine for application in a lateral-flow-test (LFIA) especially in saliva.

Materials and Methods: Blood samples were measured retrospectively from 442 renal transplant patients (Gr.1, 69 rejection episodes) (n=5210) and prospectively from 116 patients (Gr.2) after renal transplantation (n=803). 296 healthy blood donors served as normal controls. Serum (B) **and** saliva (S) were compared in normal controls and in Gr.2 with 24 rejection episodes (BPAR).

Results: Test-recovery rate was 97-99,8%, intra-assay variance 1,53% and inter-assay variance 2,77% (B) and 94,6-97,9%, 2,24% and 3,26% for (S). Values in normal controls were 2,7±0,4 µM for serum and 0,7±0,4 µM for saliva. Mean values in patients with rejection (BPAR) was 17,4±8,4 µM in (B) and 4,7±1,6 µM in saliva (S) compared to uneventful patients 4,4±1,5 µM (B) and 1,3±0,6µM (S). The proportion rate was equal between normal controls, uneventful patients after transplantation and rejection (3,8:1). We found a) a significant correlation of Kyn and rejection episodes (BPAR) early in the beginning without a false negative result (B+S), b) a predictive information concerning the long-term run of the transplant (n=138; up to 144 mos, only B) and c) excellent tool for monitoring therapeutic interventions. In (B) testing we could differentiate significantly between steroid-sensitive, steroid-resistant and antibody mediated rejection and infection (CMV). There was no difference concerning age, gender and circadian behavior.

Conclusion: This test fulfills the given prerequisites for a biomarker especially under screening test conditions. It is a safe and reliable method, is easy and quick to perform and not costly. The LFIA set-up is under evaluation and further development. A first prospective clinical observational and interventional study is underway. This test is an opportunity for a tailored immunosuppressive therapy.

458.9

Molecular and functional immune assays as non-invasive diagnostic tools to assess the risk of acute subclinical rejection after kidney transplantation

Oriol Bestard[1,2], Tara Sigdel[1], Silke Roedder[1], Elena Crespo[2], Josep Cruzado[2], Szu-Chuan Hsieh[1], Tim Tran[1], Josep Grinyó[2], Minnie Sarwal[1].
[1]Surgery, University of California San Francisco, San Francisco, United States; [2]Kidney Transplant Unit, Bellvitge University, Barcelona, Spain.

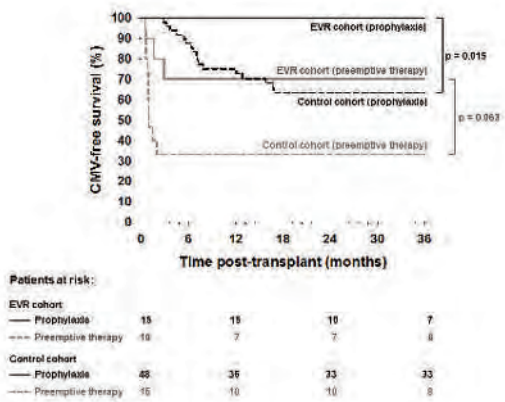
Body: Subclinical allograft rejection (sc-AR) is still a main cause of allograft loss that can only be assessed through invasive surveillance biopsies. We sought to investigate whether the combination of novel non-invasive biomarkers at the molecular and immune functional level could help identifying patients at risk of sc-AR after kidney transplantation.
Methods: We combined the assessment of the donor-specific IFN-γ T-cell ELISPOT with a highly sensitive and specific transcriptional biomarker test in peripheral blood samples predicting kidney solid organ clinical rejection (KSORT) using qPCR, in a group of 75 consecutive kidney transplant patients at the time of 6-month protocol biopsies.
Results: All patients received CNI-based IMS and either rATG or basilimab. 22/75(29%) patients showed histological lesions of sc-AR (18 TCMR, 5 ABMR and 1 mix TCMR/ABMR), 22 showed borderline (BL) lesions and 31 a stable (STA) parenchyma. The KSORT showed a high-risk (HR) and low-risk (LR) scores in 23% and 77% patients, respectively. The T-cell ELISPOT was positive in 41% of patients, whereas negative in 59%. 82% and 70% patients with BL lesions showed a LR KSORT and negative T-cell Elispot, respectively. The KSORT correctly classified as LR 98% STA and as HR 70% sc-AR ($p<0.001$), further discriminating as HR all (100%) sc-ABMR and 62.5% sc-TCMR ($p<0.001$). The d-s T-cell ELISPOT accurately ruled out the presence of sc-TCMR in 72% and predicted its presence in 83.3% ($p<0,001$), whereas it did not discriminate patients with sc-ABMR (p=NS). Transplant recipients with a positive T-cell ELISPOT showed significantly higher tubulitis (at) and interstitial infiltrates (ai) than patients with a negative ELISPOT test, whereas those patients with a positive KSORT showed higher at, ai as well as higher glomerulitis (ag) and peritubular capillaritis (ptc).
Conclusions: Combining a non-invasive molecular and functional cellular immune assay allows an accurate identification of patients developing sc-AR, dissecting the main alloimmune effector mechanism responsible for it.

459.1

Impact of everolimus and low-dose ciclosporin on cytomegalovirus replication and disease in pediatric renal transplantation

Britta Höcker[1], Sebastian Zencke[1], Lars Pape[2], Kai Krupka[1], Lennart Köster[1], Luca Dello Strologo[2], Isabella Guzzo[2], Rezan Topaloglu[2], Birgitta Kranz[2], Jens König[2], Martin Bald[2], Nicholas J.A.Webb[2], Aytul Noyan[2], Hassan Dursun[2], Stephen Marks[2], Zeynep B.Ozcakar [2], Florian Thiel[2], Heiko Billing[2], Martin Pohl[2], Henry Fehrenbach[2], Lutz T.Weber[2], Reinhard Feneberg[2], Paul Schnitzler[2], Thomas Bruckner[2], Thurid Ahlenstiel-Grunow[2], Burkhard Tönshoff[1].
[1]University Children's Hospital, Department of Pediatrics I, Heidelberg, Germany; [2]CERTAIN, Research Community, Germany.

Purpose: Preclinical studies indicate an anti-cytomegalovirus (CMV) activity of the mammalian target of rapamycin inhibitor (mTORi) everolimus (EVR), but data in pediatric renal transplant recipients are lacking.
Methods: In the framework of the Cooperative European Paediatric Renal Transplant Initiative (CERTAIN) Registry, we thus analyzed the impact of EVR-based immunosuppressive therapy on CMV replication and disease in a large cohort (n = 301) of pediatric kidney allograft recipients.
Results: The EVR cohort (n = 59), who also received low-dose ciclosporin, was compared with a control cohort (n = 242), who was administered standard-dose ciclosporin or tacrolimus and an anti-metabolite, mostly mycophenolate mofetil (91.7%). Multivariate analysis revealed an 83% lower risk of CMV replication in the EVR cohort than in the control cohort ($p = 0.005$). In CMV high-risk (D+/R-) patients (n = 88), the EVR-based regimen was associated with a significantly lower rate of CMV disease (0% vs. 14.3%, $p = 0.046$) than the standard regimen. In patients who had received chemoprophylaxis with (val-)ganciclovir (n = 63), the CMV-free survival rates at 1 year and 3 years post-transplant (100%) were significantly ($p = 0.015$) higher in the EVR cohort (n = 15) than in the control cohort (n = 48; 1 year, 75.0%; 3 years, 63.3%) (Fig. 1).



Conclusions: Our data suggest that in patients at high risk of CMV infection the use of EVR and low-dose ciclosporin is associated with a lower incidence of CMV disease compared to a standard-dose calcineurin inhibitor-based regimen.

459.2

Vaccination status and titers before and after pediatric renal transplantation: an analysis of the CERTAIN Registry

Britta Höcker[1], Martin Aguilar[1], Paul Schnitzler[2], Lars Pape[2], Luca Dello Strologo[2], Nicholas J.A.Webb[2], Martin Bald[2], Gurkan Genc[2], Heiko Billing[2], Jens König[2], Markus J.Kemper[2], Stephen Marks[2], Martin Pohl[2], Marianne Wigger[2], Rezan Topaloglu[2], Alexander Fichtner[1], Susi Rieger[1], Kai Krupka[1], Burkhard Tönshoff[1].
[1]University Children's Hospital, Department of Pediatrics I, Heidelberg, Germany; [2]CERTAIN, Research Community, Germany.

Purpose: Prevention of systemic viral and bacterial infections by effective vaccination in kidney allograft recipients is of vital importance, but data on the vaccination status in pediatric patients are scarce.
Methods: In the framework of the Cooperative European Paediatric Renal Transplant Initiative (CERTAIN) Registry, we therefore performed a multicenter, cross-sectional study in 254 patients (mean age 10.0 ± 5.6 years) and analyzed their vaccination status and titers before and after renal transplantation (RTx).
Results: Only 2/3 of patients had a complete country- and era-specific vaccination status for most vaccines prior to RTx. In particular, the vaccination rate against human papillomavirus (HPV) (27.3%), pneumococcal (42.2%) and meningococcal (47.9%) infections was low. As few as 58.1% of patients developed a hepatitis B titer (HBsAb titer) > 100 I.U./L before RTx. 39.6% of patients showed a secondary vaccination titer loss post-transplant, especially against the life-virus vaccines varicella and mumps as well as the inactivated vaccines tetanus, diphtheria, pneumococcus and hepatitis B. Patients with a HBsAb titer between 10 and 100 I.U./L prior to RTx experienced significantly ($p < 0.05$) more often a hepatitis B vaccination titer loss post-transplant than patients with a HBsAb titer > 100 I.U./L prior to RTx. The revaccination rate post-transplant was low and failed to induce protective titers in a considerable amount of patients: only 37.5% developed a sufficient HBsAb titer, and as few as 14.3% of patients showed a protective pertussis titer after revaccination.
Conclusions: The vaccination coverage rate of CKD patients prior to RTx is insufficient, particularly against HPV, pneumococcal and meningococcal infections. Recommended revaccinations post-transplant are performed deficiently. Given the rate of secondary vaccination titer loss after RTx, the recommendations of the American Society of Transplantation to measure vaccination titers against tetanus, hepatitis B and pneumococcus at regular intervals post-transplant should be followed in order to induce timely revaccination and thus avoid the development of vaccine-preventable diseases.

459.3

Pediatric kidney transplant outcomes: A single-center experience according to transplant era

Marta Monteverde[1], Juan C.Lopez[1], Juan P.Ibanez[1], Gerardo Nyman[1], Alexia Diaz Moreno[1], Liliana Bisigniano[2].
[1]Nephrology, Hospital de Pediatria JP Garrahan, Buenos Aires, Argentina; [2]Scientific and Technical Secretary, Incucai, Buenos AIres, Argentina.

Background: Improvements in management of kidney transplantation have led to better outcomes. In Argentina there are no pediatric data comparing transplant outcomes in children over different eras. The purpose of this study was to evaluate patient and graft survival in children with a kidney transplant (KT) from living related (LRD) and deceased donors (DD) in 2 periods and to analyze risk factors of graft loss and causes of death in the whole cohort.
Methods: All pediatric KT performed between 1988 and 2015 were included (n=773); 446 from DD and 327 from LRD. As in 2001 major management practices were generalized (induction therapy, MMF/MPS maintenance therapy, and all rejections confirmed by biopsy), we divided the analysis into two periods: 1988-2000 and 2001-2015.
Results: Causes of ESRD were not different between periods. Age at transplant time was slightly higher in the second era: 11(IQR: 7-14) vs 11.8 (IQR: 8.8-15) years (p=0.048). Time on dialysis before KT increased from 17 (IQR: 9-31) to 28 (IQR: 17-48) months ($p <0.001$). At 1, 3, 5, 7, and 10 years, in DD KT recipients, patient survival was 97%, 93%, 90%, 89%, and 87% in 1988-2000 vs. 100%, 99% , 98%, 98%, and 98% in 2001-2015 ($p <0.001$). Graft survival was 83%, 75%, 68%, 64%, and 52% vs. 95%, 87%, 83%, 76%, and 61%, respectively ($p <0. 001$). In LD KT, patient survival at 1, 3.5, 7, and 10 years was 99%, 99%, 98%, 95%, and 95% in the first and 100% , 96%, 96%, 96%, and 96 %, in the second era ($p = 0.74$); graft survival was 97%, 91%, 85%, 78%, and 67% in the first vs 95%, 88%, 85%, 81%, and 76% in the second cohort ($p = 0.81$). Delayed graft function (DGF) declined from 33% to 18% ($p=<0.001$) in DD, but remained unchanged (12% vs 9%; p=0.5) in LRD recipients. Prevalence of early acute rejection (1st 90 days post KT) diminished both in DD and LRD recipients (68% to 12%; $p=<0.001$, and 65% to 21%, $p=<0.001$, respectively). The first cause of graft loss was chronic rejection (61% and 62%); the second was patient death with a functioning graft (16% vs 8%; p=0.1), and the third was thrombosis (14% vs. 5%; $p = 0.04$). Crude mortality rate dropped from 7.6% to 1.3% ($p<0.001$). The first cause of death was bacterial infection (56% vs 67%; p= 0.63). Predictors of graft loss for DD KT were: DGF (HR: 4.4; 95% CI 1.6-3.3), late acute rejection (HR: 3.7; 95% CI 1.4- 3), FSGS as cause of ESRD (HR: 2.5; 95% CI 1.6-4), and early acute rejection (HR: 2.2, 95% CI 1.1-2.2); and for LRD recipients: DGF (HR: 4.8; 95% CI 2.5-9.2), age at transplant >12 years (HR: 2.7; 95% CI, 2.4-5), and late acute rejection (HR: 2.1; 95%: 1.2-3.8).
Conclusion: DD KT improved patient and graft survival in the second period. Chronic rejection remained the leading cause of graft loss. Mortality rate diminished. DGF was the main predictor of graft loss in DD and LRD KT.

459.4

Perception, diagnosis, and management of BK polyomavirus replication and disease in paediatric kidney transplant recipients in Europe

Lars Pape[1], Burkhard Toenshoff[2], Hans H.Hirsch[3,4,5].
[1]Pediatric Nephrology, Hannover Medical School, Hannover, Germany; [2]Department of Paediatrics I, University Children's Hospital Heidelberg, Heidelberg, Germany; [3]Transplantation & Clinical Virology, Department Biomedicine (Haus Petersplatz), University of Basel, Basel, Switzerland; [4]Infection Diagnostics, Department Biomedicine (Haus Petersplatz), University of Basel, Basel, Switzerland; [5]Infectious Disease & Hospital Epidemiology, University Hospital Basel, Basel, Switzerland.

Introduction: BK polyomavirus (BKPyV)-associated nephropathy remains a challenge to the success of kidney transplantation, but its impact varies in different transplant programs.
Methods: We investigated current practice through a web-based questionnaire made available by the European Society for Paediatric Nephrology (ESPN).
Results: A total of 90 physicians (23% of 391 active members) from 27 countries participated in the study. BKPyV-associated nephropathy is seen in 1-5% of patients annually with treatment success in 30-60%, and graft loss in 10%. Quantitative BKPyV load testing is available to >90% of physicians. Screening is performed in urine alone in 26%, in urine and blood in 37%, and in blood alone in 37%. Most physicians (47%) screen at month 1, 2, 3, 6, 9 and 12 post-transplant. For patients with baseline renal function and plasma BKPyV loads of 10'000 – 1'000'000 copies/mL, 50% report performing renal biopsies prior to intervention. Intervention consists of reducing immunosuppression first with mycophenolate (Myc) in 40%, first with calcineurin inhibitors (CNI) in 29%, or with both in 31%. Changing immunosuppressive drugs is considered mainly for biopsy-proven nephropathy consisting of discontinuation of Myc in 75%, and switching from CNI to mTOR inhibitors (52%). Cidofovir, intravenous immunoglobulin G, leflunomide, and fluoroquinolones are used in less than one third of this group. 66% of participants see a need for new antiviral drugs and new immmunosuppressive strategies, and almost 90% are willing to participate in future observational and interventional trials.
Conclusion: This ESPN survey suggests that prompt translation of a positive screening test into reducing immunosuppression could improve outcomes. Most participants of the survey are willing to participate in future observational and interventional studies on BKPyV.

459.5

Pubertal development in pediatric kidney transplant patients receiving mammalian target of rapamycin inhibitors or conventional immunosuppression

Julia Förster[1], Thurid Ahlenstiel-Grunow[1], Antonia Zapf[2], Martin Mynarek[3], Lars Pape[1,3].
[1]Pediatric Nephrology, Hannover Medical School, Hannover, Germany; [2]Department of Medical Statistics, University Medical Center Göttingen, Hannover, Germany; [3]Department of Pediatric Hematology and Oncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

Introduction: Data regarding the onset of puberty in children receiving mammalian target of rapamycin (mTOR) inhibitors are limited.
Methods: Kidney transplant patients aged <14 years were analyzed retrospectively to a maximum age of 18 years, with a minimal observation period of one year. Immunosuppression comprised (i) standard CNI-based regimen or (ii) low-exposure mTOR inhibitor with reduced-exposure CNI, initiated either de novo or in the maintenance phase.
Results: Of 108 children analyzed, 67 received an mTOR inhibitor (56 everolimus, 11 sirolimus) and 41 did not. The age at which girls reached Tanner stage P2 was similar with mTOR inhibitor therapy (median 11.6 years) or without (median 11.1 years) (p=0.262), as was age at stage B2 (median 11.6 versus 11.2 years; p=0.753). In boys, both the age of attaining Tanner stage P2 (median 12.9 versus 13.0 years; p=0.796) and Tanner stage G2 (median 13.1 versus 12.9 years; p=0.344) were also similar with or without an mTOR inhibitor. Age at menarche in girls, and age at spermarche in boys, did not differ between the two groups.
Conclusions: In this retrospective analysis, sexual maturation ages and reproductive hormone levels were comparable in adolescent kidney transplant patients receiving low-exposure mTOR inhibitors and reduced CNI therapy or conventional CNI-based immunosuppression.

459.6

Transitional care and adherence of adolescents after KTX in Germany and Austria: A binational observatory census within the TRANSNephro trial

Martin Kreuzer[1], Jenny Prüfe[1], Reinhard Brunkhorst[5], Dirk Bethe[4], Martina Oldhafer[3], Marie-Luise Dierks[2], Lars Pape[1].
[1]Pediatric Nephrology, Hannover Medical School, Hannover, Germany; [2]Department of Epidemiology, Social Medicine and Health System Research, Hannover Medical School, Hannover, Germany; [3]German Society of Transition Medicine, Hanover, Germany; [4]Division of Pediatric Nephrology, Center for Child and Adolescent Medicine, Heidelberg University Hospital, Heidelberg, Germany; [5]KfH Center of Nephrology, Hospitals of the Hannover Region, Hannover, Germany.

Introduction: Transition from child to adult-oriented care is widely regarded a challenging period for young people with kidney transplants and is associated with a high risk of graft failure.
Material and Methods: We analyzed the existing transition structures in Germany and Austria using a questionnaire and retrospective data of 119 patients transferred in 2011-2012.
Results: Most centers (73%) confirmed agreements on the transition procedure. Patients' age at transfer was subject to regulation in 73% (18 years). Median age at transition was 18.3 years (16.5 – 36.7). Median serum creatinine increased from 123 to 132 µmol/l over the 12 month observation period before transfer ($p = 0.002$). 25/119 patients showed increased creatinine ≥20% just before transfer. Biopsy proven rejection was found in 10/119 patients. Three patients lost their graft due to chronic graft nephropathy.
Mean coefficient of variation (CoV) of immunosuppression levels was 0.20 ±0.1. Increased creatinine levels ≥20% just before transfer were less frequently seen in patients with CoV <0.20 ($p = 0.007$).
Conclusions: The majority of pediatric nephrology centers have internal agreements on transitional care. More than half of the patients had CoV of immunosuppression trough levels consistent with good adherence. Though, 20% of the patients showed increase in serum creatinine close to transfer.

459.7

Health and wealth in children and adolescents with kidney transplants (K-CAD study)

Madeleine Didsbury[1], Anita Van Zwieten[1], Kerry Chen[1], Meredith Medway[1], Allison Tong[2], Robin Turner[3], Fiona Mackie[4], Steven McTaggart[5], Anna Francis[2,5], Tonya Kara[6], Amanda Walker[7], Sarah White[8], Kirsten Howard[2], Siah Kim[1], Jonathan Craig[1,2], Germaine Wong[1,2,9].
[1]Centre for Kidney Research, The Children's Hospital at Westmead, Sydney, Australia; [2]Sydney School of Public Health, The University of Sydney, Sydney, Australia; [3]Department of Biostatistics, University of New South Wales, Sydney, Australia; [4]Department of Nephrology, Sydney Children's Hospital at Randwick, Sydney, Australia; [5]Child & Adolescent Renal Service, Lady Cilento Children's Hospital, Brisbane, Australia; [6]Department of Nephrology, Starship Children's Hospital, Auckland, New Zealand; [7]Department of Renal Medicine, The Royal Children's Hospital, Melbourne, Australia; [8]Sydney Medical School, The University of Sydney, Sydney, Australia; [9]Centre for Transplant and Renal Research, Westmead Hospital, Sydney, Australia.

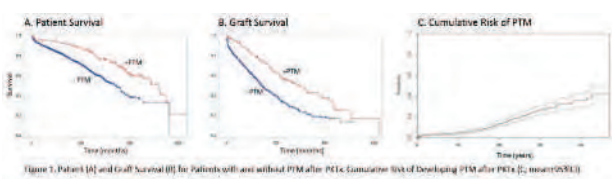
Background: Poverty and social inequality are major barriers for achieving optimal health outcomes in children, but their impact on outcomes in children after kidney transplantation remains unclear. The K-CAD study is anAustralian multicentre longitudinal cohort study that aims to describe the prevalence of economic hardship among caregivers and to determine the relationship between socioeconomic status of caregivers and self-rated health of children with chronic kidney disease (CKD).
Method: Two hundred and ninety-three children aged 6-18 years with CKD [stage 1-2 (n=69), stage 3-5 (n=77), dialysis (n=35), transplant (n=112)] were recruited from four tertiary children's hospitals across Australia. Comparisons by quintile of socioeconomic status for nominal self-rated health outcomes among children with CKD were analysed using adjusted multinomial logistic regression.
Results: The mean ages of the caregivers and children were 43.5 years (SD: 8.0) and 12.5 (SD:3.9), respectively. More than 50% of all households earned less than $1250AUD per week. Only 20% (n=60) of caregivers engaged in fulltime employment and 30% (n=89) had completed tertiary education. Across the cohort, carers who reported higher levels of financial difficulty were twice as likely to report that their child's health was poor than those with lower levels of financial difficulty (OR 2.01, [95% CI 1.2-3.3], $p<0.01$). Compared to children with pre end-stage disease, parents of children with a kidney transplant were more than twice as likely to report that their income had decreased since the diagnosis of their child's disease (OR 2.4, [95% CI 1.4-4.3]), and parents of children on dialysis were nearly five times as likely (OR 4.8, [95% CI 2.1-10.9], $p<0.05$). Socioeconomic status did not have a significant effect on access to pre-emptive transplantation or graft outcomes.
Conclusion: Socioeconomic status of caregivers appears to have a profound impact on the self-rated health in children with CKD. Families of children with end stage renal disease experience more financial hardship compared to those with less severe disease. Longitudinal follow-up will help delineate the cause of socioeconomic disadvantage in these children and the long-term effects on disease progression and wellbeing outcomes.

459.8

Incidence of post-transplant malignancy (PTM) after pediatric kidney transplantation (PKTx)

Oscar Serrano[1], Ananta Bangdiwala[2], David Vock[3], William D.Payne[1], Ty B.Dunn[1], Srinath Chinnakotla[1], Erik B.Finger[1], Raja Kandaswamy[1], Timothy L.Pruett[1], Arthur J.Matas[1], Blanche Chavers[4].
[1]Surgery, University of Minnesota, Minneapolis, MN, United States; [2]Biostatistics and Bioinformatics Core, University of Minnesota, Minneapolis, MN, United States; [3]Biostatistics, University of Minnesota, Minneapolis, MN, United States; [4]Pediatrics, University of Minnesota, Minneapolis, MN, United States.

Purpose: Post-transplant immunosuppression is associated with an increase in cancer rates. To date, the long-term incidence of PTM among PKTx recipients has not been well-characterized.
Methodology: Between 1963 and 2015, 881 pediatric (age: 0-17 years old) recipients received 1055 KTx at our institution. The incidence and type of malignancy in this cohort was analyzed. Patient outcomes were compared and the relative risk of developing PTM after PKTx was determined.
Results: After a median follow-up of 33 years, 202 PTM occurred in 136 (15.4%) patients at a median age of 29 years old. The 3 most common malignancies were cutaneous malignancies (55.0%), post-transplant lymphoproliferative disease (PTLD; 23.3%) and non-skin carcinomas (16.8%). On multivariate analysis, factors associated with increased risk of developing PTM included: increasing age at KTx (aHR, 2.69 [1.59, 4.54]), receiving an allograft from a living unrelated donor (aHR, 2.74 [1.13, 6.64]), and induction therapy with thymoglobulin (aHR, 2.09 [1.11, 3.96]). Having a rejection episode was found to be associated with decreased risk of developing PTM (aHR, 0.51 [0.29, 0.91]). Maintenance immunosuppression, rejection treatment, or graft status were not found to be associated with development of PTM. Unadjusted analysis shows excellent patient and graft survival (Fig. 1A, B), but patients had to survive long enough to develop PTM. Median time to develop a PTM after PKTx was 18 years (Fig. 1C). Of PTM patients who died during the study time period, 30/47 (63.8%) died of malignancy. The most common cause of death was PTLD (50%). Time-dependent analysis of PTM patients demonstrated a significant risk of death (aHR, 26.0 [8.0, 85.2]) and death-censored graft failure (aHR, 1.8 [1.1, 3.0]).
Conclusion: Increasing age at transplant, receipt of a graft from a living unrelated donor, and thymoglobulin induction increases the risk of developing PTM among PKTx recipients, and this has a significant adverse effect on patient and graft survival long-term.

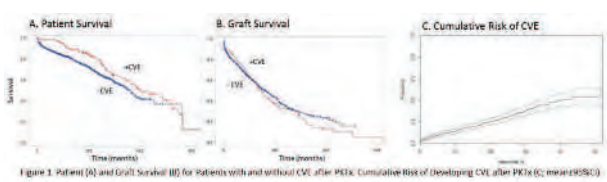


459.9

Incidence of cardiovascular disease after pediatric kidney transplantation

Oscar Serrano[1], Ananta Bangdiwala[2], David Vock[3], William D.Payne[1], Ty B.Dunn[1], Srinath Chinnakotla[1], Erik B.Finger[1], Raja Kandaswamy[1], Timothy L.Pruett[1], Arthur J.Matas[1], Blanche Chavers[4].
[1]Surgery, University of Minnesota, Minneapolis, United States; [2]Biostatistics and Bioinformatics Core, University of Minnesota, Minneapolis, United States; [3]Biostatistics, University of Minnesota, Minneapolis, United States; [4]Pediatrics, University of Minnesota, Minneapolis, United States.

Purpose: Kidney transplant (KTx) recipients have increased risk of cardiovascular disease (CVD), related to time on dialysis and post-KTx immunosuppression. To date, the incidence of post-KTx cardiovascular events (CVE) among pediatric KTx recipients (PKTx) has not been well-characterized.
Methodology: Between 1963 and 2015, 881 pediatric (age: 0-17 years old) recipients received 1055 KTx at our institution. The incidence and type of CVE (angina, arrhythmia, stroke/cerebrovascular accident, cardiomyopathy, congestive heart failure, coronary artery disease, myocardial infarction, peripheral vascular disease, thrombotic disease, valve dysfunction) was assessed. Patient outcomes were compared and the relative risk of developing CVE after PKTx was determined.
Results: After a median follow-up of 16 years, 340 CVE occurred in 164 (18.6%) patients at a median age of 18 y.o. Thrombotic/embolic events and arrhythmias accounted for 20.0% and 14.7% of CVE, respectively. On multivariate analysis, factors associated with increased risk of developing CVE included: pre-KTx CVD (aHR, 2.34 [95% CI 1.55-3.54]), graft failure (aHR, 3.63 [95% CI 2.61-5.03]), having an acute rejection episode (aHR, 2.19 [95% CI 1.56-3.08]), induction therapy with OKT3 (aHR, 2.54 [95% CI 1.01-6.41]), ATG (aHR, 2.31 [95% CI 1.42-3.75]) or thymoglobulin (aHR, 2.54 [95% CI 1.6-4.02]), maintenance immunosuppression with azathioprine/prednisone (aHR, 1.85 [95% CI 1.15-2.97]) or mycophenolate/calcineurin-inhibitor/prednisone (aHR, 7.02 [95% CI 2.95-16.7]). Overall, patient survival was excellent in patients who developed CVE (Fig. 1A; $p<0.001$); however, patients needed to survive long enough to develop disease. Graft survival was similar among patients ($p=0.26$; Fig. 1B). The median time to developing a CVE after PKTx was 10 years (Fig. 1C). Of patients who developed CVE and died, 53/81 (65.4%) died of CVE causes. Time-dependent analysis of CVE patients demonstrated a significant risk of death (aHR, 2.5 [95% CI 1.6-4.0]).
Conclusion: Pre-KTx CVD, graft failure, acute rejection, induction with OKT3, ATG or thymoglobulin, and maintenance immunosuppression with azathioprine/prednisone or mycophenolate/calcineurin-inhibitor /prednisone are independent risk factors for post-KTx CVE in PKTx recipients. Post-transplant CVD increases the risk of death after PKTx.



470.1

The role of HLA class II antibodies in chronic kidney rejection

Gabriel Cantanhede[1], Jeremy Palmer[1], Keith Giles[2], Vaughan Carter[2], John Kirby[1], Simi Ali[1].
[1]Institute of Cellular Medicine, University of Newcastle, Newcastle upon Tyne, United Kingdom; [2]National Health Service Blood Transfusion, NHSBT, Newcastle upon Tyne, United Kingdom.

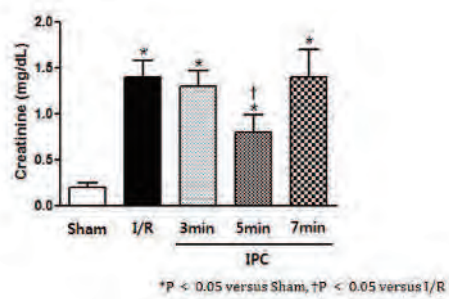
Background: Antibody-mediated rejection is one of the major causes of chronic rejection. This is mediated by endothelial cell activation, a process of endothelium microvascular inflammation and leukocyte recruitment. Despite evidence pointing to the relevance of HLA class II antibody in graft rejection, the role of antibody-endothelium interaction in the absence of complement is not fully understood. This project seeks to identify signalling cascades activated by HLA class II antibodies and mechanisms mediating endothelial cell – leukocyte interaction.
Methodology: Stably-transfected Ea.hy926 class II transactivator and HMEC-1 cells were stimulated with HB145, a pan-HLA class II mouse monoclonal antibody. Cell signalling pathways were examined using a R&D Human phosphokinase array, Western Blot and RT-qPCR. The expression of ICAM-1 and VCAM-1 was determined using flow cytometry. Wound healing assays were performed using µ-slide cell culture inserts. Eahy.926 CIITA and HMEC-1 cells were genotyped for HLA class I and II epitope expression using PCR-SSP. HB145 was characterised for class I and class II by Luminex. Antibody from multiparous women and sensitised transplant kidney patients have been used to verify cell signalling cascades and functional expression of adhesion markers contributing to leukocyte trafficking via statically via flow cytometry and dynamically via a microfluidics platform manufactured by Cellix (Dublin, Ireland).
Results: Interpreted HLA Types were Ea.hy926 CIITA DR11(5), 13(6); DQ6(1),7(3) and HMEC-1 DR12(5), 18(3); DQ4, 5(1). HB 145 was found a comprehensive pan-class II antibody. Eahy.926 cells stimulated with HB145 showed time-dependent phosphorylation of p-ERK, p-AKT, p-mTOR, and p-AMPK α1,α2. Phosphorylation of p-mTOR was abrogated by Rapamycin at 20nM. Stimulated Ea.hy 926 CIITA upregulated the expression of mRNA encoding Cyclin D1 ($p<0.001$) after 24h of treatment, while levels of c-myc remained unchanged. In HMEC-1 cells, HB145 induced a 3-fold expression of ICAM-1 after 72h of treatment ($p<0.001$). Stimulation of HMEC-1 cells using both HLA class I and HLA class II monoclonal antibodies (after subsaturation with IFN-gamma) led to increased adhesion of monocytes through a microfluidics platform. In Eahy.926 CIITA cells, HB145 treatment alone also increased the overall wound closure rate, reaching faster wound closure (11h vs. 14h; $p<0.005$) when compared to a HLA-class I monoclonal antibody (W6/32). Sensitised tranplanted patient samples produced similar phosphorylation cascades seen with monoclonal antibody and ICAM-1 upregulation.
Conclusions: Exposure of endothelial cells to HLA class II antibody induces an activation of signalling cascades characteristic of endothelial cell activation. This activation is further responsible for upregulation of adhesion molecules, which facilitate the interaction of donor endothelium and recipient leukocytes. Strategies to block endothelium-leukocyte interaction might reduce the incidence of allograft rejection and improve allograft survival.

470.2

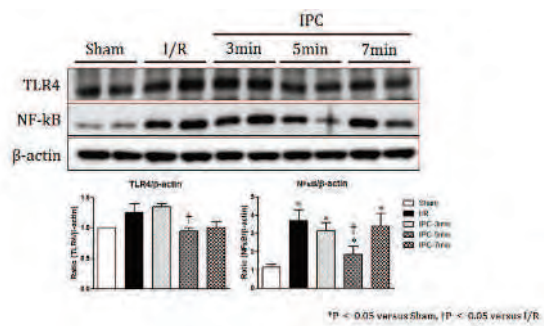
The proper ischemic preconditioning attenuates against renal ischemia-reperfusion injury by inhibiting toll-like receptor 4/NF-kB signaling pathway in mice

Jeongkye Hwang[1], Hyunsu Choi[2], Ilyong Shin[1], Kyungjai Ko[4], Kangwoong Jun[4], Mi-Hyeong Kim[4], Sangdong Kim[5], Suncheol Park[3], Jiil Kim[4], Insung Moon[4].
[1]Surgery, Daejeon St.Mary's hospital, Daejeon, Korea; [2]Clinical Research Institute, Daejeon St.Mary's hospital, Daejeon, Korea; [3]Surgery, Uijeongbu St.Mary's hospital, Uijeongbu, Korea; [4]Surgery, Seoul St. Mary's Hospital, Seoul, Korea; [5]Surgery, Incheon St.Mary's Hospital, Incheon, Korea.

Purpose: Ischemic preconditioning (IPC) is a potent renoprotective strategy which underlying mechanisms are not completely understood. In this study, we analyzed the hypothesis that proper IPC downregulates the expression of Toll-like receptor 4 (TLR-4) resulting in attenuation of renal ischemia-reperfusion (I/R) injury.
Methods: Male C57BL/6 mice were randomized into four groups according to IPC times; control (non-IPC), 3 min (group I), 5 min (group II), 7 min (group III). After right nephrectomy, mice were subjected to IPC and then 10 min reperfusion before 30 min ischemia. We assessed serum creatinine, microscopically morphology, and mRNA expression of tumor necrosis factor-alpha (TNF-a), IKK-beta, IL-1b and MCP-1. Renal expression of TLR-4 and nuclear factor kappa-B (NF-kB) was determined by western blotting and immunohistochemistry.
Results: The expression of intra-renal TLR4 and NF-kB protein were increased by renal I/R injury, and significantly decreased by IPC. ($P = 0.031$, 0.012, respectively). Among the four groups, there was significant difference in serum creatinine levels ($P = 0.042$).



Serum creatinine levels were 1.4±0.18 (non-IPC), 1.3±0.17 (group I), 0.8 ±0.19 (group II), 1.4 ±0.30 (group III). In 5min IPC group (group II), tubule-interstitial abnormality was clearly slighter compared with the other groups ($P < 0.001$). IKK-b, IL-1b and MCP-1 transcription levels were significantly less compared with the non-IPC group ($P<0.05$).



Conclusion: These data demonstrated that proper IPC (5min/10min) could attenuate renal I/R injury by inhibition of TLR-4/NF-kB pathway.

470.3

Relationship between B cell-rich tertiary lymphoid organs and systemic as well as local humoral alloresponses in a murine kidney allograft model

Anna Nowocin, Lucy Meader, Kathryn Brown, Raul Elgueta, Wilson Wong.
DTIMB, King's College London, London, United Kingdom.

Introduction: Tertiary lymphoid organs (TLO) are formed at sites of chronic inflammation such as an allograft, and are thought to contribute to the immune response. The analysis of their involvement in promoting or regulating the alloresponses has resulted in contradicting conclusions. We have characterised the structure and function of TLO in solid organ transplantation to elucidate their contribution in the alloresponses in a mouse model of kidney transplantation
Materials and Methods: Fully allogeneic DBA/2 (H-2d) kidney grafts were transplanted into C57BL/6 (H-2b) recipients. B cells infiltrating TLO in allografts were analysed histologically. The presence of antibody secreting cells on the isotopic differentiation of the alloantibodies was evaluated.
Results: DBA/2 allografts survive in C57BL/6 recipients with varying degree of dysfunction. We show that TLO can be associated with a beneficial rather than a destructive response in a kidney allotransplantation model. B cell subsets within tertiary lymphoid organs in mouse kidney allografts consist of naive, plasma and memory B cells which are mostly grouped within or in close proximity of TLO (Figure 1). Staining for intracellular IgG showed that some of these B cells within tertiary lymphoid organs are capable of producing antibodies. Although allospecific antibodies are found in the serum of recipient mice, and are deposited in the transplanted kidneys, graft function was not affected in this model. However more specific IgG isotope profiling reviled that the levels of IgG1 and IgG2b, but not total IgG or IgG2c and IgG3 in sera could be used to predict graft faith (Figure 2). This suggesting that additional protective mechanisms are at work to prevent donor specific antibodies from damaging the graft.
Discussion: We tried to establish whether TLO presence and/or graft function are affected by alloantibody presence in circulation or in the graft, and how can be this linked with local infiltration by B cells and antibody producing cells. The results presented here are reflective of the ability of TLO to provide an environment for either a stimulatory or a regulatory response, in the same way as secondary lymphoid tissues.
Conclusions: Our data indicates that tertiary lymphoid organs are capable of supporting the B cell response and contribute to the humoral arm of the alloresponses. The presence of cellular infiltrates or high levels of alloantibodies was not a prerequisite of kidney rejection.

470.4

Kidney transplant recipients after solid organ transplantation other than kidney show low alloreactivity but an increased risk of infectious complications

Thomas Schachtner[1,2], Maik Stein[2], Petra Reinke[1,2].
[1]Nephrology and Internal Intensive Care, Charité University Medicine Berlin, Berlin, Germany; [2]Berlin-Brandenburg Center for Regenerative Therapies, Charité University Medicine Berlin, Berlin, Germany.

Introduction: The number of kidney transplant recipients (KTRs) after solid organ transplantation (SOT) other than kidney is increasing to approximately 3-5% of all kidney transplantations. Knowledge on patient and allograft outcomes, infectious complications, and immunological characteristics, in KTRs after SOT other than kidney, however, remain scarce.
Material and Methods: Here, we studied 40 KTRs from 2004 to 2013, who underwent a previous SOT other than kidney. 15 of 40 KTRs (37%) underwent a previous liver transplantation and 25 of 40 KTRs (63%) underwent a previous heart or lung transplantation. 720 single KTRs of a first kidney allograft and 119 retransplant KTRs were used for comparison. Samples were collected before transplantation and at +1, +2, +3 months posttransplantation. Alloreactive T-cells and CMV-specific T-cells were measured using an interferon-γ Elispot assay.
Results and Discussion: KTRs after SOT other than kidney show comparable patient survival, allograft survival, and allograft function compared with first KTRs. In contrast, KTRs after SOT other thank kidney show a tendency for superior patient and allograft survival, and superior allograft function compared with retransplant KTRs ($p<0.05$). Interestingly, KTRs after SOT other than kidney show less preformed panel reactive antibodies, less preformed alloreactive T-cells, and less acute cellular rejection compared with retransplant KTRs ($p<0.05$). KTRs after SOT other than kidney, however, show a significantly higher incidence of EBV viremia and development of PTLD compared with first and retransplant KTRs ($p<0.05$). KTRs after SOT other than kidney show a significantly higher incidence of septic complications and death from sepsis compared with first and retransplant KTRs ($p<0.05$). A tendency of impaired CMV-specific immunity was associated with more CMV-replication compared to retransplant KTRs.
Discussion: Our results suggest patient and allograft outcomes in KTRs after SOT other than kidney comparable to KTRs of a first kidney allograft. The observed low alloreactivity may be attributed to the effects of maintenance immunosuppression. Caution should be taken in KTRs after SOT other than kidney regarding overimmunosuppression with development of EBV viremia and septic complications.

470.5

Aging effect on immunity implying to minimize immunosuppression in elder transplant recipients in a murine model

Wei-Chen Lee, Kun-Ming Chan, Yu-Chao Wang, Tsung-Han Wu, Chih-Hsien Cheng, Chen-Fang Lee, Ting-Jung Wu, Hong-Shiue Chou.
Liver and Transplantation Surgery, Chang-Gung Memorial Hospital, Taoyuan, Taiwan.

Background: Aged people to have solid organ transplantation are increasing. Clinically, aged recipients have a higher incidence of complications and lower rejection rate than young recipients. However, the difference of immunity between aged and young recipients is not really clear. The understanding of aging effects on immunity will be helpful for post-transplant care in transplant recipients.
Methods: C3H mice were employed as donor. Old (12-month-old) and young (10-week-old) C57BL/10J mice were employed as recipients. The phenotypic cells in old and young were examined by flow cytometry. The allogeneic proliferation capacity and cytotoxicity were performed to examine the aging effect on immunological reactions.
Results: The spleen cells from old and young mice were stained with CD11b and Gr-1 and analyzed by flow cytometry. The result showed that the population of bone marrow-derived suppressor cells was higher in old than in young mice. The proliferation capacity of old T-cells was lower than that of young T-cells in mixed lymphocyte reaction (0.350±0.003 O.D. versus 0.430±0.017 O.D. at responders/stimulatory cells = 100/1). T-cells derived from old mice also had a lower Ag-specific cytotoxic ability than T-cells derived from young mice (21.2±3.0% versus 39.3±4.8% at target cell/effector cells = 1/100, p=0.003). When T-cells were activated for 3 days, a higher frequency of regulatory cells was induced in T-cells derived from old mice than young mice (7.87±3.42% versus 5.04±2.71%, p=0.023). Allogeneic skin transplantation was performed and the result showed that the skin transplanted to young mice was rejected earlier.
Conclusion: The immunity was altered along aging process. The aged mice had a higher population of immunosuppressive cells including bone marrow-derived suppressor cells and regulatory cells. These aging effects on immunity implied that immunosuppressive agents could be reduced for aged recipients to achieve survival.

470.6

CD3+CD8+GranzymeB+ cytotoxic T lymphocytes play role in chronic antibody mediated transplant rejection

Narayan Prasad[1], Brijesh Yadav[1], Vikas Agarwal [2], Vinita Agarwal [3], R. K. Sharma[1].
[1]Nephrology and Renal Transplantation, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India; [2]Clinical Immunology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India; [3]Pathology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India.

Introduction: Chronic antibody associated transplant rejection (CABMR) accounts for 20-63% graft loss in late transplant period, often associated with C4d deposition in peritubular capillaries, circulating donor specific antibody, duplication of glomerular capillary wall, proteinuria, and hypertension. CABMR is also associated with glomerular and interstitial inflammation. Besides antibody mediated complement activation and injury to the graft, the role of infiltrating cytotoxic T cell and simultaneous changes in circulating Cytotoxic T cell profiles has not studied in same cohorts in CABMR.
Aim: We undertook this study the changes icytotoxic T cell in circulating blood and Intragraft tissue of patient and their association with CABMR.
Methods: From our cohort of renal allograft recipients, total 42 (CABMR-32, and stable functioning graft SGF-10) patients were included. SGF was defined as stable graft function for 6 months without proteinuria and <10% cortical surface area with IF/TA on biopsy; and CABMR was defined as per Banff's classification 2013. We have analyzed peripheral blood cytotoxic T cell frequency by flow cytometry, Soluble and biointact Granzyme B (GzmB) level in serum and PBMCs culture supernatant with ELISA; and tissue Granzyme-B mRNA transcript, in renal biopsy specimen by Taqman real time PCR and protein expression by Immunohistochemistry, in both groups of patients. Non parametric continuous variables were analyzed with Mann Whitney U test.Fold change was calculated by 2-^^Ct methods.
Results: The mean age of patients in CABMR and SGF group was 40.90±12.68 and 37.93±12.78 years; post-transplant time at biopsy was 70.18±34.48,and 49.70±22.71 months; S.creatinine was 2.40±0.80,and 1.23±0.18 mg/dl; and daily urine protein 2.94±1.54,and 0.13±0.10 g respectively.
Peripheral blood CD3+CD8+ T cell frequencyin CABMR and SGF was 27.55±6.53% and 19.89±4.37% (p=0.001), respectively. The peripheral cytotoxic T cell (CD3+CD8+GzmB+) was significantly low in CABMR group (15.38±3.29%), as compared to SGF (27.96±4.86%; p<0.001). Soluble GzmB level in serum was significantly high in CABMR group (177.82±48.66) as compared to SGF(100.82±22.41; P<0.001).However, Biointact GzmB level was significantly low in CABMR group (82.69±19.88) as compared to SGF (109.41±33.27; p<0.003). The Intragraft mRNA transcript expression of Granzyme-B in CABMR was (3.59±1.45) fold higher as compared to SGF (1.01±0.04; P<0.001). Number of GzmB+cell/mm^2, in CABMR was high (8.03±3.76) as compared to SGF patients (1.75±2.45; p<0.001).
Discussion: Granzyme B is Serine/Threonine proteases that activates MMPs, Procaspase, IL-1β and cleaves cytoskeleton, nuclear lamin protein and generates reactive oxygen species formation. This leads to allograft injury and induces apoptosis in endothelium/podocytes cell of microcappilary and ultimately leading to proteinuria. Higher intragraft granzyme suggest CABMR is associated with Granzyme mediated injury. Low frequency of cytotoxic T cell and high Granzyme serum level in CABMR suggest release of Granzyme B in serum. Simultaneous high intragraft expression suggest sequestration of cytotoxic cells into the graft from circulation.Therapy against cytotoxic T cell may be benficial in cases of CABMR.
Conclusion: CD3+CD8+GzmB+ Cytotoxic T cell is asociated with chronic antibody mediated rejection.
Brijesh Yadav is a DST Inspire fellow getting fellowship from Department of Science and Technology, Govt. of India

471.1

Body mass index (BMI) is associated with microalbuminuria in the late renal transplant period

Boris Zingerman[1,2], Ruth Rahamimov[1,2], Arie Erman[1,2], Avry Chagnac[1,2], Benaya Rozen-Zvi[1,2].
[1]Nephrology and Hypertension, Rabin Medical Center, Petach-Tikva, Israel; [2]Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel.

Introduction: Microalbuminuria is an established risk factor for cardiovascular morbidity and mortality in the general population. In renal transplant recipients it also predicts graft loss. Obesity is an independent predictor of chronic kidney disease in the general population, but its effect on microalbuminuria in renal transplant recipients has not yet been studied. The aim of the present investigation was to evaluate the relationship between obesity and microalbuminuria in the renal transplant population.
Materials and Methods: This cross-sectional prospective study was performed on adult renal graft recipients with a daily urinary albumin excretion rate of up to 300 mg. Measurements were performed at least 6 months after renal transplantation on accurately carried out 24-hour urine collections. Patients were divided into two groups: the early period - 6 months to 3 years after transplantation and the long term period - more than 3 years after transplantation. A multivariate regression analysis was performed in order to identify predictors of microalbuminuria (albumin excretion rate of 30 to 300 mg/day).
Results and Discussion: Two hundred and seven patients (66.7% males, 38.2% diabetics, mean age 53.7±12.5 years) were studied. There were 111 patients in the early group with median time from transplantation of 1.1 years (range 0.6-2.9) and 97 patients in the long term group with median time of 7.7 years (range 3-25). BMI was 27.3±5.1, waist circumference was 97.5±16.6 cm and albumin excretion rate was 22.9 (3-295) mg/24 hr.
For the early group there was no association between BMI and the risk of albuminuria by univariate analysis (Hazard Ratio (HR) 1.001 per kg/cm2, 95% Confidence Interval (CI) 0.94-1.09, p=0.81). In contrast, in the long term group the risk of albuminuria was associated with increased BMI by univariate (HR 1.18 per kg/cm2, 95% CI 1.07-1.31, p=0.001) and multivariate analysis (HR 1.25 per kg/cm2, 95% CI 1.09-1.44, p=0.001). In a similar manner, waist circumference was not associated with albuminuria in the early group, but was significantly associated with this outcome in the long term group (HR 1.04 per cm, 95% CI 1.01-1.07, p=0.018 and HR 1.06 per cm, 95% CI 1.02-1.11, p=0.005, in univariate and multivariate analysis, respectively).
BMI and waist circumference are independent predictors of microalbuminuria in the late post-transplant period. These variables are not associated with microalbuminuria in the early post-transplant period. Considering the established causal relationship between obesity and albuminuria in the general population, the findings of the present study suggest that a 3-year lag time must elapse for an increased adiposity to affect albumin excretion rate in the transplant population.
Conclusion: Adiposity is an independent predictor of microalbuminuria in the late- but not early-period after renal transplantation.

471.2

Posttransplant hyperuricemia as a cardiovascular risk factor: Correlation between cardiovascular risk factors

Siren Sezer[1], Emre Tutal[1], Zeynep Bal[1], Mehtap Erkmen[1], Gokhan Moray[2], Turan Colak[1], Mehmet Haberal[2].
[1]Department of Nephrology, Baskent University Faculty of Medicine, Ankara, Turkey; [2]Department of General Surgery, Baskent University Faculty of Medicine, Ankara, Turkey.

Introduction: Hyperuricemia is common after renal transplantation (RT) and a strong evidence supports its inverse prognostic role in graft function[1]. Patients with chronic kidney disease (CKD) are under high risk of cardiovascular disease (CVD)[2]. Chronic inflammation, disturbed calcium-phosphorus-parathyroid hormone axis, increased fibroblast growth factor-23 (FGF-23), increased advanced glycation end-products (AGE) and decreased Klotho gene activity are some of the possible factors that will play a role in progression of kidney disease and cardiovascular disease in renal disease population[3]. In this study we aimed to analyze if there is a relationship between hyperuricemia, AGE, FGF-23 and Klotho activity in a group of RT recipients.
Materials and Methods: A total of 100 patients (28 female, 39.2 ± 11.2 years aged) who underwent RT at least 12 months ago were included in this observational cross-sectional study. Demographic characteristics of patients and mean of creatinine, calcium, phosphorus, parathyroid hormone, CRP and uric acid (UA) levels of the last 3 months were collected from patient charts. A plasma sample was studied for Klotho activity, FGF-23 and AGE levels in each subject. Patients were grouped according to UA levels as hyperuricemic (≥ 6 mg/dL, n: 50) and control (n:50) groups for statistical analysis.
Results and Discussion: Hyperuricemic patients had higher serum CRP [5.7 (7) vs 4.5 (4), p:0.009], AGE [1.6 (3.5) vs 0.6 (0.9), p:0.0001] and FGF-23 [71.6 (187) vs 43 (36), p: 0.008] levels while they also had lower Klotho [0.5(0.9) vs 1.1 (4.7), p0.0001]activity. Uric acid levels were positively correlated with serum CRP (r:0.237, p:0.018), FGF-23 (r:0.352, p:0.0001) and AGE (r: 0.481, p:0.0001) levels and negatively correlated with Klotho activity (r: -0.364, p:0.0001). Hyperuricemic patients also had lower creatinine clearance (53.8 ± 23.1 vs 80.6 ± 23.7 mL/min). A linear regression analysis revealed that AGE and FGF-23 levels were significant determinants of UA levels (p: 0.0001, 0.012 respectively). Hyperuricemia is closely related with decreased Klotho activity, increased FGF-23, AGE levels and inflammation.
Conclusion: Prognostic influence of hyperuricemia may depend on strong association with the cardiovascular risk factor in renal transplant recipients.
References:
[1] Kalantar E et al. Hyperuricemia after renal transplantation. Transplant Proc. 2011 ;43:584-5.
[2] Tonelli M et al. Chronic kidney disease and mortality risk: a systematic review. J Am Soc Nephrol. 2006;17:2034-47.
[3] Gutierrez OM et al. Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. N Engl J Med. 2008; 359: 584–592.

471.3

Definite (dBKPVN) and presumptive (pBKPVN) polyomavirus associated nephropathies cannot be distinguished clinically but viral clearance in pBKPVN depends on renal function at the time of the biopsy.

CinthiaB. Drachenberg, John C.Papadimitriou, Muhammad Chaudhry, Richard Ugarte, Beje Thomas, Manju Mavanur, Nadiesda Costa, Charles Cangro, Abdolreza Haririan.
Pathology and Medicine, University of Maryland School of Medicine, Baltimore, MD, United States.

Introduction: Persistent, increasing BK viremia is considered a marker of renal parenchymal disease (BK polyomavirus associated nephropathy, BKPVN), however, histological evaluation of the kidney in these patients may or may not reveal the infection. The term presumptive BKPVN (pBKPVN) was coined to classify patients with persistent BK viremia (typically with concurrent BK viruria) but a biopsy without viral inclusions and negative for SV40 staining. Early intervention in patients with BK viremia is considered ideal, in order to prevent irreversible organ damage, however, the exact impact of clinical intervention in pBKPVN versus BKPVN is not known.
Materials and Methods: We retrospectively evaluated biopsy and clinical findings in 73 consecutive patients with sustained (>3 weeks) BK viremia (viral copies/ml) who underwent protocol or for cause renal transplant biopsies. The biopsies were classified as dBKPVN or pBKPVN based on the presence or absence of SV40 staining infected cells, respectively. The patients were 56 males/17 females (M:F 3.2/1) with a mean age of 55.6 (31-87). The patients racial composition was 35 AA, 25 Caucasian, 5 Hispanic and 2 Asian/Pacific.
Results: Overall, 24.6% (n=18) of the biopsies in this cohort were classified as pBKPVN based on the presence of sustained BK viremia but negative SV40 biopsy staining. The pBKPVN represented 44.4% (8 of 18) and 27% (10 of 37) of the protocol (surveillance) and for cause biopsies, respectively. There were no significant differences between pBKPVN and dBKPVN in the protocol and for cause biopsy groups with respect to serum creatinine at the time of the biopsy, level of BK viremia and demographics.

Biopsies with normal-baseline creatinine
26 "protocol" Bx

	dBKPVN	pBKPVN	
Number of bx: 26	18	8	
Serum Cr at Bx	1.37 mg/dl (±.25)	1.04 mg/dl (±.24)	(NS)
Viremia	Mean 784,000 Median 39,000 Range 3,400-12,000,000	Mean 259,000 Median 18,500 Range 5,500-1,900,000	(NS)
Time of biopsy (days post-tx)	Mean 152 (median 120)	Mean 215 (median 139)	(NS)
SV40%	1-85%	0% (per definition)	
Follow up post Tx	1220 days (±502)	1142 days (±395)	(NS)
Last creatinine Graft loss*	2.14 mg/dl (±1.31) 1 of 18: mixed ACMR and AMR	1.42 mg/dl (±.48) 0 of 8	(NS)
Viral clearance	69.23%	100%	p=.0308

Biopsies with increasing creatinine
47 for cause bx

	dBKPVN	pBKPVN	
Number of bx: 47	37	10	
Serum Cr at Bx	2.45 mg/dl (±1.1)	2.37 mg/dl (±.85)	(NS)
Viremia	Mean 71,000 Median 50,000 (±2,000 500) Range 1600-7,500,000	Mean 48,580 Median 46,000 (±71,900) Range 2500-220,000	(NS)
Time of biopsy (days post-tx)	Mean 344 (median 171)(±381)	Mean 407 (median 123) (±627)	(NS)
SV40%	1-75%	0% (per definition)	
Follow up post Tx	1217.7 (±738)	839 days (±509.2)	(NS)
Last creatinine Graft loss*	3.61 mg/dl (±2.67) 7 of 37 (18.9%)	2.66 mg/dl (±1.27) 0 of 10	(NS) (NS)
Viral clearance	62.16%	50%	(NS)

After a minimum of 36 weeks of follow-up, viral clearance occurred in 100% of the patients with pBKPVN and normal renal function at the time of the biopsy (protocol biopsy). In contrast only 50% of the patients with pBKPVN and increased creatinine achieved viral clearance in the same time period. There was no difference in viral clearance between dBKPVN in protocol and for cause biopsies (69.23% and 62.16% respectively).
Conclusions: Definite and pBKPVN apparently cannot be distinguished clinically, these two pathological categories appear to represent true sampling variations in a quantitative spectrum rather than a biologically different process. On the other hand, pBKPVN had better outcome (viral clearance) when the renal function was intact at the time of the biopsy. These findings support the current recommendations for early diagnosis (e.g. bu the usage of pBKPVN diagnosis) and prompt decrease of the immunosuppression before the renal function is altered.

471.4

The preventative effect of mTOR inhibition by anti-A/B antibody ligation and mTOR-inhibitor on anti-HLA-DR antibody mediated complement dependent cytotoxicity

Kenta Iwasaki[1], Yuk Miwa[1], Kazuharu Uchida[1], Takaaki Kobayashi[2].
[1]Kidney Disease and Transplant Immunology, Aichi Medical University School of Medicine, Nagakute, Japan; [2]Renal Transplant Surgery, Aichi Medical University School of Medicine, Nagakute, Japan.

Background: Although de novo Donor-specific antibody (DSA) against HLA class II have been well recognized to cause antibody mediated rejection (CAMR), the mechanistic insight of HLA-class II DR expression on endothelial cell remain unclear.. The condition of no injury even in the presence of anti-donor antibody has been referred to as "accommodation", which would be one of the key factors for successful long-term graft survival. The aim of this study was to analyze the beneficial effect of anti-A/B antibody ligation on endothelial cells against HLA-DR antibody-mediated, complement-dependent cytotoxicity (CDC).
Method: Blood group A/B-expressing endothelial cells EA.hy926 or Human Umbilical Vein Endothelia Cells (HUVEC) were incubated with IFNg in the presence or absence of anti-A/B antibody or mTOR inhibitor (mTOR-i) for 48 hours. The effects on signaling pathway, HLA expression, complement regulatory factors, and CDC were investigated.
Results: Pre-incubation with anti-A/B antibody had resistance to HLA-DR antibody-mediated CDC against IFNg-treated cells in a concentration-dependent manner. This finding was ascribed to decreased expression of HLA-DR by post-translational regulation and increased expression of CD55/59, which was related to ERK and mTOR pathway inhibition. mTOR-i also inhibited HLA-DR expression by itself. Furthermore, the combination of mTOR-i and anti-A/B ligation had an additive effect in preventing HLA-DR antibody-mediated CDC.
Conclusion: Anti-A/B antibody might play a preventive role in CAMR through the inhibition ERK and mTOR signaling pathway. Inhibition of mTOR signaling pathways by anti-A/B antibody or drugs including direct mTOR inhibitor may partially contribute to the development of a novel treatment in the maintenance period after transplantation.

471.5

Outcome of renal transplantation in obese recipients

Mahir Kirnap, Ebru H.Ayvazoglu Soy, Aydincan Akdur, Gokhan Moray, Mehmet Haberal.
Transplantation Center, Baskent University, Ankara, Turkey.

Background: In literature there are conflicting results regarding to effect of obesity on kidney transplantation outcome. The aim of our study is to summarize our obese kidney recipients outcomes and investigate the association between continuous and categoric values of BMI and graft survival as an independent risk factor in renal transplantation.
Materials and Methods: Between October 1985 and December 2015 we performed 2248 kidney transplants at our centers. We excluded patients with insufficient data. We retrospectively reviewed 1125 kidney transplant recipients to evaluate graft and patient survivals between nonobese (BMI <30) and obese (BMI ≥30) patients at the time of transplantation, considering BMI as a categoric variable.
Results: We followed 1125 kidney transplant recipients for 10.6 ± 4.2 years. Among these 1125 patients, 987 patients were nonobese and 138 patients were obese. When we compared obese and non obese patients there was no significant difference in graft survival at the first year of transplantation. However, at the third year of transplantation there was a significant difference in graft survivals between obese and non obese recipients; 23.2% of obese recipients and 5.3% of nonobese recipients lost their grafts (p=0.003). First year's (8.1% vs 2.2%; p=0.024) and third year's (16.9% vs 4.3%; p=0.035) mortality rates were statistically higher in obese patients. Overall graft loss was significantly higher among obese compared with nonobese recipients.
Conclusions: Here in this study we confirmed that BMI higher than 30 kg/m² is an independent risk factor for graft survival and patient mortality. Careful patient selection and weight loss before transplantation may be useful to reduce early posttransplantation complication and improve long term outcomes of kidney transplantation.

471.6

Decreased number of plasma cells in peripheral circulation is associated with acute rejection in renal transplantation

Mukut Minz[1], Ravi Dhital[1], Ranjana Minz[2], Ashish Sharma[1], Ritambhara Nada[3], Sarbpreet Singh[1], Deepesh Kenwer[1].
[1]Renal Transplant Surgery, Post Graduate Institution of Medical Education and Research, Chandigarh, India; [2]Immunopathology, Post Graduate Institution of Medical Education and Research, Chandigarh, India; [3]Histopathology, Post Graduate Institution of Medical Education and Research, Chandigarh, India.

Background: Plasma cells are antibody producing cells and are the major cells that regulate humoral immunity. After their differentiation, plasma cells primarily reside in bone marrow and are a major source of persistent donor specific allo- antibody production in sensitized patients. Therefore we aim to estimate the percentage of plasma cells in the peripheral blood at the time of acute rejection.
Methodology: Percentage of Plasma cells in peripheral blood was quantified by flowcytometry using monoclonal antibodies against CD20, CD27, CD138 and CD38. Phenotypes with CD27-CD138+CD38+ gated over CD20- lymphocyte population were considered plasma cells. Percentage of plasma cells were compared among 38 cases of acute renal allograft rejection (Rejection group), 40 cases of stable graft function (Non Rejection group) at corresponding time and 40 cases of healthy controls. Rejection group was further classified as acute cellular (ACR, n= 21), acute antibody mediated (ABMR, n= 8) and mixture of both (Mixed, n= 9). Percentage of plasma cells was compared among rejection types.
Results: No significant difference in percentage of plasma cells was noticed between patients with end stage renal disease and healthy controls. Post transplantation, a significant decrease in the percentage of plasma cells was noticed at the time of rejection ($P<0.0001$) whereas, no significant change was noticed in non rejection group at the corresponding time points. When the rejection group was further classified, the decrease of plasma cells during rejection was equally significant in all the types of rejection ($P<0.0001$).
Conclusion: Decrease of plasma cells from peripheral circulation is associated with acute rejections and indicates their possible localization in rejecting grafts. The phenomenon is equally prominent in cellular rejection. The finding suggests the importance of elimination of plasma cells in the treatmen of acute cellular rejection.

472.1

Towards a standardized informed consent procedure for live donor nephrectomy: What do surgeons tell potential donors?

Kirsten Kortram, Jan N.M.Ijzermans, Frank J.M.F.Dor.
Surgery, Erasmus MC University Medical Center, Rotterdam, Netherlands.

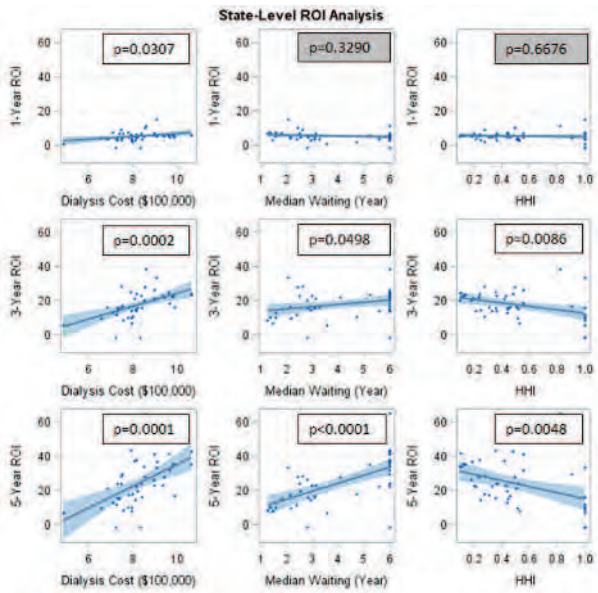
Background: A recent systematic review demonstrated that there is no consensus on how the informed consent procedure in live donor nephrectomy should be arranged and practices vary. The aim of this survey study was to evaluate the surgical informed consent procedure for live donor nephrectomy, with special regards to disclosed complications.
Methods: A web-based survey was sent to all surgeons in the Netherlands who were believed to be, or had been in the past, involved in live kidney donation (n=50). Surgeons were asked if, and how often they mentioned 23 items regarding short- and long-term complications. In addition, questions were included regarding the informed consent practices in each transplant center. Center- and surgeon characteristics (e.g. center volume, surgeons' gender, specialization, experience) were compared to assess whether information disclosure was related to any of these features.
Results: The response rate was 98% (N=49), and responses were obtained from all eight kidney transplant centers. Of these 49 respondents, 32 were still involved in living kidney donor education. Surgeons had the following subspecializations: transplant surgery (50%), vascular surgery (31%), abdominal surgery (13%), and urology (6%). Informed consent procedures vary between centers, ranging from assumed to signed consent. Some respondents from the same center report different procedures. Bleeding was the only complication every surgeon mentioned. Risk of death was always mentioned by 16 surgeons (50%), sometimes by 12 (37.5%), four surgeons (12.5%) never disclosed this disastrous complication. Reported mortality rates ranged from 0.003% to 0.1%. Mentioning frequencies for all other complications varied per individual surgeon, but also per center.
High volume surgeons reported more complications than low volume surgeons. The risk of postoperative pain was more often disclosed by consultant surgeons than fellows, and the risk of death was more often disclosed by surgeons with a differentiation in transplant surgery.
Conclusion: Important complications are not always disclosed during the surgical informed consent process for live donor nephrectomy. Informed consent procedures vary. To optimally prepare living kidney donors for the procedure, a standardized informed consent procedure for live donor nephrectomy is highly recommended.
References:
[1] Kortram K, Lafranca JA, JN IJ, Dor FJ. The need for a standardized informed consent procedure in live donor nephrectomy: a systematic review. Transplantation. 2014; 98: 1134-43.

472.2

Analysis of dialysis cost and median waiting time on return on investment (ROI) of the US National Living Donor Assistance Center (NLDAC) program

Robert M. Merion[1,2], Jiawei Xing[2], Barry Hong[3], Akinlolu O. Ojo[1], Patricia H. Warren[4], Kimberly A. Gifford[4], Amit K. Mathur[5,6].
[1]University of Michigan, Ann Arbor, MI, United States; [2]Arbor Research Collaborative for Health, Ann Arbor, MI, United States; [3]Washington University School of Medicine, St. Louis, MO, United States; [4]American Society of Transplant Surgeons, Arlington, VA, United States; [5]Mayo Clinic Arizona, Phoenix, AZ, United States; [6]Robert D. and Patricia E. Kern Center for the Science of Healthcare Delivery, Mayo Clinic, Rochester, MN, United States.

Introduction: The US National Living Donor Assistance Center (NLDAC) program is a means-tested federally funded program that has provided reimbursement of travel and other out-of-pocket expenses to living kidney donors since 2008. Compared with deceased donor transplant, living donor kidney transplant results in earlier transition from dialysis to transplant. However, there is marked geographic variation in maintenance dialysis and transplant costs in the US. Therefore, we analyzed the association between NLDAC program return on investment (ROI) and state-based predictors including dialysis cost, waiting time, and transplant program competition.
Methods: ROI was calculated using NLDAC program costs from 2012-2015, USRDS estimates of state-specific dialysis and transplant costs, and SRTR-based calculations of state-level median waiting time. Transplant program competition was calculated using Hirschman-Herfindahl Index (HHI), where lower HHI indicates more competition. Univariable and multivariable linear regression models were fitted to test associations of predictors with 1-, 3-, and 5-year NLDAC ROI.
Results: In univariate analyses, higher state-specific annual dialysis cost was significantly associated with 1-year ROI, and the effect increased monotonically at 3 and 5 years (Figure). Longer median waiting time showed similar associations, and were significant at 3 and 5 years. More state-level transplant program competition (lower HHI) was associated with significantly higher ROI at 3 and 5 years. Transplant cost was strongly correlated with dialysis cost at the state level (r=0.77; p<0.0001).



In multivariable linear regression models, state-specific dialysis cost was strongly associated with a progressive increase in ROI at 1, 3, and 5 years (Table). Median waiting time was a significant independent predictor of ROI only at 5 years. HHI was not significant in any multivariable ROI model, suggesting that program competition may be accounted for by its relationships to dialysis cost and waiting time.

ROI Model	Parameter	Estimate	95% Confidence Interval	p-value
1-year	Dialysis Cost (per $10,000)	0.82	0.08 - 1.56	0.0307
3-year	Dialysis Cost (per $10,000)	3.76	1.91 - 5.61	0.0002
5-year	Dialysis Cost (per $10,000)	4.40	1.72 - 7.07	0.0019
	Median Waiting Time (per year)	3.77	2.11 - 5.43	<.0001

Conclusions: Dialysis cost is a strong driver of ROI for the NLDAC program for living kidney donors. Longer waiting time for deceased donor transplant is also independently associated with long-term NLDAC ROI. These findings suggest that enhanced marketing of NLDAC donor expense reimbursement to kidney programs in states with higher dialysis cost and longer waiting time may further enhance the effectiveness of this program aimed at removing financial disincentives to live organ donation.
Supported by HRSA grant U13-HS-07689

472.3

The increasing number of unspecified living kidney donors

Willij Zuidema[1], Jacqueline Wetering van de[1], Emma K.Massey[1], Sohal Ismail[3], Jan N.M.IJzermans[2], Michiel G.H.Betjes[1], Willem Weimar[1].
[1]Internal Medicine, Nephrology and Transplantation, Erasmus MC, Rotterdam, Netherlands; [2]General Surgery, Erasmus MC, Rotterdam, Netherlands; [3]Psychology and Psychiatry, Erasmus MC, Rotterdam, Netherlands.

Background: An emerging strategy to bridge the gap between supply and demand for kidneys is the use of unspecified living kidney donors. Unspecified donation characterizes donation to an anonymous and unspecified recipient without any emotional link. There is no material benefit for the donor. Over the years the general public became more aware of this type of donation through reports in the media. We wondered whether this affected the actual number of unspecified donations.
Methods: From 2000-2015, we prospectively collected data on individuals who approached our center (adherent population 2.3 million) with the intention to donate a kidney in an unspecified way.
Results: In total 317 candidate donors contacted the coordinator in our center for information. They received a DVD and written information about the procedures and consequences of living unspecified donation. Two hundred and eleven (211) donor candidates embarked on the screening procedure which included a medical and psychological evaluation. One hundred and twenty eight of them (128/211, 62%) have donated. There were slightly more female than male (67 vs 61) and their age was 57 (median), range 21-89 year.
Unspecified donors donated to 72 recipients of incompatible couples in domino paired procedures, and to 56 patients on the waitlist, while 87 bridge donors (donors of the incompatible couples) donated either to recipients of incompatible couples (15) or to waitlist recipients (72). The 128 unspecified donations resulted in a total of 215 kidney transplants.
The number of unspecified donations increased over the years: 2000-2004: 5, 2006-2010: 52, 2011-2015:71, resulting in 6, 97, and 112 kidney transplants respectively. In 2015 22/114 (19.1%) of the living kidney transplants were donated by unspecified donors.
Conclusion: The number of unspecified living kidney donors is increasing over the years. Both patients on the deceased donor waiting list and those participating in a kidney exchange program stand to benefit from unspecified donation. Given that other research has shown no differences in physical or mental health outcomes between these donors and other living donors, this type of living donation should be encouraged.

472.4

Chinese adult perception towards organ donation in Hong Kong

Wah Chun LI[1], John Albarran[1], Issy Bray[1], Chi Shing LI[2].
[1]Faculty of Health & Life Sciences, University of the West of England, Bristol, United Kingdom; [2]School of Public Health, University of Hong Kong, Hong Kong, Hong Kong.

Introduction: Organ transplantation is a treatment of choice for most end-stage organ failure patients. However, organ transplant rates in Asia remain the lowest around the world. According to the International Registry on Organ Donation and Transplantation (2015), the organ donation rate in Hong Kong was 5.4 deceased donors per million of the population (pmp) in 2014, which is a far lower than Western countries like Spain of 36 donors pmp. The number waiting for organ transplantation has increased from 1,628 in 2007 to 2,113 in 2014 (Hong Kong Department of Health, 2015). The reasons for low donation rates remain a major challenge for healthcare providers, and a burden for those on waiting lists. Having a better understanding of organ donor's decision making processes and related behaviours in Asian populations is important for developing effective strategies to promote organ donation among the public.
Aim: A qualitative descriptive study was designed with the aim of investigating the current values, beliefs and experiences of Adult Hong Kong Chinese people around organ donation.
Methods: Two semi-structured focus group interviews were carried out in December 2015 with members of the public. A total of 15 participants who are registered or not registered as organ donors, were included. Participants are aged 18 or above without cognitive impairments, who have ordinarily resided in Hong Kong for a continuous period of not less than seven years and with an ability to speak fluent Cantonese. Translation and backward translation together with thematic analysis was used to analyse interview data.
Results: Both registered and not registered groups demonstrated an understanding that organ donation could help to save lives. However, culture and traditional beliefs hamper a commitment towards organ donation. In addition, participants dare not to talk or think of organ donation in front of elderly, as this would be disapproved. Previous unpleasant experience on the last journey of a relative, inadequate understanding of brain death, worry about deceased person will suffer from pain during organ removal [IB1] and disfigurement of the deceased body were the factors affecting their willingness to donate their own or family members' organs after death. Four registered donors misinterpreted that a missing or lost donor card meant that individuals would no longer be eligible to donate. Lack of follow-up after donor registration had a negative impact on willingness to donate in the future. However, public campaigns with catchy slogans or regular media promotion were believed to be helpful in reaching more potential donors.
Conclusion: It is apparent from the study that the public of Hong Kong are well aware of the benefits of organ donation. Despite various public events in promoting organ donation (e.g. forums, sports events, media and education in schools), the donation rate remains low. A major step forward is registered donor follow-up, and strategies to increase the coverage of organ donation in the media are important in meeting the ever-increasing need for donor organs.
References:
[1] Hong Kong Department of Health (2015). Organ Donation – Statistics (Milestones of Hong Kong Organ Transplantation). Available from: http://www.organdonation.gov.hk/eng/statistics.html [Accessed 2 February 2016].
[2] International Registry on Organ Donation and Transplantation. (30 December 2015). IRODaT Database. Available from: www.irodat.org. [Accessed 1 February 2016].

472.5

Kidney transplantation from donors with rhabdomyolysis and acute renal failure: a report of 16 cases

Xiaopeng Yuan, Chuanbao Chen, Changxi Wang, Jian Zhou.
Organ Transplant Center, First Affiliated Hospital of Sun Yat-sen University, Guangzhou, People's Republic of China.

Objectives: To explore the clinical effect of kidney transplantation from donors with terminal acute renal failure (ARF) and rhabdomyolysis.
Methods: Kidney transplantation from donors with ARF and rhabdomyolysis from January 2012 to December 2015 was retrospectively reviewed. ARF was defined by RIFLE criteria. Rhabdomyolysis was defined by creatinine kinase (CK) level higher than five times normal (> 1000U/L in conjunction with high serum myoblobin.
Results: Eight donors suffered from rhabdomyolysis and ARF with peak CK 10623 ± 3692U/L, peak serum myoblobin 20618 ± 7959μg/L and peak serum creatinine 483 ± 176μmol/L. Three donors received plasmapheresis therapy (1～3 sessions), 2 donors received continuous renal replacement therapy and 1 donor received extra-corporeal membrane oxygenation treatment. Kidneys from these donors were transplanted into 16 recipients with 4 cases (25.0%) of delayed graft function, 4 cases (25.0%) of slow graft function and 8 cases of normal graft function. With a follow-up of 6 - 46 months, both patient and kidney graft survival were 100%. Mean 6 and 12-month glomerular filtration rate were 65.4 ± 13.5 and 71.2 ± 14.3 mL/(min•1.73m2) respectively.
Conclusions: Kidneys from donors with terminal ARF caused by rhabdomyolysis have excellent short-term outcomes. Screening for rhabdomyolysis should be recommended in deceased donors with acute renal injury.

472.6

Increased consented-not recovered (CNR) cases as a consequence of aggressive donor pursuit: Impact and implications

Jeffrey P. Orlowski[1], Chad C. Muse[1], Janice F. Whaley[1], Jan Foutch[1], Ronald A. Squires[1,2].
[1]LifeShare Transplant Donor Services of Oklahoma, Oklahoma City, OK, United States; [2]Department of Surgery, University of Oklahoma Health Science Center, Oklahoma City, OK, United States.

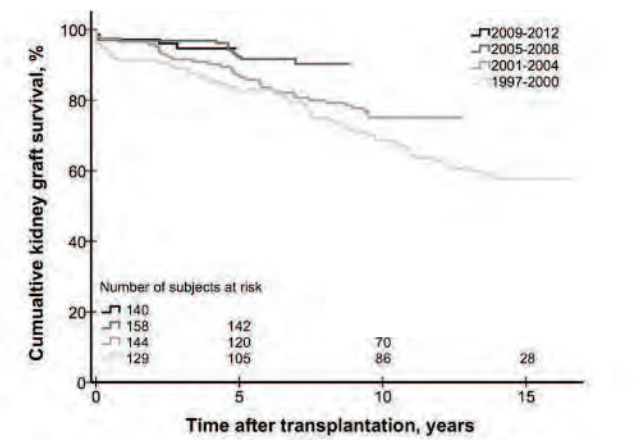
Introduction: One U.S. organ procurement organization (OPO) achieved significant growth in the number of deceased donors for the years 2014 and 2015 as compared to historical performance. One key strategy in achieving this growth was "aggressive pursuit" of any donor with a potentially transplantable organ. The OPO noted that as donors increased, so too did "consented-not recovered" (CNR) cases, or cases authorized and worked up for donation in which organs ultimately were not recovered for transplant.
Methods: A retrospective review of recovered and CNR cases was conducted. The 24 month cohort of 2014/2015 was compared to the prior 24 month cohort of 2012/2013. Donors, organs transplanted, donor types (DBD, DCD), CNR type and frequency data, and hours worked on CNR cases were compared.
Results and Discussion: In 2012/2013, 183 deceased donors were recovered (DBD and DCD combined); in 2014/2015, 315 deceased donors were recovered (+72.1%). Comparing the same periods, organs transplanted increased from 612 to 924 (+51.0%) and CNR increased from 41 to 92 (+124.4%). CNR in intended DBD cases increased from 25 to 52 (+108.0%) and in intended DCD cases from 16 to 40 (+150.0%). Total CNR case length (from authorization to case shut-down) increased from 633.5 hours in 2012/2013 (15.5 hours per case) to 1495.9 (16.3 hours per case) in 2014/2015 (+136.2%).
Conclusions: Aggressive pursuit of DBD cases, which may be older or have significant co-morbidities, combined with aggressive pursuit of DCD cases determined to have any likelihood of arrest has allowed our OPO to dramatically increase deceased donors recovered and organs transplanted. However, this aggressive pursuit has increased CNR at an even more dramatic rate when compared to growth in donors (124.4% vs. 72.1%). While we have accepted this as a consequence necessary to achieve a 51% increase in organs transplanted (lives saved), the primary implication is a 136.2% increase in case time spent on CNR (i.e., non-productive cases). The total financial cost of the extra manpower, testing, and ICU time is difficult to measure but is no doubt significant and it should be understood by OPOs and Transplant Centers alike that aggressive pursuit of deceased donors will result in increased cost per transplantable organ. Prospectively, the OPO will be seeking to quantify and categorize the costs associated with CNR separate from recovered donors.

473.1

Pediatric renal transplantation in the scandiatransplant region 1997-2012

Lars Wennberg[1], Anna Bjerre[3], Marie Larsson[1], Juuso Talnio[2], Helle C.Thiessson[4], Hannu Jalanko[2], Søren Schwartz Sørensen[5], Timo Jahnukainen[2].
[1]Department of Transplantation Surgery, Karolinska University Hospital, Stockholm, Sweden; [2]Department of Pediatric Nephrology and Transplantation, Childress Hospital, Helsinki, Finland; [3]Department of Pediatrics, Oslo University Hospital, Oslo, Norway; [4]Department of Nephrology, Odense University Hospital, Odense, Denmark; [5]Department of Nephrology, Copenhagen University Hospital, Copenhagen, Denmark.

Introduction: The Nordic Pediatric Renal Transplantation Study Group (NPRTSG) has registered data on all pediatric renal transplants (RTx) performed in the Scandiatransplant region (Denmark, Finland, Norway and Sweden) since 1994. Data from 430 RTx performed 1982-1996 was published 1998[1].
Methods: NPRTSG include all patients <16 years and is updated annually. We have studied factors affecting patient- and graft survival in 602 RTx performed 1997-2012. We also performed a comparison to the 1982-1996 cohort
Results and Discussion: The number of transplants increased by 30% and the number of recipients <2 years was doubled. Patients with glomerular disease decreased from 25% to 12%. The proportion of LDs increased from 54% to 60%, but differed between countries. The use of TAC and MMF increased from practically 0% to 50% and 40% respectively. Graft survival at 1 and 3 years was improved significantly ($p<0.001$) since 1997, especially among the youngest recipients with deceased donors.
For RTx performed 1997-2012, graft survival was significantly better for young recipients (<2 years) and for patients transplanted 2005-2012. Patients with ≤1 HLA A, B and DR mismatch had significantly better graft survival. There was a tendency towards better graft survival with living donors. Cold ischemia time, type of CNI or induction therapy did not affect graft survival. Acute rejection occurred in 149 patients (26%) but the incidence was reduced with time from 44.4% to 5.6%. Induction therapy did not reduce the incidence of acute rejection.
Conclusion: Between 1997 and 2012, 602 pediatric RTx was performed in the Scandiatransplant region. When compared with 1982-1996, results was significantly improved.



References:
[1] 1. TYDÉN G & BERG U. Pediatric renal transplantation in the Nordic countries: a report of the Nordic Pediatric Renal Transplant Study Group. Pediatr Transplantation 1998: 2: 240-243.

473.2

Results of the first kidney transplant in pediatric patients in Argentina: Period 1998-2014

Liliana Bisigniano[1], Viviana Tagliafichi[1], Daniela Hansen Krogh[2], Marta Monteverde[4], Maria del Carmen Bacque[3].
[1]Scientific and Technical Direction, INCUCAI, Buenos Aires, Argentina; [2]Informatic Direction, INCUCAI, Buenos Aires, Argentina; [3]Presidence, INCUCAI, Buenos Aires, Argentina; [4]Pediatric Nephrology, Hospital de Pediatraia J. P. Garrahan, Buenos Aires, Argentina.

Objective: To describe both patient and graft survival after kidney transplant, using living and cadaveric donor, in patients under the age of 18 in Argentina.
Material and Methods: patients under the age of 18 at the time of transplant (TX) between January 1, 1998, and December 31, 2014.The results were reported as a percentage of categorical variables and the median of numerical variables To estimate survival the Kaplan-Meir curve was used.Variables included in the Cox model were age and gender of the donor, cause of death of the donor (CVA, head trauma, and other), cold ischemia time (CIT) (< or > 12hours), years on waiting list (< or > 0.55) and years on dialysis (< or > 2)at the time of transplant , etiology on listing, and donor-recipient compatibility.
Results: 1255 patients were assessed in 48 centers, 932 (74%) received a kidney from a cadaveric donor (CD) and 233 (26%) received a kidney from a related living donor (LRD).
Median age of the recipient was 13.0 years (CI 95% 12.6-13.4); 55 % of the recipients were males. Median time in years on waiting list and on dialisys at the time of transplant was 0.5 (CI95% 0.4-0.6) and 2.0 (CI95% 1.9-2.19) respectivily. Both patient and graft survival after transplant with a cadaveric donor was 98% and 92%; at 3 years, 96%, and 83%; at 5 years 94% and 78%, respectively. The median time in years on waiting list ad dialisys at the time of transplant with LRD was 0.14 (CI95% 0.10-0.19) and 0.33 (CI95% 0.21-0.47 respectivily. Patient and post-transplant graft survival with LRD respectively was at 1 year 98% and 96%, at 3 years 97%, and 92%, at 5 years 95% and 88%. In recipients over 12 using CD, age was associated with post transplant mortality, and was statistically significant with a HR 2.21 (1.18 and 4.15). Graft survival as associated with age over 12, (HR 1.47; CI 1.13 and 1.91), glomerulonephritis, (HR 1.36; 1.02 and 1.83) and years on dialysis to TX with CD (HR 1.34; CI 1.03 and 1.75), adjusted with patient variables.
Conclusions: in recipients over 12 using CD, age was a risk factor in terms of post transplant graft survival. In the case of CD, glomerulonephritis as an etiology and time on dialysis before transplant were risk factors for post transplant graft survival.

473.3

Enbloc kidney transplant in pediatric recipients - The Miami experience

Vincent J.M. Chia, George Burke, Gaetano Ciancio, Linda J. Chen.
Miami Transplant Institute, University of Miami/ Jackson Memorial Hospital, Miami, United States.

Introduction: Enbloc renal transplantation (EBKT) is not commonly performed in pediatric recipients due to its potential complications. There is very limited published literature on this form of transplantation.
Methods: A retrospective review of all pediatric patients who received EBKT was performed.
Results: Between 2004-2014, 25 patients had EBKT, including 7 patients who had liver-kidney transplants. The recipient median age was 4.6 (range,0.9- 17.0) years and weight was 17.9 (range, 5.6-70.0) kg. The median donor age was 22 (range,2-108) months and weight 12.0 (range,5.8-21.1) kg. There are 7 patients who were preemptively transplanted. During the perioperative period, 4 patients required surgical intervention for complications;1 patient had a succesful thrombectomy and repair of urine leak, 1 patient required allograft repositioning/ureteric reimplantation, 1 patient had allograft ischemia,1 patient had kidney rupture. The last 2 patients had graft loss eventually. There was 1 intraoperative mortality from a liver-kidney patient. Patient and graft survival at 1 year is 96% and 88% respectively. The median serum creatine at 1 year, 3yr and 5yr was 0.42mg/dL, 0.51mg/dL and 0.62mg/dL respectively.
Conclusion: This paper represents the largest series of EBKT in pediatric recipients. Our center experience shows that EBKT is an acceptable form of transplantation in pediatric recipients.

473.4

When the vena cava inferior is missing: kidney transplantation in children with venous drainage to the portal vein

Udo Vester[1], Anja Büscher[1], Jürgen Treckmann[2], Peter Hoyer[1], Andreas Paul[2].
[1]Pediatric Nephrology, University Hospital, Essen, Germany; [2]Transplant Surgery, University Hospital, Essen, Germany.

Introduction: Kidney transplantation requires patent vascular access with the recipients circulation. Vascular malformations or thrombotic complications are major challenges in transplant surgery. We report the outcome of kidney transplantation in two boys after complete thrombosis of the vena cava inferior (VCI) who were transplanted using the portal circulation.
Case 1: A boy with congenital nephrotic syndrome and extensive thrombosis of the caval and iliacal veins received a pre-emptive kidney transplant (DD) at the age at of 5 4/12 years. Venous anastomosis was achieved with the portal vein. Post-operative recovery was uneventful with stable graft function. After follow-up of 5 years, eGFR is 58 ml/min/1.73m2. No signs of portal hypertension or splenomegaly have been observed since transplant.
Case 2: A boy suffered from septicemia with renal failure and caval and bilateral renal vein thrombosis at the age of 3 weeks. Peritoneal dialysis was performed until kidney transplant (DD) at the age of 2 9/12 years using the portal vein. Within the first 24 h after transplantation, portal vein thrombosis due to portal distortion requires surgical revision with complete recovery. After follow-up of 2 years, eGFR is 73 ml/min/1.73m2. On ultrasound, no evidence of portal hypertension or splenomegaly was seen.
Conclusion: In children with complete thrombosis of the VCI, successful renal transplantation can be achieved using the portal vein of the recipient. Despite increased portal blood flow, no signs of portal hypertension were observed.

473.5

Nationwide conversion to generic tacrolimus in paediatric kidney transplant recipients

Derisha Naicker, Tonya Kara, William Wong, Chanel Prestidge.
Paediatric Nephrology, Starship Children's Hospital, Auckland, New Zealand.

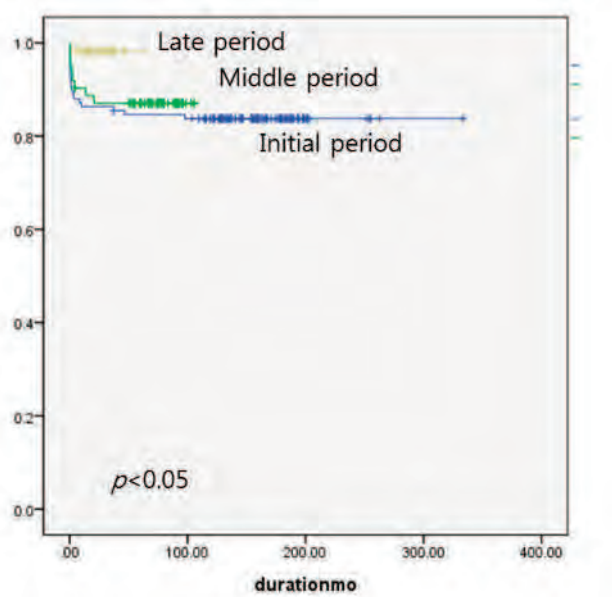
Introduction: PHARMAC, the New Zealand government agency which regulates access to and funding of pharmaceuticals, awarded sole supply of tacrolimus to Tacrolimus Sandoz® in March 2014. This necessitated conversion in all renal transplant recipients from the previously used brand Tacrolimus Prograf® to Tacrolimus Sandoz®. Bioequivalence has been demonstrated in adult subjects but clinical experience and safety data for generic tacrolimus in solid organ transplantation is limited, and particularly so for paediatric transplant recipients[1],[2]. The aim of this study was to evaluate the safety of conversion from Tacrolimus Prograf® to the generic preparation Tacrolimus Sandoz® in paediatric renal transplant patients. The primary outcome measurement was change in mean tacrolimus concentration after generic conversion. Additionally changes in renal function pre- and post- conversion, the need for dose adjustments and episodes of biopsy proven rejection in the 12months following the change were evaluated.
Methods: We conducted a retrospective cohort study in 37 paediatric renal transplant recipients in New Zealand who had been switched from Tacrolimus Prograf® to Tacrolimus Sandoz® over a 3 month period in 2014. We analysed the mean pre- and post-conversion tacrolimus trough concentrations and eGFR using the updated Schwartz equation. The 3 pre-conversion tacrolimus trough concentrations were recorded over no more than the prior 3 month period. The 3 post-conversion tacrolimus trough concentrations were measured on Day 3, Day 10 post conversion and the next subsequent level.
Results: Thirty-seven patients (41% females, age 3-19 years) who were a median of 3 years post transplantation were included. The mean trough tacrolimus concentration pre-conversion was 5.02µg/L and post-conversion 5.05µg/L. The median creatinine was 83µmol/L with an eGFR of 65.5ml/min/1.73^2 pre-conversion compared to the median creatinine of 79µmol/L with an eGFR of 69ml/min/1.73^2 post-conversion. There was no difference between the number of drug dose changes per patient for the 12months pre- and post- conversion. Three patients had biopsy proven acute rejection within the 12months post-conversion compared to 1 patient within the 12month period pre-conversion.
Conclusion: This study finds that paediatric renal transplant patients can be safely converted from Tacrolimus Prograf® to Sandoz® with no difference in the tacrolimus trough concentration, immediate allograft function or an increased requirement for dose alterations. Of some concern was the increased number of biopsy proven acute rejection episodes in the 12 month period post-conversion, however non-adherence contributed in at least one of these episodes. This difference was clinically and statistically not significant.
Meredith Foster; Jane Ronaldson
References:
[1] Alloway R.R, Isaascs R, Lake K, Hoyer P, First R, Helerman H, Bunnapradist S, Leichtman A, William Bennett M, Tejani A, Takemoto S.K. Report of the American Society of Transplantation Conference on Immunosuppressive Drugs and Use of Generic Immunusuppressants *American Journal of Transplantation* 2003;3: 1211-1215
[2] Duong S.Q, Ashwin K.L, Rujuta J, Feingold B, Venkataramanan R. Transition from brand to generic tacrolimus is associated with a decrease in trough blood concentration in pediatric heart transplant recipients. *Pediatric Transplantation* 2015: 19: 911-917

473.6

Recent advancements in the pediatric liver transplantation: A single-center study of 235 patients over 27 years

Nam-Joon Yi, Kyung Chul Yoon, Suk Kyun Hong, Hyo-Sin Kim, Hyeyoung Kim, Kwang-Woong Lee, Kyung-Suk Suh.
Surgery, Seoul National University College of Medicine, Seoul, Korea.

Background: Pediatric liver transplantation (PLT) has been the key therapy for end stage liver disease and the outcome has been excellent. However, still surgical complication associated with small recipient is the main cause of graft loss. In the present study, we assessed recent advances in outcome of PLTs through our experience.
Methods: A total of 235 PLTs performed between Mar 1988 and July 2015 were analyzed. Three chronological periods were investigated: the initial period (1988-2007, n=117), the mid-term period in which our PLT management protocol was settled down (2008-2011, n=62), and the period in which surgical procedures were refined for small children (2012-2015, n=56).
Results: The grafts' (84% vs. 82% vs. 98%) and patients' (84% vs. 87% vs. 98%) survival have been improved ($p<0.05$), whereas the number of biliary atresia (31% vs. 45% vs. 50%) deceased and the proportion of deceased donor (24% vs. 36% vs. 52%) and split PLT (7%vs. 16% vs. 46%) increased ($p<0.05$). The number of re-LT (3% vs. 9% vs. 2%) has been changed. ABO incompatible PLT has introduced on the last period (9%).
Conclusion: The quality of the PLT has recently been standardized through a large volume of experience, and the operation has been proven to improve the survival outcome. However, a constant evaluation of our experience is critical for further progress.



474.1

Immunosuppression at first hospital discharge after transplantation may not accurately predict risk of cancer in solid organ transplant recipients

Claire Vajdic[1], Angela C. Webster[2], Andrew E. Grulich[3], Nicola S. Meagher[4], Geoffrey W. McCaughan[5], Anne M. Keogh[6], Renhua Na[4], Maarit ALaaksonen[1].
[1]Centre for Big Data Research in Health, UNSW Australia, Sydney, Australia; [2]School of Public Health, University of Sydney, Sydney, Australia; [3]Kirby Institute, UNSW Australia, Sydney, Australia; [4]Prince of Wales Clinical School, UNSW Australia, Sydney, Australia; [5]The Centenary Research Institute, University of Sydney, Sydney, Australia; [6]Victor Chang Cardiac Research Institute, St Vincent's Hospital Sydney, Sydney, Australia.

Introduction: Organ transplant recipients can experience marked changes in the prescribed type and dose of immunosuppressive agents over time. For rare post-transplantation outcomes like cancer, population-based cohorts may be the only source of evidence of associations with specific immunosuppressive drugs. Longitudinal immunosuppression data can be very challenging to collect at the population level. As a consequence, most risk factor analyses of major health outcomes are based on immunosuppression at first hospital discharge after transplantation ("discharge"), and assume exposure is unvarying after that.
Materials and Methods: We conducted a population-based cohort study of all adult Australian liver, heart and lung transplant recipients 1984-2006 (n=4141). We abstracted risk factor data including immunosuppression at discharge and regular intervals thereafter from hospital medical records. We ascertained incident melanoma (n=48), lip cancer (n=58), and late (>1 year after transplant) non-Hodgkin lymphoma (NHL, n=61) and deaths by probabilistic record linkage between transplant registries and the Australian Cancer Database and National Death Index. We estimated adjusted hazard ratios (HR) using the Fine and Gray proportional subdistribution hazards model, accounting for death as a competing risk. We compared findings from two modeling approaches, (i) time-dependent immunosuppressive agents and (ii) immunosuppressive agents at discharge.
Results: The median time from transplantation to cancer diagnosis was 6.9, 6.1 and 3.6 years for lip cancer, late NHL and melanoma respectively. In fully adjusted models using time-dependent immunosuppressive agents, higher mean daily dose of azathioprine increased the risk of lip cancer (HR 2.28, 95%CI 1.18-4.38) and late NHL (HR 1.78, 95%CI 1.12-2.84) but not melanoma (HR 0.55, 95%CI 0.29-1.04). In fully adjusted models using immunosuppressive agents at discharge, higher mean daily dose of azathioprine was not associated with risk of lip cancer (HR 1.33, 95%CI 0.87-2.04), late NHL (HR 1.23, 95%CI 0.92-1.81) or melanoma (HR 0.71, 95%CI 0.44-1.13). No other immunosuppressive agents were significantly associated with risk of any of the cancers using either modeling approach.
Discussion: Assessments of cancer risk that rely solely on discharge immunosuppression data can differ from those that capture changes over time in the dose and type of immunosuppression.
Conclusion: With over two-decades of follow-up and detailed longitudinal data on individual immunosuppressive agents, this study provides the first evidence that observational risk factor analyses using discharge immunosuppression data may generate spurious findings due to misclassified drug exposure. Our findings have implications for clinical guidelines that draw from such evidence.

474.2

Longitudinal immunosuppression data reduces exposure misclassification and improves outcome prediction among solid organ transplant recipients

Maarit Laaksonen[1], Angela C.Webster[2], Andrew E.Grulich[3], Nicola S. Meagher[4], Geoffrey W. McCaughan[5], Anne M. Keogh[6], Renhua Na[4], Claire M.Vajdic[1].
[1]Centre for Big Data Research in Health, University of New South Wales, Sydney, Australia; [2]Sydney School of Public Health, University of Sydney, Sydney, Australia; [3]Kirby Institute, University of New South Wales, Sydney, Australia; [4]Prince of Wales Clinical School, University of New South Wales, Sydney, Australia; [5]Centenary Institute of Cancer Medicine & Cell Biology, University of Sydney, Sydney, Australia; [6]Victor Chang Cardiac Research Institute, St Vincent's Hospital Sydney, Sydney, Australia.

Introduction: Most observational studies on the effect of immunosuppressive agents on clinical outcomes among solid organ transplant recipients have relied on discharge immunosuppression data, which assumes the immunosuppressive regimen remains unchanged throughout follow-up. We studied the changes in immunosuppressive regimens of Australian liver, heart and lung transplant recipients over time and compared survival outcomes for individual agents and common drug combinations.
Material and Methods: We performed a cohort study in Australia using population-based liver and cardiothoracic transplant registries (1997–2006; n=2330). We recorded immunosuppressive agents (cyclosporine, tacrolimus, azathioprine, and mycophenolate) at first hospital discharge after transplantation and at 1 and 5 years, and classified transplant recipients according to the individual agents and combinations they were prescribed at discharge and whether they remained on those regimens during the follow-up period. We identified deaths from all causes by record linkage with the National Death Index. We estimated the risk of death from exposure to individual immunosuppressive agents and compared the risks between different dual therapies, as defined by discharge immunosuppression data (discharge analysis) versus longitudinal immunosuppression data (longitudinal analysis), using hazard ratios (HR) from Cox proportional hazards models adjusted for age, sex, organ type, and year of transplantation.
Results: During the 10-year follow-up, the immunosuppressive regimens of 660 (28%) transplant recipients were altered, most often involving removal of azathioprine, and 463 (20%) recipients died. Of the individual drugs, receipt of mycophenolate or tacrolimus was associated with a significant reduction in risk of death in longitudinal analysis (HR (95% CI): 0.69 (0.52, 0.93) and 0.75 (0.57, 0.98), respectively) whereas in discharge analysis the results for these agents were only suggestive of reduced risk (0.77 (0.58, 1.01) and 0.80 (0.61, 1.03), respectively). Recipients on mycophenolate and tacrolimus dual therapy had a lower risk of death compared to those on azathioprine and cyclosporine dual therapy in longitudinal (0.55 (0.33, 0.92)) but not in discharge (0.67 (0.42, 1.08)) analysis whereas the other dual therapies did not differ in their risk.
Discussion: As expected, we observed notable changes in the immunosuppressive regimen during follow-up, especially among those who started on azathioprine. Innovatively, accounting for those changes in longitudinal analysis revealed significant differences between drug regimens which were not identified by the discharge analysis.
Conclusion: Use of longitudinal immunosuppression data will help avoid exposure misclassification and improve prediction and comparison of outcomes related to immunosuppressive therapy, which may affect choice of immunosuppressive regimens, and conclusions about the pathogenicity of different agents.

474.3

National transplantation pregnancy registry (NTPR): 25 years of data collection

Michael J. Moritz[1,2], Lisa Coscia[2], Dawn P.Armenti[2], Serban Constantinescu[2,3].
[1]Surgery, Lehigh Valley Health Network, Allentown, PA, United States; [2]National Transplantation Pregnancy Registry (NTPR), Gift of Life Institute, Philadelphia, PA, United States; [3]Medicine, Temple University, Philadelphia, PA, United States.

The year 2016 marks 25 years of continuous data collection by the National Transplantation Pregnancy Registry (NTPR). Since 1991, the NTPR has studied pregnancies of transplant recipients in North America. The NTPR is the largest study of its kind. Data are collected via telephone interviews and review of medical records. The NTPR follows participants and their children indefinitely. To date, 1465 female solid organ transplant recipients, including over 1000 kidney recipients, participate in the NTPR (Table). The majority of children are reported healthy and developing well. At least 125 grandchildren now comprise a second generation providing the ability to study theoretical concerns about potential far-reaching effects of in utero exposure to immunosuppressive medications. NTPR analyses have contributed to clinical recommendations regarding advisability and timing of pregnancy, medications, comorbid conditions and other aspects of post-transplant motherhood. The NTPR also serves as a resource for recipients who are making family planning decisions. Pregnancy considerations vary depending on the organ transplanted, but the most significant factors for successful outcomes for mother and child are stable prepregnancy graft function, avoidance of mycophenolic acid during the 1st trimester, and close follow-up during pregnancy and postpartum. To provide the most complete information to the transplant community, NTPR will expand in 2016 to include recipients treated or transplanted beyond North America.

NTPR: Pregnancy Outcomes in Female Transplant Recipients

	Recipients	Pregnancies	Additional Multiple Birth Outcomes	Total Outcomes	Estimated Conception Date Range	Pregnancies in Progress	Fetal Losses*	Live Offspring
Kidney	1024	1842	65	1907	1967-2015	19	399	1395
Liver	247	467	15	482	1985-2015	14	115	345
Heart	84	143	5	148	1987-2015	3	40	95
Kidney-pancreas	60	107	7	114	1989-2015	1	32	77
Lung	30	40	2	42	1992-2014	0	16	20
Liver-kidney	9	12	1	13	1993-2014	0	1	12
Heart-lung	5	5	0	5	1994-2007	0	0	5
Pancreas alone	4	8	0	8	2000-2013	0	4	4
Small bowel	2	3	0	3	2008-2012	0	1	2
Total/Overall	1465	2627	95	2722	1967-2015	37	608	1955

*miscarriages, terminations due to fetal anomalies, stillbirths, neonatal deaths, and child deaths due to congenital issues

Conclusions: The NTPR is a reliable resource for the worldwide transplant community. For many recipients, pregnancy after organ transplantation is possible with the majority of the pregnancies resulting in a healthy live birth. Healthcare providers who counsel transplant recipients about parenthood and transplant recipients themselves are encouraged to contact the NTPR.

The NTPR is supported by grants from Astellas Pharma, US Inc., Bristol-Myers Squibb Co. and Pfizer Inc.

474.4

Prospective randomized controlled study evaluating the relationship between electronic adherence data and tacrolimus concentration data

Lisa Potter[1], Brenna Kane[1], Mark Lockwood[1], Adam Bodzin[1], Diane Covington[2], Kimberly Mayer[2], J. Michael Millis[1].
[1]Transplant Surgery, University of Chicago, Chicago, IL, United States; [2]Innovative Clinical Research, Pheonix, AZ, United States.

Purpose: The SIMpill® Medication Adherence System (MAS) is being evaluated in transplant recipients in a prospective 3-year study. The MAS captures patient medication adherence data in real-time. This report describes preliminary results combining adherence data and tacrolimus levels.
Methods: 89 solid organ transplant recipients were randomized to 1 of 4 groups: 2 intervention groups (I1 and I2) and 2 control groups (C1 and C2). Subjects in I1 or I2 received a MAS, a medication-dispensing device that communicates timing of openings or doses taken to a secure server. If a dose is missed, a text reminder message is sent to the subject (I1). In addition to patient reminders, providers were notified if consecutive doses were missed over a 72 hour period (I2), enabling intervention. C1 received a MAS but feedback was not provided to the patient or provider. C2 did not receive a MAS device. The MAS utilizes patient dose timing data with a one-compartment pharmacokinetic (PK) model to project individual tacrolimus drug level plots. The individual tacrolimus blood levels are plotted on drug curves based on the adherence data.
Results: PK modeling utilizing MAS data combined with tacrolimus levels resulted in 4 adherence profiles: A) patients with consistent dose timing with a blood draw time close to patient's planned dose time, B) patients with irregular day to day dose timing with a blood draw time close to patient's planned dose time, C) patients with consistent dose timing with a blood draw after the dose time resulting in a low trough level, and D) patients who do not withhold their dose before the blood draw resulting in the perception of a high trough level [figure 2].
Conclusions: Utilizing a MAS system may improve the interpretation of actual serum tacrolimus levels. Preliminary results indicate that the MAS may provide useful PK projections of tacrolimus serum levels. Irregular dose administration results in variable and/or inaccurate tacrolimus trough levels.

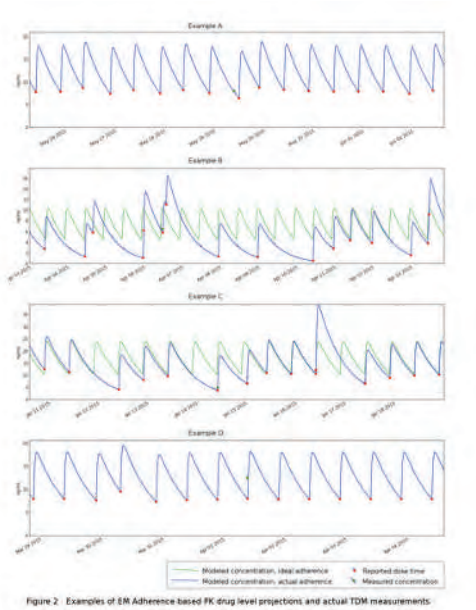


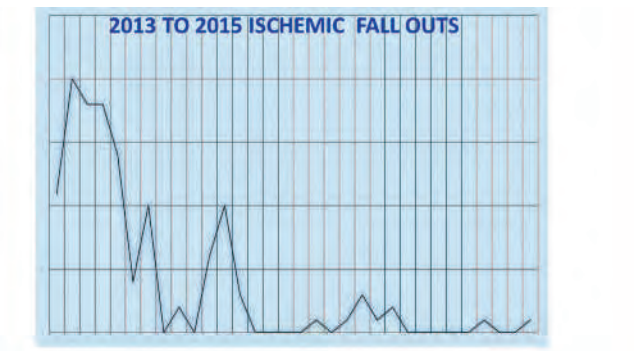
Figure 2 Examples of EM Adherence-based PK drug level projections and actual TDM measurements

474.5

Automatic ischemia time calculation in the intra-operative nursing record

Carmen Sierra, Marie Telfort.
Transplant Living Donor, Jackson Memorial Hospital/Miami Transplant Institute, Miami, United States.

Introduction: Reducing ischemia time calculation during transplant surgey leads to better outcomes for graft survival. Inaccurate calculation in ischemia time may lead to erroneous quality reporting. One source of inaccurate data is manual ischemia time calculation.By implementing an automatic ischemia time calculation segment in the electronic intra-operative nursing record,perioperative nurses can reduce wrong ischemia time from being calculated by way of data entry or inaccurate mathematical computation. Utilizing technology to facilitate mathematical computation with minimal errors in data entry and calculation leads to better reporting of ischemia time calculation and improved quality measures.
Purpose: The goal of this study is to reduce the amount of mathematical errors made by the circulating nurse in calculation ischemia time. By reducing the errors in manual arithmetic, accurate quality data becomes available to make necessary quality improvements.
Methodology: Daily autdits of the ischemia time calculated and entered in the intra-operative nursing record over the course of twenty-three months was performed. A total of 307 chart audits prior to implementation were performed and 388 chart audits after implementation.
Results: There were 40.4% errors or 59.6% accuracy with manual calculation. This implementation demonstrated a 99.5% accuracy in ischemic time calculation with the implementation of automatic ischemia time calculation segment in the eletronic nursing medical record. Manual ischemia times increase probability of errors.
Conclusion: Improving quality data reporting through the application of technology has proved to be instrumental in providing accurate data. There is a significant correlation in the manual entry of ischemia times and errors versus a significant decrease in errors when ischemia time calculation is automatically calculated by the eletronic intra-operative nursing record. Having accurate quality data will provide quality improvement measures when technology is appropriately applied.



Jason Bankersingh; John Maguire; Analucia Scheneegans
References:
[1] Perez Valdivia MA et al Transplant Proc. 2011 Jul-Aug

474.6

Impacts of ureteric stent removal timing on post renal transplant major urological complication. A single centre experience

Syed Raza[1], Abbas Ghazanfar[2], Ibrahim Alahmadi[1], Hani Alahdal[1], Jens Brockmann[1], Dieter Broring[1], Amira Abassi[1], Hassan Aleid[1].
[1]Department of Kidney and Pancreas Transplantation, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia; [2]Department of transplant Surgery , St Georges Healthcare NHS Trust, London, United Kingdom.

Background: Renal transplant (RTx) has high allograft success rate but is also associated with some potential complications. Urinary leak, ureteric stenosis and stricture are few such major urological complications (MUC) that can result from transplant ureteroneocystostomy. To prevent such complications different techniques of ureteroneocystostomy are used some with Double J ureterovesical stents (JJ-stent) and some without. The timing of postoperative removal of these stents varies considerably between 7 days to six weeks. In King Faisal Specialist Hospital (KFSH) this is a common practice to remove urinary catheter with JJ-stents on 41h postoperative day. In this present study we describe our experience with JJ-stent removal on 4'h post opt day following Adult RTx.
Material and Methods: In this retrospective study we analyzed outcome of patients for MUC between Jan 2004 and Dec 2013. In our renal database all the information are prospectively logged including all the demographics, outcome and complications. We divided the patient into two cohorts; cohort A where JJ-stent was removed in 4 days and cohort B where the stent was left longer then 4 days. We compared risk incidence of MUC between the two cohorts. We used SPSS 21 for statistical analysis and a P value of <0.05 was considered statistically significant.
Results: A total of 39 (3.06%) MUC including 8 ureteric leaks and 31 ureteric stenosis were recorded among 1271 Adult RTx. Majority of patients had their stents removed within 4 postoperative days (cohort A n=1052) compared with more than 4 days (Cohort B n=219). There was no significant MUC risk recorded between two cohorts [RR 1.1406; 95% CL 0.8591 to 1.8966; P=0.7638]. General demographics and complications are in Table 1. There was no significant survival or hazard function difference between the two groups [P=0.4051].
Conclusion: Removal of JJ-stent at 4'h postoperative day is a safe option with not increased risk of MUC

550.1

Prospective treatment and novel biomarkers of BK polyomavirus associated nephropathy

Minal Borkar[1], Vikas Srivastava[2], Ryan Cunnington[1], Tejeswin Sharma[1], Lee Anne Tibbles[1].
[1]Faculty of Medicine, University of Calagry, Calgary, AB, Canada; [2]CSIR-Indian Institute of Toxicology Research, Lucknow, India.

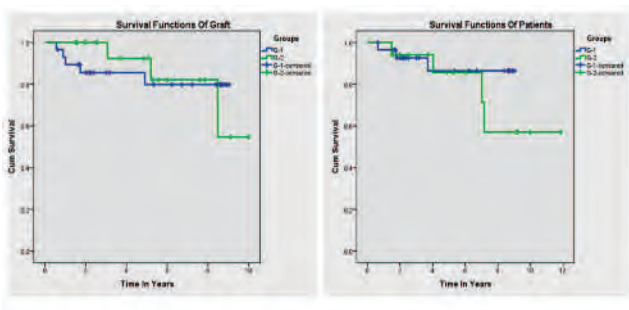
Background: BK polyomavirus assoiciated nephropathy (BKVAN) is documented as an important cause of graft failure in renal posttransplant. To date, the mechanisms involved in the progression of fibrosis associated with BKV nephropathy are poorly understood and an effective treatment against BKV is unavailable.
Objective: To determine the key epigenetic mechanism BKV uses to induce fibrosis and necroptosis, and to assess DNA methyl transferase 1 (DNMT1) as a potential therapeutic target.
Methodology: Human Proximal Tubular Epithelial Cells (HPTCs) and CCD1105 cell lines were infected with BKV. A low drug dose (5um) of RG108 (DNMT1 inhibitor) was used to treat BKV infected cells and RNA/DNA was isolated. Methylation Specific PCR (MSP) and Bisulfite Sequencing (BS) assays were performed to assess the hypermethylation of promoter of genes and expression study was carried out using real-time PCR. Further, Western blotting and Flow cytometry were executed to confirm necroptosis.
Results: BKV infection results into significant downregulation of epithelial cell markers E-cadherin (CDH1) and collagen IV (COLIVA1) gene expression, whereas, fibrotic marker collagen I mRNA level was increased suggesting that BKV infection causes cells to undergo epithelial to mesenchymal transition (EMT). MSP confirmed silencing of those genes through DNA methylation mechanism by demonstrating hypermethylation of promoters of CDH1 and COLIVA1 genes. RG108 treatment had shown altered COLIVA expression and decrease in methylation of promoter, as demethylating agent verified that BKV may uses epigenetic mechanism (DNA methylation) for inducing EMT and eventually fibrosis.
The expression based study at both mRNA and protein level confirmed increase in Receptor-interacting serine/threonine-protein kinase 3 (RIPK3), phospho-Mixed Lineage Kinase Like pseudokinase (pMLKL) and High Mobility Group Protein B1 (HMGB1). The treatment with RG108 potentially reduced the expression of these necroptotic genes, demonstrating that BKV may induce necroptosis epigenetically in renal tubular cells.
BS, MSP and real-time results had shown that BKV also hypermethylates the RB1 gene promoter to silence it and instigate host cell division for its own replication however, followed by RG108 treatment results had demonstrated significant decrease in BKV DNA (p-value<0.037).
Conclusion: This study confirms that BKV epigenetically induces EMT and necroptosis for pathogenesis hence, hypermethylated genes could act as potential biomarkers for early diagnosis. RG108 inhibits DNMT1, prevents these epigenetic events and blocks BKV replication, therefore, may act as potential drug for treating BKVAN however, further studies targetting DNA methylation to lessen BKV induced fibrosis are warranted.

550.2

BK Virus nephropathy in renal transplantation- A Pathologist's perspective

Kamlesh Suthar, Aruna V.Vanikar, Rashmi D.Patel, Kamal V.Kanodia, Lovelesh A.Nigam.
Department of Pathology, Lab. Medicine, Transfusion Services and Immunohematology, Institute of Kidney Diseases & Research Centre and Institute of Transplantation Sciences (ITS), Ahmedabad, India.

Introduction: BK Polyoma virus nephropathy (BKVN) occurs in 1-10% of allografts leading to 30-50% graft loss in renal transplant (RT) recipients. We present our experience of BKVN in RT.
Material and Methods: We analysed BKviral load in urine/blood and correlated with renal graft pathology. Totally 2932 biopsies performed between 2006 and 2015 were evaluated and divided in 2 groups on basis of timeline of BKVN. Group-1 included BKVN ≤ 1 year RT and group-2 included BKVN > 1 year RT. Formalin fixed allograft biopsies were processed, stained by standard techniques and evaluated following modified Banff-'13 guidelines[1]. Urine was screened for decoy cells. Blood and urine samples were simultaneously tested for BKV-DNA by polymerase chain reaction (PCR) in a subset of patients. There were 29 recipients (25 males) in group-1 and 17 (14 males) in group-2. Mean patient age (years) was 38.05 in group-1 and 33 in group-2, with mean HLA match of 2 in each. Mean donor age (years) was 48 in group-1 and 46 in group-2. One patient in group-1 and 3 in group-2 had persistent BKVN. All patients received antiviral therapy with modified immunosuppression.
Results and Discussion: BKVN incidence was 1.6% (39 males, 7 females), 1% at mean 0.31 years post-RT in group-1 and 0.6% at 3.3 years post-RT in group-2. Decoy cells were observed in 13.8% of group-1 and 11.8% of group-2. In group-1, BKV-DNA was observed in 16.7% blood and 33.3% urine and in group-2, 33.3% blood and 66.7% urine samples. Histopathology stage-A was observed in 48.3% of group-1 and 41.2% of group-2, stage-B in 44.8% of group-1 and 47.1% of group-2, stage-C in 6.9% of group-1 and 11.8% of group-2. Associated rejection was observed in 20.7% of group-1 and 17.7% biopsies of group-2. Mean SCr (mg/dL) at 1, 5, 7 and 9 years post-RT was 2.06, 1.79, 1.82 and 2.34 in group-1 and 1.41, 2.12, 3.05 and 4.87 in group-2 respectively. Patient loss was 10.3% in group-1 and 23.5% in group-2 mainly due to septicemia. There was 13.8% graft loss in group-1 and 23.5% loss in group-2. Kaplan Meier analysis revealed better outcome in group-1.



Our study had lower incidence of BKVN compared to others[2]. Our observations are similar to 15%, 25-75% and >80% risk of graft loss with patterns A, B and C respectively reported in literature[3]. Measurement of BKV load in urine/plasma helps in identifying patients at risk of developing BKVN and for monitoring response to therapy[4]. All RT recipients should be screened for BKV with quantitative plasma PCR for timely patient management[5].
Conclusion: BKVN leading to deterioration in RT outcome was observed in 1.6% biopsies in our 10 year study. BKVN before 1 year post-RT had better outcome compared to BKVN >1 year. Higher

histopathological stage had worst prognosis. Thus biopsy was crucial for patient management.

Dr. H.V.Patel, Dr. VB Kute, Mrs. Priyadarshini Shah, Mr. Paresh Dhamecha, Babubhai Patel, Pinky Bhavsar, Jaydatt Chudasma, Varsha Trivedi

References:

[1] Masutania K, Shapiro R, Basu A, et al. The Banff 2009 Working Proposal for Polyomavirus Nephropathy: A Critical Evaluation of Its Utility as a Determinant of Clinical Outcome. Am J Transplant. 2012; 12: 907–18

[2] Dharnidharka VR, Cherikh WS, Abbott KC. An OPTN analysis of national registry data on treatment of BK virus allograft nephropathy in the United States. Transplantation 2009; 87:1019-26

[3] Drachenberg CB, Papadimitriou JC, Hirsch HH, et al. Histological patterns of polyomavirus nephropathy: correlation with graft outcome and viral load. Am J Transplant 2004; 4: 2082-92

[4] Randhawa P, Ho A, Shapiro R, et al. Correlates of Quantitative Measurement of BK Polyomavirus (BKV) DNA with Clinical Course of BKV Infection in Renal Transplant Patients. Journal Of Clinical Microbiology. 2004; 42(3): 1176–80

[5] Sawinski D and Goral S. BK virus infection: an update on diagnosis and treatment Nephrol Dial Transplant 2014; 0: 1–9

550.3

The impact of recurrent glomerulonephritis after kidney transplantation in the era of contemporary maintenance immunosuppression

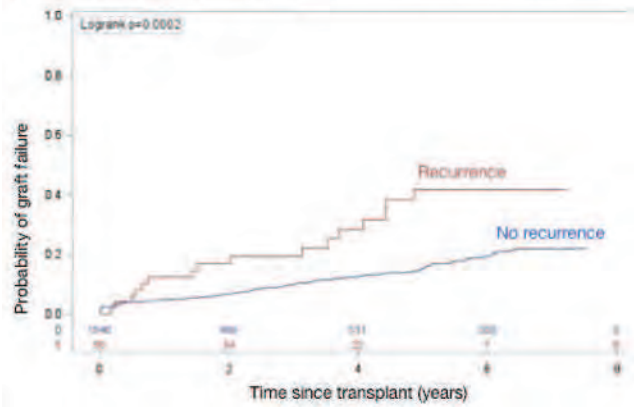
Penelope Allen[1,7], Steven J.Chadban[3,6], Jonathan C.Craig[1], Wai H.Lim[2], Richard D.Allen[3,6], Germaine Wong[1,3,4,5].

[1]Centre for Kidney Research, The Children's Hospital at Westmead, Sydney, Australia; [2]Department of Renal Medicine, Sir Charles Gairdner Hospital, Perth, Australia; [3]Renal Medicine and Transplantation, Royal Prince Alfred Hospital, Sydney, Australia; [4]Centre for Transplant and Renal Research, Westmead Hospital, Westmead, Australia; [5]Sydney School of Public Health, The University of Sydney, Sydney, Australia; [6]Sydney Medical School, The University of Sydney, Sydney, Australia; [7]The Australian National University, Canberra, Australia.

Background: Glomerulonephritis (GN) is a common indication for kidney transplantation, with recipients at variable risk of recurrence and graft loss. However, the impact of recurrence on graft outcomes in the context of contemporary immunosuppression is relatively uncertain. We aimed to determine the prevalence and estimate the excess risk of graft loss in recipients with recurrent GN.

Methods: Data was obtained from the Australia and New Zealand Dialysis and Transplant Association (ANZDATA) registry. All primary living and deceased-donor kidney transplant recipients between 2006 and 2013 whose end stage kidney disease (ESKD) was attributed to GN were included in the analyses. The association between the risk of overall and death-censored graft loss was calculated using adjusted Cox proportional regression models.

Figure 1. Cumulative incidence of overall graft loss in recipients with and without recurrent glomerulonephritis.



Results: Among the 3,739 kidney transplant recipients followed for a total of 11,057 person-years, GN was the primary cause of end-stage renal disease in 1,590 (42.5%) recipients. The most common types were IgA (n=585, 36.8%), focal segmental glomerulosclerosis (FSGS) (n=308, 19.4%) and lupus nephropathy (n=80, 50%). A total of 50 (3.1%) recipients experienced disease recurrence, with the median time to disease recurrence of 0.7 (interquartile range (IQR): 2.25) years. Of those that recurred, FSGS was the most rapidly recurring GN (0.1 years from time of transplantation, IQR: 0.52 years), followed by mesangioproliferative GN (1.09 years, IQR: 0.53) and IgA nephropathy (1.26 years, IQR: 2.34). The incidence of overall graft failure for those whose GN disease recurred was 12.7% compared to 4.09% in those without disease recurrence (p value <0.001). Adjusted hazard ratios (HRs) for overall graft loss and death-censored graft loss were 3.18 [(95%CI: 1.89 – 5.4)] and 4.52 [(95%CI: 2.55 – 8.05)], respectively. (Figure 1). Of those who experienced disease recurrence, recurrent GN was the leading cause of graft loss (70%), followed by chronic allograft nephropathy (22%) and acute rejection (2%).

Discussion: Whilst there is a small overall risk of recurrent GN in the transplanted kidney (3.1% at a median follow up time of 3 years post transplant), disease recurrence continues to have significant impact on graft survival in the contemporary era.
The Australia and New Zealand Dialysis and Transplant Registry (ANZDATA)

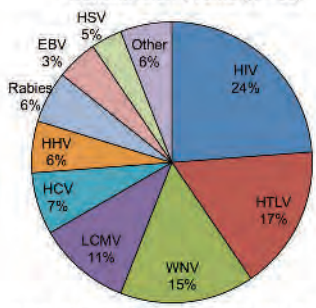
550.4

Trojan kidneys: Unexpected donor-derived viral transmission in kidney transplant recipients – a systematic review

Rashmi Shingde[1], Vaishnavi Calisa[1], Jonathan C.Craig[1,2], Allison Tong[1,2], Germaine Wong[1,2,3].
[1]Centre for Kidney Research, The Children's Hospital at Westmead, Sydney, Australia; [2]Sydney School of Public Health, The University of Sydney, Sydney, Australia; [3]Centre for Transplant and Renal Research, Westmead Hospital, Sydney, Australia.

Introduction: Unexpected donor-derived viral transmission may occur through kidney transplantation due to a false-negative donor test or a lack of pre-donation donor testing. Although rare, it is associated with significant morbidity and mortality. We aimed to evaluate the outcomes of donor-derived unexpected viral infections in kidney transplants.
Methods: We searched MEDLINE, Embase and the Cochrane Central Register of Controlled Trials (CENTRAL) to December 2015. Studies that provided evidence of 'proven' or 'probable' unexpected donor-derived viral transmission events were included. Recipient survival estimates were calculated using the Kaplan-Meier method.
Results and Discussion: We identified 49 studies eligible for inclusion and all were case reports or case series. In total, there were 57 renal transplant donors and 85 recipients. Of the donors, 43 (75.4%) were deceased and 31 (54.4%) were female. The average donor age was 36.7 years (SD=13.2). Of the recipients, 48 (56.5%) were male and the mean age at time of transplant was 42.3 years (SD=12.8). The recipients varied by the region of origin: North America (n=41), Europe (n=31), Asia (n=5), Australia & NZ (n=5) and South America (n=3).
Human Immunodeficiency Virus (HIV) was the most common (23.5%) unexpected donor-derived viral infection in kidney transplants, followed by Human T-cell Lymphotropic Virus (HTLV) (16.5%) and West Nile Virus (WNV) (15.3%) (See Figure 1). Time from transplantation to symptomatic disease onset ranged from 0.7 months (SD=0.2) for WNV to 5 months amongst Hepatitis C (HCV) recipients (SD=3). HIV had a median time to disease onset at 1.7 months (IQR=12.6), compared to 2.7 months for HTLV (IQR=23.5).

Figure 1. Frequency of Unexpected Donor-derived Viral Transmission Events (n = 85)

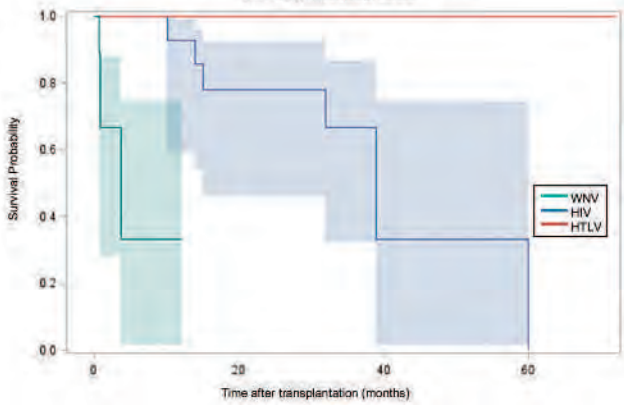


HIV = Human Immunodeficiency Virus, HTLV = Human T-cell Lymphotropic Virus, WNV = West Nile Virus, LCMV = Lymphocytic Choriomeningitis Virus, HCV = Hepatitis C Virus, HHV = Human Herpesvirus, HSV = Herpes Simplex virus, EBV = Epstein - Barr Virus, Other = B19, Arenavirus, CVB3

All five rabies-infected recipients died from rabies-related complications. Prior to death, all 3 rabies-infected donors had altered mental status with fever. Patients with WNV experienced the worst prognosis, with more than 66.7% recipients dying within 12 months, followed by 7.1% of HIV-infected recipients dead in a 12-month period (See Figure 2). The median survival times for recipients with WNV and HIV were 0.88 months (IQR=1.49) and 23.5 months (IQR=25) respectively.
Treatment data was available for 54 out of the 85 recipients. Approximately one third of the patients received antiviral therapy (28.2%) and had immunosuppression withdrawn (29.4%).
Conclusion: The most frequently reported unexpected donor-derived viral infections in kidney transplants were HIV, HTLV and WNV.

Neuroinvasive viruses were associated with the worst recipient prognoses. These data suggest that donors with unexplained death and/or neurological symptoms should be deferred. By understanding the prognoses of such unexpected donor viral transmission events, the safety of organ recipients can be maintained whilst expanding the pool of potential donors.

Figure 2. Recipient Survival Estimates
With 95% Confidence Limits



550.5

Sequential biopsy findings of Polyomavirus associated nephropathy

Handan Özdemir[1], Nurhan Özdemir[2], Aysen Terzi[1], Sebnem Ayva[1], Eda Yılmaz Akçay[1], Hande Arslan[3], Gökce Özdemir[1], Mehmet Haberal[4].
[1]Pathology, Baskent University, Ankara, Turkey; [2]Nephrology, Baskent University, Ankara, Turkey; [3]Infectious Disease, Baskent University, Ankara, Turkey; [4]Transplant Surgery, Baskent University, Ankara, Turkey.

Introduction: The sequential biopsy findings of Polyomavirus nephropathy (PVN) has not been investigated in detail before. The aim of this study is two folds; first to find out the difference of histopathological findings in sequential biopsies of patients with PVN, second to investigate the impact of histopathological findings on graft survival.
Material and Method: Total 70 of 580 patients (12%) had sustained BKV viremia. Only 53 patients whom had at least 2 sequential biopsies (BXs) after initial biopsy with PVN were included to study. All sequential BXs (n:184) of 53 patients (M/F: 43/10) with a mean age of 35,5±16,2 years were re-evaluated. Both initial diagnostic biopsy and the sequential 2nd BXs of all patients were scored according to Banff criteria. The mean interval between initial BX and the 2nd BX was 3,1±1,4 months. HLA-DR expression of tubules was noted both in initial 1st and 2nd BXs. Development of interstitial fibrosis (IF) was investigated throughout in all sequential biopsies. Clinical data and viral loads of each patient were retrieved from patients' records.
Results: The mean interval between the diagnosis of PVN and transplant was 12,5±17 months. Most patients received tacrolimus (75,5%). At the same time with PVN, CMV viremia was found in 23 patients. SV-40 was positive in 26 patients (49,1%) in 1st BX and it was positive in 32 patients (60,4%) in 2nd BX. Glomerular SV40 was positive in 7 cases. BXs containing both cortex and medulla had higher rate of SV-40 staining ($p<0.001$). Statistically significant differences was found between 1st and 2nd BXs in regards of stage of PVN and PVL ($p<0.001$ for both). The stage of 1st BXs was increased to higher stage in 25 cases in the 2nd BXs. Also PVL was increased to higher degree in 16 cases in the 2nd BXs. The tubulitis score and interstitial inflammation significantly increases in the 2nd BXs compared to 1st BXs. The rate of acute humoral and cellular rejection at the same time with PVN was 24,5%. Thrombotic microangiopathy was found in 9 cases (17%). When we compared 1st and 2nd BXs in regards of tubular HLA-DR expression, we noted that expression was increased from 28,3% to 47,2% with increasing degree of interstitial inflammation ($p<0.001$). The development of IF in sequential BXs was found in 28 cases (52,8%) in a mean time of 5,5±3,4 months. Patients whom had glomerular SV40, CMV viremia, higher degrees of inflammation and PVL showed higher risk of IF. The risk of graft loss was found to be increased with increasing PVN stage and PVL ($p<0.01$). Thrombotic microangiopathy, IF, glomerular SV-40 have also shown a negative influence on graft lost.
Conclusion: The rate of negative SV40 can be reached to 50,9% especially in the early BXs and there was a significant differences between two sequential BXs. Therefore it is important to follow-up these patients with sequential BXs in order to diagnose early, to schedule their therapy and to evaluate the prognostic parameters. We also showed that histological findings were correlated well with graft outcome and PVN is morphologically indistinguishable from interstitial rejection.

550.6

Polyomavirus (PV) replication and smoking are independent risk factors for bladder cancer after renal transplantation

John C. Papadimitriou, Sandy Liu, Borislav Alexiev, Cinthia B. Drachenberg.
Pathology, University of Maryland School of Medicine, Baltimore, MD, United States.

Solid organ transplant recipients are at increased risk for polyomavirus nephropathy (PVAN), particularly BK, as well as for developing malignancies, primarily due to immunosuppression. The cancer risk that is associated with PV replication and the concurrent effect of other carcinogenic factors has not been assessed. The aim of this study was to investigate the incidence of PV replication in kidney transplant recipients and assess its correlation with the development of specific malignant neoplasms, alone or in association with smoking.
A retrospective case-control study was conducted for PV replication in all kidney biopsies and urine cytologies received between 1998 and 2014 from kidney transplant recipients. PV replication positive patients (n=943) were defined as having a kidney biopsy with PVAN or any urine cytology with "decoy" (PV infected) cells. PV negative patients (n=943) were defined as never having evidence of PV replication. Incidence of any malignancy (excluding non-melanoma skin tumors) was determined in these 1,886 patients and correlated with demographic data and smoking.
The overall cumulative incidence of PVAN in out total population (3612 patients)t was 8.1% and the cumulative incidence of any decoy cell shedding was 25.2%. There was a 8% incidence of malignant tumors after a mean post-transplant follow-up of 7.9 years.
There was a total of 149 solid organ and hematologic cancers among the 1,886 transplant patients (7.9% incidence); one patient had synchronous urothelial and prostate cancers. In the PV positive group, there were a total of 85 non-skin malignancies/carcinomas: 11 bladder, 14 kidney, 10 prostate, 8 lung, 8 colon, 5 breast, 3 anus, 3 cervix, 3 metastatic carcinomas of unknown primary, 2 stomach, 2 head and neck, 2 liver, 1 thyroid, 1 ovary, 1 pancreas, and 1 esophagus carcinomas, and 8 leukemias/lymphomas.
In the PV negative group, there was a total of 64 non-skin malignancies/carcinomas: 2 bladder, 13 kidney, 8 prostate, 11 lung, 6 colon, 4 breast, 3 endometrial, 2 pancreas, 2 esophagus, 1 metastatic carcinomas of unknown primary, 1 head and neck, 1 cervix, 1 vagina, and 1 thyroid carcinomas, and 8 leukemias/lymphomas.
Only bladder carcinoma, among all cancer subtypes, was significantly associated with PV replication. PV replication and smoking were both independent risk factors. By multivariate analysis, PV replication and smoking incrased the risk of bladder cancer by 11.73 fold (p=0.0013) and 5.62 fold (p=0.0053), respectively.
Conclusions These findings suggest that kidney transplant recipients with PV replication and smoking history are at particular risk to develop bladder carcinomas, and further support the need for long term cancer surveillance in these patients.

550.7

BK virus-associated nephropathy in Scotland: evidence of better transplant survival but variation in incidence, management and outcome between renal units

Michaela Petrie[1], Colin Geddes[2], David Walbaum[3], Nicola Joss[6], Andrew Henderson[4], Annette Alfonzo[5], Lorna Henderson[1].
[1]Renal and Transplant Unit, Edinburgh Royal Infirmary, Edinburgh, United Kingdom; [2]Renal and Transplant Unit, Queen Elizabeth University Hospital, Glasgow, United Kingdom; [3]Renal Unit, Aberdeen Royal Infirmary, Aberdeen, United Kingdom; [4]Renal Unit, Ninewells Hospital, Dundee, United Kingdom; [5]Renal Unit, Victoria Hospital, Kirkaldy, United Kingdom; [6]Renal Unit, Raigmore Hospital, Inverness, United Kingdom.

Introduction: BK virus associated nephropathy (BKVAN) is a significant cause of renal transplant graft loss. The benefit of screening and optimum management once diagnosis is confirmed remains unclear. The study aim was to assess the incidence, management and outcome of BKVAN in a national cohort, looking for variation and potential improvement in practice.
Methods: All kidney or simultaneous kidney/pancreas recipients transplanted in Scotland between 1/1/2009 and 31/12/2013 (n=1044) were identified. Scotland has two transplanting units but six renal units providing follow-up, all of whom contributed complete data. Details of biopsy-proven BKVAN cases diagnosed up to 21/12/15 were collected. All patients had a minimum two years post-transplant follow-up.
Results and Discussion: Of 1044 patients transplanted, 49 (4.7%) developed BKVAN at median time from transplant of 398 days (inter-quartile range (IQR) 122-587, range 30-1839 days). The incidence varied by unit from 1.4% (the only unit screening for BK viraemia) to 8.5% (p=0.09) and between patients transplanted in the West of Scotland (3.4%) versus East of Scotland (6.3%) (p=0.06). There were no differences between units in immunosuppression regimen or patient characteristics. All BKVAN cases had immunosuppression reduced and 27/49 (55%) patients were prescribed "anti-viral" medicines with no observable benefit versus immunosuppression reduction alone. Six patients (12%) suffered transplant loss secondary to BKVAN (no transplants lost to rejection) with median time to failure of 322 days (IQR 197-377, range 20-1612 days). The remainder had stable transplant function with median loss of 1 ml/min in eGFR (IQR -6 - +4.5, range -31 - +26 ml/min) by two years since diagnosis (figure 1).

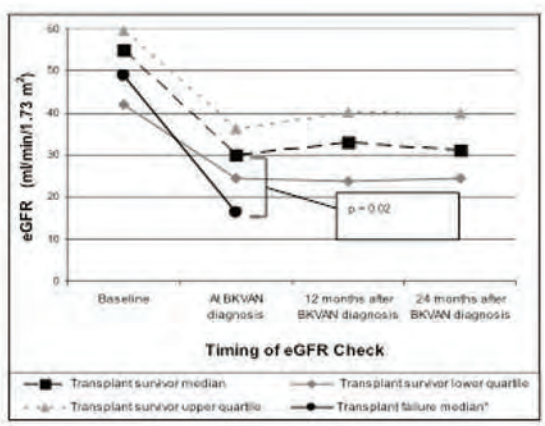


Figure 1. Change in eGFR at BKVAN diagnosis until 2 years follow-up (* only 1 patient in the graft failure group had graft survival beyond 11 months)

The BKVAN-associated transplant failure rate varies by unit from 0 - 25% (p=0.07). The median BK viral level at diagnosis was 171,325

copies/ml (IQR 135,000-1,120,663) for transplant failure cases compared to 100,000 copies/ml (IQR 33,643-599,338) for those with ongoing transplant survival (p=0.006). Greater degree of fibrosis and atrophy on biopsy (PyVAN-C)[1] was associated with greater risk of transplant failure (p=0.01) but patients with lesser fibrosis/atrophy (PyVAN-A and B) had better graft survival than previously published outcome data[1].

Conclusion: BKVAN affects almost 1 in 20 patients in this national cohort, but the difference in incidence and outcome between units warrants further assessment, particularly of BK viraemia screening practice and potential differences in immunosuppression burden. Routine prescription of "anti-viral" drugs did not appear to offer benefit over immunosuppression reduction alone in this observational study. In the absence of severe tubular atrophy or fibrosis on biopsy, BKVAN outcome was better than previously described[1].

References:

[1] Drachenberg CB et al.Histological patterns of polyomavirus nephropathy: correlation with graft outcome and viral load.*Am J Transplantation*.2004;4:2082-2092

550.8

High numbers of intragraft blood dendritic cell antigen-1 (BDCA-1)-positive myeloid dendritic cells during BK polyomavirus–associated nephropathy

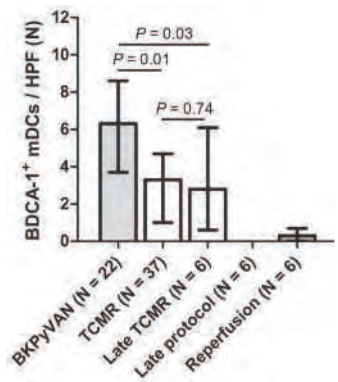
Unsal Yapici[1], Jesper Kers[1], Ivana Slavujevic-Letic[1], Geurt Stokman[1], Joris J.T.H.Roelofs[1], Michiel C.van Aalderen[1], Jaap W.Groothoff[2], Onno J.de Boer[1], Karlijn A.M.I.van der Pant[1], Nike Claessen[1], Luuk B.Hilbrands[3], Frederike J.Bemelman[1], Ineke J.M.ten Berge[1], Sandrine Florquin[1,3].

[1]Academic Medical Center, Amsterdam, Netherlands; [2]Emma Children's Hospital, Academic Medical Center, Amsterdam, Netherlands; [3]Radboud University Nijmegen Medical Center, Nijmegen, Netherlands.

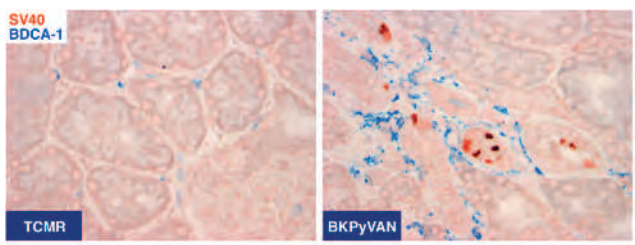
Introduction: Although both polyomavirus infection and T cell–mediated rejection (TCMR) are characterized by tubulointerstitial inflammation in the renal allograft, these conditions are treated with opposing therapeutic regimens. The aim of our study was to gain more insight into the differences between antiviral and alloimmune responses.

Materials and Methods: We performed a case-control study, in which we immunophenotyped the inflammatory infiltrates in renal biopsy specimens with BK polyomavirus-associated nephropathy (BKPyVAN) and specimens with TCMR (T cells, B cells, plasma cells, type II and total macrophages and mature, plasmacytoid and DC-SIGN$^+$ and BDCA-1$^+$ myeloid dendritic cells).

Results and Discussion: Compared with TCMR, BKPyVAN was diagnosed later after transplantation; therefore, BKPyVAN specimens showed more signs of chronic damage than TCMR specimens showed. However, TCMR and BKPyVAN specimens had comparable levels of tubulointerstitial inflammation. Adjustment for confounders in various multivariable models revealed more blood dendritic cell antigen-1$^+$ (BDCA-1$^+$) myeloid dendritic cells (mDCs) present during BKPyVAN (adjusted odds ratio, 2.31; 95% confidence interval, 1.03 to 5.16; P=0.04) than during TCMR.



Double immunostaining for SV40 and BDCA-1 showed that, during BKPyVAN, BDCA-1$^+$ mDCs were localized in proximity to the polyomavirus–infected tubular epithelial cells.



We ensured that time of biopsy after transplantation was not a confounding factor by including additional specimens with late TCMR

and protocol biopsy specimens matched for biopsy time. These additional specimens showed amounts of BDCA-1$^+$ mDCs comparable with those in the early TCMR specimens.
Conclusion: These results suggest that BDCA-1$^+$ mDCs, known to be involved in the antiviral immune response during various viral infections, have a pivotal role during BKPyVAN infection in the renal allograft.

550.9

Outcome after Eculizumab therapy to prevent recurrence of atypical hemolytic uremic syndrome: experience in twelve renal transplant recipients

Charlene Levi[1], Marion Rabant[1,2], Veronique Fremeaux-Bacchi[2,3], Anne Scemla[1], Julien Zuber[1,2], Christophe Legendre[1,2], Rebecca Sberro-Soussan[1].
[1]Department of Renal Transplantation, Necker Hospital, Paris, France; [2]Universite Paris Descartes, Sorbonne Paris Cité, Paris, France; [3]Department of Immunology, Georges Pompidou European Hospital, Paris, France.

Atypical hemolytic uremic syndrome (aHUS) is a rare disease with a high recurrence rate after kidney transplantation. aHUS is associated with histological lesions of thrombotic microangiopathy that mainly leads to graft loss. The successful use of Eculizumab (Ecu) to prevent or to treat post-transplantation aHUS recurrence has been scarcely reported. In this study, we describe 12 patients who received a renal transplantation for aHUS and who were treated by Ecu for renal transplantation.
Twelve renal transplants recipients with aHUS on their native kidney received Ecu at our center between 2010 and 2015. Ten patients received prophylactic Ecu at day 0. Two were treated at time of recurrence (day 6 and 25). We reviewed clinical, genetic testing and histological data, and posttransplant course. Median follow-up was 24,6 (4,5 – 68,2) months.
Five patients had at least one previous transplantation that failed secondary to recurrent aHUS. A genetic mutation was identified in eleven patients (H factor (4) ; I factor (3), CFH-CFHR1 hybrid gene (1) ; C3 (1), CFHR1 deletion and anti-H-factor antibody (2)). No patient experienced biological thrombotic microangiopathy (TMA) recurrence under treatment. We found transient histological lesions in the 2 patients with later Ecu introduction. One patient lost his graft of recurrence after discontinuation of eculizumab for an infectious complication. Three antibody mediated rejections (AMR) occurred during treatment including one associated with histologic TMA lesions. One patient, with aHUs on his native kidneys experienced a C3 glomerulonephritis.
These data confirm that Ecu is highly effective to prevent post-transplantation aHUS recurrence, with a good renal function. However, Ecu doesn’t prevent AMR. The best treatment duration remains to be defined.

551.1

Influence of pancreas import and cold ischemia on outcomes following pancreas transplantation

Robert Stratta[1], Jeffrey Rogers[1], Alan Farney[1], Giuseppe Orlando[1], Hany El-Hennawy[1], Muhammad Khan[1], Amber Reeves-Daniel[2], Amudha Palanisamy[2], William Doares[3], Scott Kaczmorski[3].
[1]Surgery, Wake Forest, Winston-Salem, NC, United States; [2]Internal Medicine, Wake Forest, Winston-Salem, NC, United States; [3]Pharmacy, Wake Forest, Winston-Salem, NC, United States.

Introduction: New changes in pancreas transplant (PT) allocation in the US may improve organ sharing but increase cold ischemia time (CIT).
Methods: We performed a single center, retrospective study of 202 consecutive PTs in 192 patients and analyzed outcomes according to donor site of origin and CIT. Nearly all pancreata were preserved with UW solution and PT was performed with portal-enteric drainage. All patients received either rATG or alemtuzumab induction with tacrolimus, MMF, and tapered steroids.
Results: From 11/01 to 3/13, we performed 162 simultaneous kidney-PTs (SKPT) and 40 solitary PTs; 160 were local and 42 were import organs. Solitary PT recipients were more likely to receive an import organ (47% solitary vs 14% SKPT, p<0.001). 37 PTs were performed with pancreas CITs ≥20 hours (mean CIT 21.8 hours) whereas the remaining 165 PTs had CITs <20 hours (mean CIT 14.9 hours, p<0.001). There were no other differences between the study groups except that import organs came from younger donors (mean import donor age 23 vs local 27 yrs, p=0.04) with longer pancreas CITs (mean import 17.3 vs local 15.6 hrs, p=0.02). With a mean follow-up of 7 years, actual patient survival (78.4% ≥20 vs 85.8% <20, p=0.31), kidney (51.5% ≥20 vs 78.3% <20, p=0.004) and pancreas graft (PG, 45.9% ≥20 vs 64.2% <20, p=0.04) survival rates were inferior in PTs with prolonged CITs. Death-censored kidney and PG survival rates were 63% ≥20 vs 86.3% <20 and 51.5% ≥20 vs 75% <20 (both p=0.01), respectively. The early (3 month) relaparotomy rate was 56.8% ≥20 vs 32.7% <20 (p=0.008) including an early PT thrombosis rate of 13.5% ≥20 vs 6.7% <20 (RR=2.0, p=0.18). One- and 5-year actuarial kidney graft (import 95%/83% vs local 97%/80%), PG (import 83%/58% vs local 87%/72%), and patient survival (import 98%/89% vs local 97%/92%, all p=NS) rates were comparable between recipients of import and local organs. PG survival was significantly worse in SKPT recipients from import donors (1- and 5-year PG survival for imports 78%/45% vs local 87%/73%, p=0.013) and was related to longer CIT. Technical and non-technical causes of PG failure were similar with import and local donors.
Conclusions: Prolonged pancreas CIT is associated with inferior graft survival rates. We observed that PG survival was inferior in SKPT recipients from import donors despite similar causes of PG loss with import and local organs. Strategies to decrease CIT should be deployed for improving utilization and outcomes.

551.2

Why are pancreas transplants declining in the united states in the past decade?

Robert Stratta[1], Angelika Gruessner[2], Jon Odorico[3], Jonathan Fridell[4], Rainer Gruessner[2].
[1]Surgery, Wake Forest, Winston-Salem, NC, United States; [2]Surgery, SUNY Upstate, Syracuse, NY, United States; [3]Surgery, University of Wisconsin, Madison, WI, United States; [4]Surgery, Indiana University, Indianapolis, IN, United States.

In the past decade, the annual number of pancreas transplants (PTxs) in the US has steadily declined coincident with increasing numbers of PTxs performed outside of the US.
Methods: We retrospectively reviewed data from the International Pancreas Transplant Registry (IPTR) to identify and track trends pursuant to the scope of PTx in the US.
Results: As of 12/14, >48,000 PTxs were reported to the IPTR including >29,000 from the US. The total annual number of US PTxs steadily increased until 2004 (peaking at 1484) but has since declined substantially in the past decade with only 954 PTxs performed in 2014, which is the lowest total since 1994. Since 2004, the overall number of simultaneous pancreas-kidney (SPK) transplants declined by 10% whereas decreases in PTx after kidney (PAK) and PTxs alone (PTA) were 55% and 34%, respectively. Coincident with the 35% decrease in overall PTx activity in the US, fewer patients are being added to the waiting list, waiting times have increased, and wait list mortality for SPK transplant candidates is 10%. Paradoxically, these changes occurred in the setting of improvements in graft and patient survival outcomes and transplanting higher risk patients. PTx is now associated with an extremely low mortality rate, ranging from 3-4% at 1 year to <6% at 3 years in all 3 PTx categories. At 3 years, graft survival (insulin-free) rates are 83% for SPK, 76% for PAK, and 65% for PTA recipients. The number of active US PTx centers has decreased from 132 to 114; 11 centers currently perform ≥20 PTxs per year and nearly half perform <5 PTxs annually. Only 50% of centers perform PAK and 25% PTAs. 14 centers in North America are currently certified for PTx fellowship training (minimum of 20 PTxs/year required). This national trend in diminishing numbers of PTxs is disturbing and related to a number of factors including lack of a primary referral source and acceptance by the diabetes care community, changing donor and recipient demographics, and increasing risk aversion because of regulatory scrutiny.
Conclusions: Given that the incidence of end stage renal disease secondary to diabetes remains high, a national initiative is needed to "re-invigorate" SPK and PAK transplants as preferred options for appropriately selected uremic patients taking insulin irrespective of C-peptide levels or "type" of diabetes. Moreover, many patients may benefit from PTA because all categories of PTx are not only life-enhancing but life-extending procedures.

551.3

Extended pancreas donor program – The EXPAND study, a prospective multicentre trial

Andrea Proneth[1], Andreas A.Schnitzbauer[1,2], Ines Holub[1], Florian Zeman[3], Bernhard Banas[4], Richard Viebahn[5], Peter Schenker[5], Helmut Arbogast[7], Falk Rauchfuß[6], Andreas Pascher[8], Steffen Manekeller[9], Wolf O.Bechstein[2], Peter Schemmer[10], Thomas Becker[11], Michael A.Ströhlein[12], Michael Heise[13], Silvio Nadalin[14], Edward K.Geissler[1], Hans J.Schlitt[1], Stefan A.Farkas[1,15].
[1]Klinik und Poliklinik für Chirurgie, Universitätsklinikum Regensburg, Regensburg, Germany; [2]Klinik für Allgemein- und Viszeralchirurgie, Universitätsklinikum Frankfurt, Frankfurt am Main, Germany; [3]Zentrum für Klinische Studien, Universitätsklinikum Regensburg, Regensburg, Germany; [4]Abteilung für Nephrologie, Universitätsklinikum Regensburg, Regensburg, Germany; [5]Chirurgische Universitätsklinik, Universitätsklinikum Knappschaftskrankenhaus Bochum der Ruhr-Universität Bochum, Bochum, Germany; [6]Klinik für Allgemein-, Viszeral- und Gefässchirurgie, Universitätsklinikum Jena, Jena, Germany; [7]Klinikum für Allgemeine, Viszeral, Transplantations- Gefäß- und Thoraxchirurgie, Klinikum der LMU München-Großhadern, München, Germany; [8]Klinik für Allgemein-, Visceral- und Transplantationschirurgie, Charité Campus Virchow Klinikum, Universitätsmedizin Berlin, Berlin, Germany; [9]Klinik und Poliklinik für Allgemein-, Viszeral-, Thorax- und Gefäßchirurgie, Universitätsklinikum Bonn, Bonn, Germany; [10]Abteilung für Allgemein-, Viszeral- und Transplantationschirurgie, Universitätsklinikum Heidelberg, Heidelberg, Germany; [11]Klinik für Allgemeine Chirurgie, Viszeral-, Thorax, Transplantations- und Kinderchirurgie, Universitätsklinikum Schleswig-Holstein - Campus Kiel, Kiel, Germany; [12]Klinik für Viszeral-, Gefäß- und Transplantationschirurgie, Kliniken der Stadt Köln, Krankenhaus Merheim , Köln, Germany; [13]Klinik für Allgemein-, Viszeral- und Transplantationschirurgie, Universitätsmedizin der Johannes Gutenberg-Universität Mainz, Mainz, Germany; [14]Klinik für Allgemeine, Viszeral- und Transplantationschirurgie, Klinikum der Universität Tübingen, Tübingen, Germany; [15]Allgemein- und Viszeralchirurgie, St. Josefs-Hospital Wiesbaden, Wiesbaden, Germany.

Introduction: Simultaneous Pancreas Kidney transplantation (SPK) or Pancreas Transplantation after Kidney (PAK) are the only curative treatment options for patients with type 1 diabetes and impaired renal function. Severe diabetic complications such as nephropathy, retinopathy, neuropathy and angiopathy can be avoided by pancreas transplantation. According to the existing allocation system in the Eurotransplant area only organs from donors age <50 years and BMI <30 could be allocated for transplantation. While the waitlists are increasing, there is a decrease of organs meeting the currently accepted criteria.
Materials and Methods: The aim of this study was to extend the organ pool by using organs with extended donor criteria either from donors age 50 to 60 or with a BMI 30 to 34. For the study a new allocation system was implemented. All patients on the waitlist for primary SPK or PAK were evaluated. The study was conducted as a prospective, multicentre, single-blinded, non-randomized, two-arm trial. The primary endpoint was insulin-free pancreas allograft survival rate after 3 months. Rejection episodes, kidney function and quality of life were secondary endpoints. A patient who agreed to participate in the trial and received an extended pancreas allograft (EDC) was included as study group patient. A patient who received a normal criteria organ was followed-up as a control-group patient. Patients remained blinded during the study period and received standard immunosuppressive treatment. Follow-up was 1 year.
Results: A total of 79 patients were included in 12 German centres from July 2011 to April 2015. 18 received an EDC organ and 61 a standard criteria organ. Recipient demographics were similar in both groups.

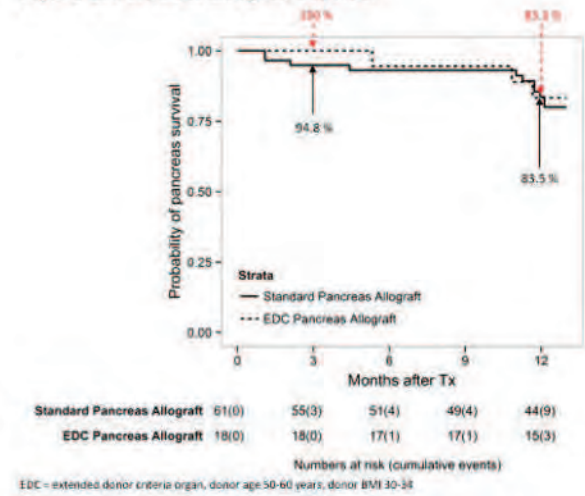
Table 1: Patient (recipient) demographics

	Standard group	EDC group	p-value
Number of patients (male / female in %)	61 (67.2 / 32.8)	18 (66.7 / 33.3)	0.965
Age [years ± SD]	43.7 ± 9	47.7 ± 8	0.085
Body weight [kg ± SD]	73.5 ± 12	76.9 ± 15	0.316
Time of diabetes [years ± SD]	**28.3 ± 9**	**32.3 ± 9**	0.103
Daily dose of insulin [IU ± SD]	33 ± 19	33 ± 21	0.940
Medical History [no. of patients in %]			
Nephropathy	100	94.4	0.228
Retinopathy	77.0	77.8	1.000
Neuropathy	55.7	66.7	0.409
Coronary heart disease	24.6	27.8	0.785
Peripheral arterial disease	18.0	11.8	0.540
Chronical dialysis [no. of patients in %]	90.2	88.9	0.875
Time on dialysis [years ± SD]	**2.8 ± 2**	**3.2 ± 3**	0.555
Type of dialysis [haemodialysis / CAPD in %]	83.6 / 16.4	81.2 / 18.7	0.823
Waiting time for transplantation [month ± SD]	**20.5 ± 16**	**18.7 ± 13**	0.895

EDC = extended donor criteria organ, donor age 50-60 years, donor BMI 30-34

Mean donor age was 51.4 ± 5 years in the EDC group vs. 31.7 ± 12 in the control group. Insulin-free graft survival was 83.3% for EDC organs vs. 77.6% for standard organs (p=0,75) after 3 months. Pancreas allograft survival was 100% in the EDC group after 3 months (p=0.03) and 83.3% after 1 year vs. 94.8% and 83.5% in the standard group.

Figure 1: Pancreas allograft survival



EDC = extended donor criteria organ, donor age 50-60 years, donor BMI 30-34

Kidney allograft survival was 94.4% in both groups after 1 year. Rejection episodes or quality of life did not differ between groups.
Conclusions: The EXPAND study shows for the first time in a prospective trial that extended pancreas criteria organs can be used with similar outcome. Therefore, the organ shortage can be reduced, as well as waiting times, by allocating carefully selected EDC organs without additional risk. This study substantiates the full implementation of EDC organs in the Eurotransplant area allocation system.
We would like to thank the responsible staff within Eurotransplant and the DSO for contribution to establishment of the rescue allocation system. We would also like to thank the supportive personnel at each site for contribution. Partial funding was provided by a research grant from Novartis and Astellas

551.4

Preservation of human pancreas with hypothermic machine perfusion

Diego Cantarovich[2], Karine Renaudin[1], Julien Branchereau[2].
[1]Pathology, Nantes University Hospital, Nantes, France; [2]ITUN, Institut de Transplantation, Urologie et Nephrologie, Nantes University Hospital, Nantes, France.

Pancreas transplantation is one of the best treatment options for insulin-dependent diabetes. The pancreas is high vulnerable to ischemic damage and ischemic-reperfusion injury. Graft thrombosis is the consequence of this process and accounts for the majority of graft losses. Cold storage is the mainstay preservation method applied for pancreas preservation.
Hypothermic machine perfusion (HMP) has proved its value in preserving kidney transplants. Whether HMP could prevent ischemic reperfusion damage in human pancreas transplants and therefore decrese graft failure is not known. The first pancreas pulsatile perfusion was done in canine pancreas autografts in the eighties; this was a high pressure perfusion and induced severe edema. Today, there is no clinical study designed to investigate the impact of HMP on human pancreas allografts.
In our pre-clinical study, 7 human pancreas from deceased heart beating donors rejected for clinical transplantation because of advanced age and/or other comorbidities, were used and perfused with the Wave (Waters Medical Systems) perfusion machine. Pancreas were prepared identically as for human transplantation of vascularized organs. The splenic artery and superior mesenteric artery were anastomosed to an iliac artery division. We therefore perfused all pancreas with a pressure of 25 mmHg. Tissue biopsies were collected at baseline and after 6, 12 and 24 hours of HMP.
Histology did not demonstrate edema after 6, 12 and 24 hours of HMP. We conclude that human pancreas preservation with a pulsatile machine perfusion is feasible and does not induce edema after 24 hours or perfusion under low pressure.

551.5

A proposal for new donor criteria for pancreas transplantation based on postoperative insulin secretion

Taihei Ito[1], Takashi Kenmochi[1], Minoru Oshima[1], Naohiro Aida[1], Atsushi Suzuki[2], Mamoru Kusaka[3], Hitomi Sasaki[3], Midori Hasegawa[4], Hisahiro Matsubara[5], Kiyotaka Hoshinaga[3].
[1]Department of Organ Transplant Surgery, Fujita Health University, School of Medicine, Aichi, Japan; [2]Division of Endocrinology and Metabolism, Fujita Health University, School of Medicine, Aichi, Japan; [3]Department of Urology, Fujita Health University, School of Medicine, Aichi, Japan; [4]Department of Nephrology, Fujita Health University, School of Medicine, Aichi, Japan; [5]Department of Frontier Surgery, Chiba University, Graduate School of Medicine, Chiba, Japan.

Background: Under a revision to the law in 2010, the number of pancreas transplantations from brain dead donors has been increasing in Japan. Although about 70% of donors have been defined as marginal according to Kapur's criteria, there has been no significant difference between marginal donors and non-marginal donors in terms of pancreatic graft survival in Japan. Moreover, the major criteria for brain dead donors of pancreas transplantation in Europe and the United States do not refer to HbA1c which must be measured in our country. Therefore, we establish here a new donor criteria for pancreas transplantation in Japan.
Methods: 36 recipients who received pancreas transplantation in our facility and who underwent a Glucagon tolerance test 1 month after transplantation were analyzed. These recipients were divided into the two groups according to the levels of ΔCPR (CPR6min-CPR0min, ng/ml) of a Glucagon tolerance test which was used to be an index of insulin secretion; Well function group (WF, ΔCPR ≧1.0, n=31) and Poor function group (PF, ΔCPR ＜1.0, n=4). Donor's background including age, cause of death, presence of cardiopulmonary arrest episode, BMI, HbA1c, TIT, days of ICU stay, dose of catecholamine and s-Cre level on admission and preoperatively, were compared between the two groups.
Results: 13 out of 32 (40.6%) of the donors in the WF died of cerebrovascular accidents (CVA), while all 4 donors (100%) in the PF died of cerebrovascular accidents with significant difference ($P<0.05$). There was a significant difference in terms of preoperative s-Cre level between the WF (0.82±0.62mg/dl) and the PF (2.78±3.03) ($P<0.01$). ΔCPR was significantly lower when donor age was over 50 (2.02±1.36) compared to when donor age was under 49 (3.06±1.40) ($P<0.05$). ΔCPR was also significantly lower when donor's HbA1c was over 5.6% (1.37±0.86) compared to when donor's HbA1c was under 5.5% (3.03±1.38) ($P<0.01$).
According to these results, we are proposing new donor criteria for pancreas transplantation in our country: 1. Donor's age≧50, 2. HbA1c≧5.6%, 3. Brain death of CVA, 4. Preoperative s-Cre≧1.5mg/dl.⊿CPR was 0.91±0.22 in the donors with 3 or 4 factors, 2.15±0.76 with 2 factors, 3.34±1.52 with single factor and 3.35±1.40 without any factor. When donors had multiple marginal factors, ?CPR was significant lower than with only one factor or without any factors. Two recipients out of 4 (50%) transplanted with the pancreas from the donors with 3 factors showed the low levels of⊿CPR(<1.0) The recipient transplanted with the pancreas from the donor with 4 factors 1/1 (100%) failed to achieve an insulin independency despite of positive setum CPR.
Conclusion: These results suggest that our new donor criteria for pancreas transplantation should be reasonable in terms of postoperative insulin secretion of pancreatic graft. Using these criteria, named Fujita Univ. criteria, we have to be careful for the indication for pancreas transplantation in terms of postoperative insulin secretion when the donor has more than 3 factors.

551.6

Total pancreatectomy with islet autotransplantation improves quality of life allowing for the resolution of the intractable pain with good glucose control in properly selected patients with chronic pancreatitis

Julia Solomina[1], Sabrina Matosz[1], Andres Gelrud[2], Karolina Golab[1], Celeste Thomas[2], Zehra Tekin[1], Louis H.Philipson[2], Lindsay Schenck[1], W. James Chon[2], Justyna Golebiewska[3], Sabarinathan Ramachandran[1], Martin Tibudan[1], Maciej Sledzinski[4], Jeffrey B.Matthews[1], Piotr Witkowski[1].
[1]Surgery, University of Chicago, Chicago, United States; [2]Medicine, University of Chicago, Chicago, United States; [3]Nephrology, Transplantology and Internal Medicine, Medical University of Gdańsk, Gdańsk, Poland; [4]General Surgery, Endocrinology and Transplantology, Medical University of Gdańsk, Gdańsk, Poland.

Background: There is a group of patients with chronic pancreatitis (CP) who develop malnutrition, constant pain with narcotics dependency, and extremely poor quality of life[1]. We evaluated the outcomes of total pancreatectomy with islet autotransplantation (TPIAT) in our recent cohort of such patients.
Material and Method: Total pancreatectomy with islet autotransplantation was offered to patients with intractable pain who exhausted all medical and surgical intervention. Most of the patients 7 of 13 (54%) were diagnosed with a genetic mutation (SPINK1, PRSS1, CFTR) driving chronic inflammation and progression of symptoms.
Results: Thirteen patients suffered from CP for 9.5 years (3-39). Over that time, they had multiple endoscopic or surgical procedures- 4 (2-15), with an average of 4 (1-6) episodes of acute pancreatitis per year. They experienced severe chronic pain- 9.5 (5-10) in Visual Analogue Score (VAS), which led to chronic narcotic medication use in 12 (92%) patients for 7.5 (1-110) months prior to surgery in mount of 52 (5-240) morphine equivalent doses (MED). However, narcotics allowed only for partial relief of pain to VAS of 4.5 (2-5). Three patients (23%) require central line for pain control or parental nutrition. Most patients, 9 (70%) required pancreatic enzyme supplementation and one of them insulin support. Patients received on average 211,000 (76,600-379,000) islet equivalents (IEQ) infused into their portal vein. Wound infection was recorded in 3 (23%) patients and 8 (61%) patients developed incisional hernia requiring repair.
On day 75 follow up, 6 of 13 (46%) patients were insulin free with A1c<6.5. Remaining individuals presented also good glucose control with insulin (1-18 U/day, or 0.07-0.17U/kg of body mass), and median A1c 6 (5.9-7.3). At the same time, 5 patients were able to completely wean off narcotics. Remaining individuals required lower dose- 37.5 (10-130) MED. Abdominal pain due to an incisional hernia was recorded in 4 out of 8. All patients denied diarrhea or steatorrhea after adjusted pancreatic enzyme supplementation. At 1-year evaluation, 4 of 7 (57%) patients remained insulin free with A1c 6.25 (5.6-6.5) and the rest had continuously good glucose control with A1c of 7.3 (6.3-7.5). Patient BMI declined from median 28 (20-39) prior to procedure to 24.4 (range 18.6-33.7) on day 75, and 22.7 (range 19.9 -28.4) on 1-year follow-up.
All patients expressed overall improvement in pain control and quality of life. Prior the procedure, physical health (dimension A) based on SF36 questionnaire was compromised to 24.6% (17-46.3), which improved to 33.1% (19.5-56.2) on day 75 and 44.3% on 1 year. Mental health (dimension B) was 44.3 % (17.5-60.8) and improved to 51.2% (32.7-65.8) on day 75 and 1 year to 52.8% (31.2-56.8).
Conclusion: TPIAT provides improved quality of life with pain resolution and good glucose control in selected patients suffering from chronic pancreatitis and intractable pain.
References:
[1] Witkowski P, Savari O, Matthews JB. Islet autotransplantation and total pancreatectomy. In: Cameron J (ed): Advances in Surgery, Vol 48, 2013. Elsevier, Philadelphia, PA

551.7

Validation of a new North American islet donor score for donor pancreas selection and successful islet isolation

Sabrina Matosz[1], Julia Solomina[1], Ling-jia Wang[4], Tatsuya Kin[5], Zehra Tekin[1], Karolina Golab[1], Maciej Sledzinski[2], Justyna Golebiewska[3], Omid Savari[1], Piotr Witkowski[1].
[1]Surgery, University of Chicago, Chicago, United States; [2]General Surgery, Endocrinology and Transplantation, Medical University of Gdansk, Gdansk, Poland; [3]Nephrology, Transplantology and Internal Medicine, Medical University of Gdansk, Gdansk, Poland; [4]Surgery, Cell Isolation Program, University of Illinois at Chicago, Chicago, United States; [5]Clinical Islet Transplant Program, University of Alberta and Alberta Health Services Edmonton, Edmonton, Canada.

Background: The selection of the optimal pancreas donor is one of the key factors determining the ultimate outcome of the human islet isolation. We developed and validated a new pancreas scoring system based on data from the multiple islet centers.
Method: We designed and conducted a retrospective multicenter analysis of data from eleven islet isolation centers in North America. The data set from 1,056 deceased donors was used for development of scoring system to predict islet isolation success (defined as post-purification islet yield >400,000 islet equivalents). Then we created donor-scoring systems (points from 0 to 100) consisting main donor variables influencing outcome. We plotted receiver operating characteristic (ROC) curve for each scoring system. We calculated area under the curve (AUC) (also referred as c-index) to assess the ability of scoring system to predict successful islet isolation. Finally, we identified a scoring system having the highest AUC and named it North American Islet Donor Score (NAIDS).

Table 1. North American Islet Donor Score

BSA	X<1.54	1.54≤X<1.82	1.82≤X<2	2≤X<2.18	2.18≤X
	0	5	10	20	25
Vasopressor		more than 2	double	single	none
		0	3	10	15
BMI	X<20.1	20.1≤X<28.1	28.1≤X<32.5	32.5≤X<52.0	52.0≤X
	0	2	7	10	0
Unfavorable factors	Age (y) > 20 : +75 C/T (h) ≤ 2 : +17 Body weight (kg) < 55 HbA1c (%) > 6.5	ALT (U/L) > 1070 AST (U/L) > 580 BUN (mg/dL) > 80 Amylase (U/L) > 1600		At least one	None
				0	35
Favorable factors	Body weight (kg) > 120 Own team procurement 130<Na (mEq/L)<160 Peak glucose (mg/dl) <478	None	One	Two	More than 2
		0	2	7	15

Next, we tested the validity of NAIDS on the validation cohorts in the University of Alberta, Edmonton and University of Chicago.
Results: Donor body surface area, body mass index and use of vasopressors were recognized as main donor variables included into the NAIDS. There were also two supplemental composite factors-favorable (based on serum Na and glucose, also body weight as well as own organ procuring team), and unfavorable (based on age, cold ischemia time, body weight, serum A1c, liver enzymes, BUN and amylase).
The c-index in the development cohort was as high as 0.70 [95% confidence interval 0.70 - 0.76] indicating high correlation of the score with the outcome of islet isolation. The success rate increased proportionally as NAID score increased, from 6.8% success in NAIDS < 50 points to 53.7% success in NAID score ≥ 80 points. Similar characteristics of NAID score were observed in the validation cohorts from Edmonton N=179 and University of Chicago N=70 (c-index 0.73 [95% confidence interval 0.637 - 0.788] and 0.66 [95% confidence interval 0.526-0.793] p>0.05, respectively. New NAIDS had better ability to predict the outcome of islet isolation also at University of Chicago than the previously used scoring system[1], for which c-index was 0.589 [95% confidence 0.448- 0.749].
Conclusion: Newly developed pancreas donor scoring system (NAIDS) allows for good correlation of the score with the outcome of the islet isolation; the higher score, the higher islet isolation success rate. NAID score might be a useful tool for donor pancreas selection in

the clinical and research practice allowing adjusting donor selection strategy to the center logistic and financial resources.

References:

[1] O'Gorman, D.; Kin, T.; Murdoch, T.; Richer, B.; McGhee-Wilson, D.; Ryan, E.A.; Shapiro, J.A.; Lakey, J.R. The standardization of pancreatic donors for islet isolations. Transplantation 2005; 80:801-806.

551.8

Is superior mesenteric artery so indispensable for pancreas transplant?

Alexey Pinchuk, Ilya Dmitriev, Yuri Anisimov, Nonna Shmarina, Roman Storozhev, Aslan Balkarov.
Kidney and Pancreas Transplantation, Sklifosovsky Clinical and Research Institute for Emergency Medicine, Moscow, Russian Federation.

Introduction: The critical deficiency of donor organs as a result of both strict criteria for potential donor selection and anatomic features of pancreas graft (PG) blood supply limits the widespread application of pancreas transplantation (PT). During the multiorgan grafts removal we have quite often to sacrifice the superior mesenteric artery (SMA) in favor of additional hepatic artery. In such cases the implied critical PG ischemia significantly limits its implementation. The evaluation of isolated splenic artery supply (ISAS) sufficiency of the PG had defined the purpose of this study.

Materials and Methods: From January 2008 to June 2015, 47 patients had undergone PT. The gender distribution was: 23 women (48.9%) and 24 men (51.1%). The age of the patients ranged from 25 to 51 years and averaged 35.7 ±6.36 years. 18 (38.3%) patients underwent simultaneous pancreas-kidney transplantation (SPKT) with intra-abdominal localization of PG and the formation of the duodenojejunal anastomosis (DJA), 28 (59.6%) - retroperitoneal SPKT with the formation of interduodenal anastomosis (IDA), 1 (2.1%) – pancreas after kidney (PAK) transplantation with intra-abdominal PG localization and DJA formation. In 45 cases (95.7%) we performed Y-graft arterial reconstruction, in 7 cases (15,6 %) of which the total thrombosis of the SMA developed in the early postoperative period. In 2 cases (4,3%) we performed ISAS PT as a result of the SMA cutting off during organs' explantation

Results: In 9 patients with ISAS of PG we didn't observe any ischemic pancreatic head and/or graft's duodenum necrosis. Furthermore, there were no significant differences in the PG function: □-amylase – 139 vs 146 U/l (p=0,8), pancreatic amylase – 131 vs 124 U/l (p=0.8), lipase – 152 vs 127 U/l (p=0,7), HbA1c 4.8 vs 5.4 % (p=0,27), intact insulin – 24 vs 21 mcIU/ml (p=0,84), C-peptide 5.8 vs 3.4 ng/ml (p=0,052). In addition, statistically-significant differences in glucose metabolism based on the survey results using temperature gradient measuring device and in grafts' blood supply according to CT-perfusion haven't been detected.

Conclusion: PT using grafts with ISAS is reasonable due to well-developed arterial collaterals between splenic and superior mesenteric arteries systems. The viability of the surgical technique with ISAS will allow to expand the criteria for pancreas explantation and increase the number of transplantations.

551.9

Total pancreatectomy with autologous islet transplantation for chronic pancreatitis: considerations for early operation

Joshua Jolissaint[1], Linda W.Langman[1], Claire L.DeBolt[1], Allison N.Martin[1], Andrew Y.Wang[2], Daniel S.Strand[2], Victor M.Zaydfudim[1], Reid Adams[1], KennethL. Brayman[1].
[1]Department of Surgery, The University of Virginia Health System, Charlottesville, VA, United States; [2]Division of Gastroenterology and Hepatology, The University of Virginia Health System, Charlottesville, VA, United States.

Introduction: Chronic pancreatitis (CP) places an immense burden on healthcare systems and can become a debilitating condition for those afflicted. Pain is multifactorial and often refractory to medical and endoscopic treatment. Total pancreatectomy (TP) is an attractive therapy for patients with severe CP and autologous islet transplantation has been employed to counteract the consequent "brittle diabetes," with the loss of glucagon-dependent counter-regulation. Factors leading to insulin-independence after TPIAT and optimal timing for operative intervention both are poorly understood.
Materials and Methods: Institutional Review Board approval was obtained for this single-institution, retrospective and prospective cohort case series utilizing data from the electronic medical record and a telephone-based questionnaire. Islets were isolated at a Current Good Manufacturing (cGMP) islet isolation facility using the standard Ricordi method. Pancreas transport solution and final islet preparation were cultured and release criteria prior to transplantation include a negative endotoxin release assay and gram stain of the final islet preparation. Islets were loaded into transplant bags, heparinized, and infused via portal venotomy.
Results and Discussion: 29 patients have undergone TPIAT at this institution. Mean age was 41 years, median duration of CP was 4.3 years, and mean islet equivalent (IEQ) was 189,793. 27 patients who received TPIAT were >6 months post-transplant and included in data analysis. 19/27 were successfully contacted. 7/27 (26%) are insulin-independent an average of 3.53 years post-transplantation and 5/7 are female. Pain rating based on verbal analog scale was reduced from an average of 9 to 4.6 (p<.001) and 11/19 are narcotic-free. Insulin-independent patients had higher C-peptide levels at their most recent follow up (p<0.05) and trended toward longer continuity of follow up, and shorter duration of CP, however their IEQ and IEQ/kg were comparable (Table 1). 6 patients grew bacterial contaminants in the final islet preparation. These patients had a longer duration of CP and lower islet yield compared to both the cohort and those who became insulin-independent, and currently require insulin therapy (Table 2).

Table 1.

Variable	Insulin-Independent (n=7)	Insulin-Dependent (n=12)
Patients with diabetes prior to transplant+, n (%)	3 (43%)	6 (50%)
C-peptide at time of discharge (ng/mL)	1.1	1.0
C-peptide at most recent follow-up (ng/mL)	4.0*	1.2*
Mean follow-up time (range)	2.3 years	0.82 years
Median duration of CP (years)	3.2	4.0
IEQ	202,286	198,583
IEQ/kg body weight	2,958	2,711
BMI	1.8	1.9

+Diabetes was defined as: HbA1c ≥ 6.5%, elevated glucose tolerance test, or use of pharmacological hypoglycemic agents
*p < 0.05

Conclusions: At 27%, our rate of insulin-independence is comparable to other series[1]-[3]. Patients who achieved insulin-independence did not have significantly higher islet yields compared to those who require insulin. Additionally, patients with bacterial contamination in the final islet preparation have a longer duration of CP with a lower islet yield, likely indicative of multiple interventions over several years with resultant bacterial colonization, pancreatic inflammation, and reduced islet cell mass. This study suggests that patients with severe CP might benefit from early TPIAT, potentially reducing bacterial colonization, maximize islet yield, and improve graft success rates.

Table 2.

Variable	Non-contaminated (n=21)	Insulin Independent (n=7)	Bacterial Growth (n=6)
Median duration of CP (range)	3.875 (0.2-42.4 years)	3.2 years (0.2-42.4 years)	6.3 years (2.5-16 years)
IEQ	208,143*	202,286*	120,000*+
IEQ/kg body weight	3,024*	2,958*	1,534*+

*p<0.05
+p=.068

References:
[1] Garcea G, Pollard CA, Illouz S, Webb M, Metcalfe MS, Dennison AR. Patient satisfaction and cost-effectiveness following total pancreatectomy with islet cell transplantation for chronic pancreatitis. Pancreas. 2013;42(2):322–328.
[2] Wilson GC, Sutton JM, Abbott DE, et al. Long-Term Outcomes After Total Pancreatectomy and Islet Cell Autotransplantation. Ann Surg. 2014;260(4):659–667
[3] Chinnakotla S, Beilman GJ, Dunn TB, et al. Factors Predicting Outcomes After a Total Pancreatectomy and Islet Autotransplantation Lessons Learned From Over 500 Cases. Ann Surg. 2015;262(4):610-22.

552.1

A new era for organ donation and transplantation in China

Haibo Wang[1,2].
[1]China Organ Transplant Development Foundation, Shenzhen, People's Republic of China; [2]China Organ Transplant Development Foundation, Beijing, People's Republic of China.

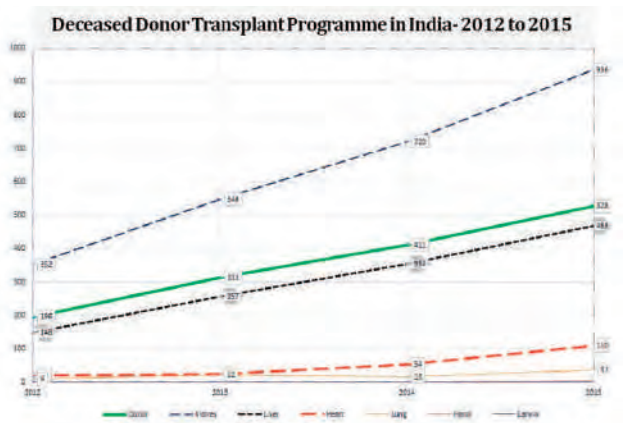
Introduction: In Dec 2014, Dr. Jiefu Huang, the director of the National Organ Donation and Transplantation Committee of China declared the total cessation of the use of death penalty prisoners' organ for transplantation in China, effective on January 1st, 2015. Therefore, the community based organ donation has become the only legitimate source of deceased organ transplantation in China since then. This action marks 2015 as the starting year of a new era for organ donation and transplantation in China. The article aims at providing an overview on the national statistics of the organ donation and transplantation of China in 2015.
Method: China Organ Transplant Response System (COTRS) is the mandatory national organ allocation system, under the authority of National Heath and Family Planning Commission (NHFPC). All of the 131 organ procurement organizations (OPOs) and 169 licensed transplant hospitals were mandated to use COTRS for organ allocation to ensure the fairness and transparency in the process, as well as the traceability of all donated organs requested by the Regulation on Human Organ Transplantation. The data of COTRS was used to generate the statistics of the deceased donors, the characteristics of transplant candidates (liver/kidney) on waitlist, organ allocation and the post-transplant outcomes of China in 2105.
Results: During the year of 2015, 12 154 kidney transplant candidates and 4,076 liver transplant candidates were registered on the waiting list of COTRS, whereas 113 for kidney and 250 for liver died respectively while waiting for transplant. The number of patients remaining on kidney wait list is 22 995 by the end of 2015 and 2,243 for liver. In 2015, there were 2766 deceased organ donation in China, a significant increase of 62.5% compared to the number of community based deceased organ donation in 2014 (1702 cases). Among them, 48.6% were donors of China Category III (Donation after brain death followed by cardiac death). Brain trauma is the most frequent-seen (48.3%) cause of death. The median age of donors is 37.5 years. There were 30.79% of the procured livers and 5.14% of the procured kidneys were shared at national level through COTRS in 2015. The discard rates were 15.2% for liver and 6.5% for kidney. The post-liver transplant survival at 3 months, 6 months and one year were 90.17%,87.62% and 81.85% respectively, which is significantly lower than those of living donor liver transplantation.
Conclusion: China has completely transformed into the community based deceased organ donation and transplantation upon the total cessation on the use of death penalty prisoners' organ for transplantation in 2015. The detailed data of the 2766 altruistic donors was captured and analyzed by COTRS, which signified China's organ transplantation has stepped into a new era in 2015.

552.2

Progress in deceased organ donor transplantation in India

Sunil Shroff[1], Sumana Navin[1], Sujatha Niranjan[1], Pallavi Kumar[1], Lalitha Raghuram[1].
[1]Trustee, MOHAN Foundation, Chennai, India; [2]Kidney Disease, Urology & Transplantation , Madras Medical Mission Hospital, Chennai, India.

Introduction: Organizing a deceased donation programme (DDP) in a developing country like India that has huge regional variations is an administrative and logistic challenge. The first 15 years of the programme saw a slow growth and acceptance but the last five years has seen very encouaging progress.
Material and Methods: Of the 35 Indian States and Union Territories five have been more proactive and helped in the growth of the programme. The central government has recntly rolled out a national programme, and has conducted many consnsus workshops, however it is still in inception and is likely to take a few years to take shape and make an impact. Of all the states the state of Tamil Nadu moved forward and has had an organ sharing network since the year 2000. The state govt. has facilitated in the implementation of the DDP and has implemented procedures to streamline the programme. Similar initiatives were taken by states of Andhra Pradesh, Kerala, Karntaka and Maharastra. MOHAN Foundation an NGO played a key role in four states for establishing the DDP that included Tamil Nadu, Kerala, Rajasthan and Andhra Pradesh. The foundation has actively been undertaking training of transplant coordinators and have trained over 1000 coordinators in the last 6 years.
Results: Since 2012 the number of deceased donors have grown steadily and the donation rate has increased 0.16 in 2012 to 0.5 in 2015 (Fig.1).



In 2012 there were 196 multi organ donors in 2012 and in 2015 there were 528 donors (Fig.2).

Deceased Organ Donation in India - 2015

State	No. of Donors	Kidney	Liver	Heart	Lung	Pancreas	Intestine	Hand	Larynx	Total Organs
Tamil Nadu	155	290	149	51	28	0	1	0	0	519
Kerala	76	132	61	14	2	1	1	4	1	216
Maharashtra	60	106	51	5	0	0	0	0	0	222
Telangana	88	160	92	16	5	0	0	0	0	263
Andhra Pradesh	9	16	7	3	2	0	0	0	0	30
Karnataka	60	91	55	11	0	1	0	0	0	158
Gujarat	3	6	3	0	0	0	0	0	0	9
Madhya Pradesh	3	6	2	1	0	0	0	0	0	9
Uttar Pradesh	4	8	0	0	0	0	0	0	0	8
Delhi-NCR	14	28	14	0	0	0	0	0	0	48
Puducherry	9	16	2	1	0	0	0	0	0	30
Chandigarh	36	69	25	1	0	2	0	0	0	97
Rajasthan	7	14	7	1	0	0	0	0	0	22
Total	528	936	468	110	37	4	2	4	1	1562

Note:
* The statistics projected in the above table is only provisional.
* The statistics for Gujarat, Andhra Pradesh and Delhi-NCR do not reflect all the deceased donations done in the region

The state of Tamil Nadu with the rate of 2.2 per million population (pmp), has almost 4 times the national average of donations. Other states like Kerala, Andhra Pradesh, Karnataka and Maharastra have also seen a rise in donation rate. The trained coordinators have been involved in almnost 50% of the donations happening in the country.
Discussion: The current progress in deceased donation programme in India over the last 5 years has opened a new avenue to overcome organ shortage. The DDP in the State of Tamil Nadu is a role model for the rest of the country. The joint venture of the State Government, transplant hospitals and the Non-Governmental Organizations (NGO) has led to the success of the state's DDP. This Programme has now been adopted by few other states and MOHAN Foundation has partnered with these states in establishing 'Organ Sharing' networks (www.knos.org.in and www.rnos.org)' and helped put procedures in place for its implementation. The placement of trained transplant coordinators has contributed immensely in capacity building and has resulted in increase in the donation rate in the country.
Conclusion: Progres of DDP in India over the last five years has taken care of some of the shortage of organs. All this has been possible due to parternership of the govt. with hospitals and NGO's like MOHAN Foundation. The rate of donation in the last 4 years had increased threefold and this is an encouraging sign for the region.

552.3

The African American journey in organ donation and the methodologies that resulted in movement from the bottom to the top

Clive Callender[1], Naoru Koizumi[2], Patrice Miles[3], J. Keith Melancon[3].
[1]College of Medicine, Howard University, Washington, DC, United States; [2]George Mason University, Arlington, VA, United States; [3]Transplant Institute, George Washington University Hospital, Washington, DC, United States.

Introduction: The shortage of donors is the number one problem in transplantation. In 1982, minorities were more than 50% of the national transplant waiting list, but represented only 15% of the donor population. Previously minorities, especially Blacks, were thought not likely to donate.
Materials and Methods: The methodology was based upon the local grass roots efforts of the D.C. Organ Donor Program (1982-1988) and the Dow Take Initiative Program (1986-1992) focusing on increasing donation rates among African Americans (A.A.) via a mass media campaign. Between 1982 and 1988 in the District of Columbia, A.A. donor card signings increased from 20/month to 750/month and Black donations doubled. A review of the compendium of data including face to face grass roots presentations combined with a nationwide Black donor media campaign was conducted. Gallup Polls in 1985 and 1990 indicated a tripling of Blacks awareness of transplantation and the number of Blacks signing donor cards. Based on these applied successful methodologies, in 1991, a national program was established targeting African American, Hispanic, Asian, and other ethnic groups. A review of the United Network for Organ Sharing (UNOS) database between 1990 and 2010 was accomplished.
Results and Discussion: Total ethnic minority organ donors per million (O.D.M.) increased from 8-10 in 1982 to 35 O.D.M (African American and Latino/Hispanic) in 2002. National minority donor percentages increased from 15% to 28.5% of donors in 2002 (minorities comprised 25% of the USA population in 2000). In 1995, organ donors per million were represented as Whites (34.2), Black (33.1), Hispanic (31.5), Asian (17.9). In 2010, Black organ donors per million totaled 35.36 versus Whites (27.07), Hispanic (25.59) and Asian (14.70). Blacks now represent 17% of the donor population (deceased and living), while representing 13% of the total U.S. population. Based on the data from UNOS, Blacks rank as the number one ethnic group in reference to organ donors per million. Blacks now represent 17% of the donor population (deceased and living), while representing 13% of the total U.S. population.
Conclusion: Based upon the data retrieved from the UNOS in 2010, Blacks were ranked above Whites and other ethnic minority populations as the number one ethnic group of organ donors per million within the U.S. This indicates that when a campaign utilizing successful methodologies such as grass roots face to face presentations and interactions along with media reinforcement is used; donation rates can be increased and sustained. Emphasis on social media with the exclusion of face to face interaction may partially explain the decrease in other ethnic groups. The recommendation to overcome the decrease in ODM in the Latino/Hispanic; White and Asian ethnic groups in the face of the rising organ donor-recipient gap makes revitalization of the face to face grass roots methodology necessary in each of these ethnic populations.

552.4

Center effect on outcome of kidney transplantation from pediatric deceased donor in China

Huanxi Zhang[1], Longshan Liu[1], Liping Chen[2], Chuanhou Sun[2], Haibo Wang[3], Bingyi Shi[2], Changxi Wang[1,4,5].
[1]Organ transplant center, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, People's Republic of China; [2]Chinese Scientific Registry of Kidney Transplantation, Beijing, People's Republic of China; [3]China Organ Transplant Response System Research Center, Shenzhen, People's Republic of China; [4]Guangdong Provincial Key Laboratory on Organ Donation and Transplant Immunology, Guangzhou, People's Republic of China; [5]Institution of Organ Donation and Transplant Immunology, Sun Yat-sen University, Guangzhou, People's Republic of China.

Introduction: The optimal use of kidneys from pediatric deceased donors remains undetermined in China. Choice of single (SK) versus en bloc (EBK) transplantation was empirically determined in different transplant centers due to lack of standard criteria. This study was designed to demonstrate the transplant outcome of single versus en bloc transplantation from pediatric deceased donors using registry data from Chinese Scientific Registry of Kidney Transplantation (CSRKT).
Subjects and Methods: Registry data (2010-2015) of primary Kidney transplantation from pediatric deceased donors in Chinese Scientific Registry of Kidney Transplantation (CSRKT) was retrospectively analyzed.
Results: There were 772 cases of primary single (n=703) or en bloc (n=69) Kidney transplantation from pediatric deceased donors. They were performed in 74 transplant centers. However, only 12 centers were capable of performing EBK and 34 centers capable of performing kidney transplantation from donors weighted less than 20kg. About 88.1% (680/772) of recipients were over 18 years old. About 71.0% (49/69) of en bloc transplantation was performed in adult. Univariate Cox regression showed that a significant increase in risk of graft loss was identified in EBK group compared with SK group (Hazard ratio, 2.66; 95% confidence interval, 1.41-5.00; P=0.002). There was also increased risk of graft loss in recipients with donor weight of 5-10kg compared with recipients with donor weight of ≥20kg (Hazard ratio, 4.32; 95% confidence interval, 2.00-9.37; P<0.001). Multivariate Cox regression controlling confounding variable such as donor weight and recipient age showed that a significant increase in risk of graft loss was identified in EBK group compared with SK group (Hazard ratio, 2.18; 95% confidence interval, 1.08-4.39; P=0.029). Graft loss rate of en bloc kidney transplant varied among transplant centers from 0 to 100% with a median of 8.0%. Subgroup analysis based on donor weight demonstrated that in 5-10kg subgroup only 10% of EBK recipients (1/10) lost their allograft function while 23.5% of SK recipients (8/34) had their kidney allograft loss at the end of follow up. Graft loss rate of single kidney transplant from donors weighted 5-10kg was also various among transplant centers from 0-100% with a median of 10%. No significant difference was seen in patient survival, incidence of DGF and 1-year post-transplant allograft function between EBK group and SK group.
Discussion and Conclusion: Outcome of pediatric EBK recipients was not as good as SK recipients in terms of graft survival. It could be partly explained by the various outcome in different transplant centers, to which surgical difficulties in EBK in adult and disparity of surgeons' technical skills were attributed. Single kidney transplantation in low-weight recipients from small pediatric deceased donors (weighted 5-10kg) was a good way to expand donor pool. However, it had difficulties in identification of indication, surgical skills as well as peri-operational management. Both EBK and single kidney transplantation from small pediatric deceased donors should be performed in experienced transplant centers.

552.5

Expanding the donor pool through intensive care treatment to facilitate organ donation: results of a Spanish multicenter study

Beatriz Dominguez-Gill[1], Elisabeth Coll[1], José Elizalde[2], Jaime Herrero[3], Teresa Pont[4], Brígida Quindós[5], Bella Marcelo[6], María A.Bodí[7], Adolfo Martínez[8], Agustín Nebra[9], Francisco Guerrero[10], José M.Manciño[11], Juan Galán[12], Miguel Lebrón[13], Eduardo Miñambres[14], Rafael Matesanz[1].
[1]Organización Nacional de Trasplantes, Madrid, Spain; [2]Complejo Hospitalario de Navarra, Pamplona, Spain; [3]Hospital Universitari Son Espases, Barcelona, Spain; [4]Hospital Universitari Vall d´Hebrón, Barcelona, Spain; [5]Hospital Universitario Central de Asturias, Oviedo, Spain; [6]Hospital Infanta Cristina, Badajoz, Badajoz, Spain; [7]Hospital de Tarragona Joan XXIII, Tarragona, Spain; [8]Hospital Ramón y Cajal, Madrid, Spain; [9]Hospital Miguel Servet, Zaragoza, Spain; [10]Hospital de Torrecárdenas, Almería, Spain; [11]Hospital Germans Trias i Pujol, Badalona, Spain; [12]Hospital La Fe, Valencia, Spain; [13]Hospital General de Málaga, Málaga, Spain; [14]Hospital Marqués de Valdecilla, Santander, Spain.

Background: The decline in the pool of potential DBD donors is challenging the progression towards self-sufficiency in transplantation in many developed countries. Intensive care treatment to facilitate organ donation (ITD) may help to increase the pool of donors and to offer more patients the chance of donating their organs upon their death. However, there is limited information published on the implementation of ITD in practice.
Objective: To describe the Spanish experience with ITD.
Patients and Methods: ACCORD-Spain was a multicentre (68 hospitals), observational and prospective study on end-of-life decision-making in patients aged 1 month-85 years dead as a result of a devastating brain injury (possible donors) during 11/1/2014-4/30/2015. Information was collected from the ACCORD-Spain database on those possible donors in whom a family interview was held to pose the option of ITD, once intensive treatment with a therapeutic purpose was deemed futile.

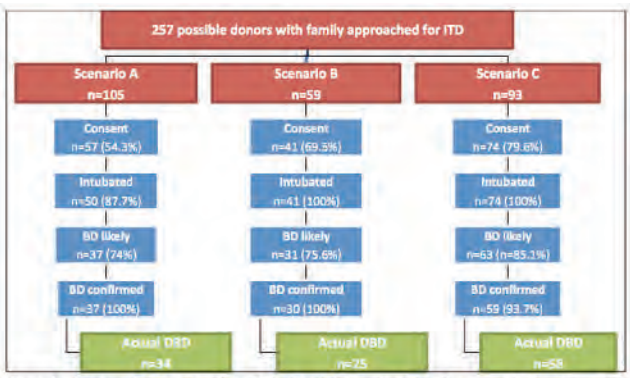


Figure 1: Process of Donation after Brain Death following a family approach to present the option of Intensive Treatment to facilitate organ Donation.
Scenario A: Family approached when intubation/ventilation not considered with a therapeutic purpose; Scenario B: Family approached with the patient intubated/ventilated when admission to an intensive care unit not considered with a therapeutic purpose; Scenario C: Family approached with the patient intubated/ventilated in the intensive care unit, when continuation of treatment is considered futile and brain death is a likely outcome.
BD: Brain Death; DBD: Donation after Brain Death; ITD: Intensive care treatment to facilitate organ donation

Results: 1970 possible donors were included in ACCORD-Spain. Interviews with the family to discuss organ donation were held in 765 cases. In 257 (34%), the family was interviewed once the decision had been made not to initiate intubation/ventilation with a therapeutic purpose (Scenario A: n=105), with the patient under intubation/ventilation but once admission to the ICU was considered not indicated with a therapeutic view (Scenario B: n=59), or with the patient intubated/ventilated in the ICU once further treatment was

deemed futile and brain death (BD) was a likely outcome (Scenario C: n= 93).
Mean age of these 257 possible donors was 69.1 (SD=14.7) years. The most frequent cause of brain injury was a cerebrovascular accident (79.8%).
Consent for ITD was obtained in 172 (66.9%) patients.
From the 172 patients with consent for ITD, 165 (95.9%) were finally intubated/ventilated, of whom 131 (79.4%) evolved to a BD condition in a median time of 1 day (IQR 1-3). BD was tested and confirmed in 126 (96.2%), of whom 117 (92.8%) transitioned to actual DBD. Main reasons why potential DBD donors did not transition to actual DBD donors were medical unsuitability (n=8), organs not suitable for transplantation (n=2), lack of appropriate recipients (n=2), haemodynamic instability with a cardiac arrest (n=1) and family decision not to continue with ITD (n=1). Results per Scenario are provided in figure 1.
From the 34 intubated/ventilated cases who did not evolve to BD, median time until death was 4 days (IQR: 2-6). DCD was considered in 5 (14.7%), 2 (40%) of them transitioning to actual DCD.
In total, 119 of the 172 (69.2%) possible donors with consent for ITD were converted into actual donors. ITD contributed to 24.2% of the total number of actual donors in the ACCORD-Spain study.
Conclusions: ITD can help to increase the donor pool and to ensure organ donation is posed at every end-of-life care pathway. A majority of approached families give consent for ITD. The practice poses significant challenges with regards to uncertainties in terms of evolution to BD and final medical suitability to organ donation. Transparent and comprehensive information to donor families is paramount.
References:
[1] De la Rosa G. Am J Transplan. 2012;12(9):2507-13
[2] Delmonico F. Lancet 2011;378(9800):1414-8
[3] De Lora P. J Med Ethics 2013; 39:145-148
[4] Baumann A. J Med Ethics 2013; 39:139-142.
[5] Coggon J. J Med Ethics 2013; 39:130-134.
[6] Gillett G. J Med Ethics 2013; 39: 149-152.
[7] Emmerich N. J Med Ethics 2013; 39: 153–157.
[8] Achieving Comprehensive Coordination in Organ Donation throughout the European Union (ACCORD). WP5 Final Report. Available at: http://www.accord-ja.eu/phases-and-project-organisation
[9] ACCORD Spain. Final report. Available at: http://www.ont.es/infesp/Paginas/ProyectosenMarcha.aspx

552.6

Deceased organ donation performance in the Chinese community: An audit of hospital medical record in Hong Kong

Chi Yuen, Simon Cheung[1], Mei Lan Pong[2], Suk Fun Au Yeung[2], Ka Foon Chau[1].
[1]Medicine, Queen Elizabeth Hospital, Hong Kong, Hong Kong; [2]Central Nursing, Queen Elizabeth Hospital, Hong Kong, Hong Kong.

Introduction: The number of actual donors per million population was the most commonly used metric to measure organ donation rates and performances in different countries. However, it is deemed inadequate because it does not take into account the potential donor pool, which is dependent on the rates and causes of death. On the other hand, medical records review appears to be the most accurate method for estimating donor potential within a hospital or region[1]. Currently nearly all studies on deceased organ donation were performed in the Western countries and there had been lack of such data in the Chinese population. This study is the first to determine the true potential for solid organ donation from deceased brain-dead donors and the reasons for non-donation in the Chinese community.
Materials and Methods: A retrospective study using the critical pathway for deceased donation[2] was conducted in Queen Elizabeth Hospital, the largest regional hospital in Hong Kong. Medical records of all hospital deaths (including ICU and non-ICU areas) between 1st January and 31st December 2014 were reviewed by our two organ donation coordinators (ODC) and clinicians. Those who had been on mechanical ventilation with documented brain injuries and aged <= 75 years were classified as possible organ donors. The likelihood of progression to brain death was based on the Glasgow Coma Scale, absence of brainstem reflexes, rapidity of deterioration and findings of cerebral tomography.
Results and Discussions: Among 3659 patient deaths, only 233 patients were put on mechanical ventilation with documented brain injury. On initial review, 112 patients were excluded due to old age (>75 years old). The remaining 121 possible organ donors were then further analyzed. The mean age was 59.4 years and 72.7% were males. Majority (88%) were from non-ICU wards. One hundred and eight patients were classified as potential organ donors after excluding 13 unlikely to fulfill brain death criteria. Finally 11 patients became actual organ donors with an overall conversion rate 10%. The conversion rate was higher in ICU than non-ICU (23% vs 8%, p=0.10). The reasons for non-donation included medical contraindication (46%), failure to identify and inform our ODC (14%), failure of donor maintenance (11%), brain death diagnosis not established (18%) and relative's refusal (11%).
Conclusions: It is possible to increase organ donation rate considerably through different areas in the process of donation. Ongoing accurate audit of current practice is necessary.
References:
[1] Christiansen CL, Gortmaker SL, Williams JM, et al. A method for estimating solid organ donor potential by organ procurement region. Am J Public Health. 1998; 88: 1645-50.
[2] Domínguez-Gil B, Delmonico FL, Shaheen FA, et al. The critical pathway for deceased donation: reportable uniformity in the approach to deceased donation. Transpl Int. 2011; 24: 373-8.

552.7

Allocation of extended criteria deceased donor kidneys based on explant biopsy and Modified Remuzzi scores optimizes their utilization

Anantharaman Vathsala[1], Terence Kee[2], Ho Yee Tiong[1], Fang Jann Lee[2], M.S. Zaini[3], Audrey Ngau[3], Xiu Xian Koh[3], Sally Kong[3].
[1]National University Centre for Organ Transplantation, National University Hospital, Singapore, Singapore; [2]SingHealth Transplant, Singapore General Hospital, Singapore, Singapore; [3]National Organ Transplant Unit, Ministry of Health, Singapore, Singapore.

Introduction: Despite Singapore's adequate legislation and excellent infrastructure for deceased(DD) and living donor(LD) renal transplantation, the transplant rates are low at 16.2 pmp. While Extended Criteria Donors(ECD) are crucial to expand the DD pool, allocation of ECD kidneys is challenging due to difficulty in predicting donor quality and post-transplant(TX) outcomes.
Methods and AIms: Since 2009, all potential kidney DDs' are stratified as ECD if Age >60 yrs(ECD_Age); or if Age 50-59 yrs and meeting 2 of 3 criteria: medical cause of brain death, terminal creatinine >133 µmol/L or hypertension (ECD_UNOS); or if Diabetic and Hypertensive regardless of age (ECD_DM) or other clinical conditions (ECD_Others). The remainder were classified as Standard Criteria Donors (SCD). ECD evaluation included wedge explant biopsy of both kidneys, rapid processing of histology specimens and evaluation by a pathologist. Modified Remuzzi (mRemuzzi) scoring was performed fo each kidney in 4 domains (Glomeruli, Tubules, Interstitium and Arteries) to derive a Total Remuzzi Score (TRScore). Kidneys with TRScore of 0-4 were allocated as Single Implants; kidneys with TRScores of 5-7 were transplanted as Dual Implants.This study evaluated the impact of our allocation strategy on the DD pool and 1-yr graft and patient survivals and renal function.
Results: Of 85 DD from 2009-2014, 31(36.4%) were ECD. 15(48.4%), 8(25.8%), 4(12.9%) and 4(12.9%) were ECD_Age, ECD_UNOS, ECD_DM and ECD_Others respectively. Proportion of male and mean age was higher among ECD (71% vs 48%; P=0.034; 56.5yrs vs 39.3yrs; P=0). After evaluation, kidneys from 1 ECD were discarded; the remainder were implanted as Single(n=35, 52%) and Dual(n=14, 45%) respectively. Median glomeruli number per ECD kidney was 26 and median TRScore was 4. Of 54 ECD kidneys with adequate glomeruli for scoring, 21(38.8%), 32(59.3%) and 1(1.9%) had TRScore of <4, 4-7 and >7 respectively.
There were no significant differences in recipient demographics for Single vs. Dual Implants. Overall 1-year survival was 93.5%, with 1 graft loss due to primary non function and 1 patient death each due to AMI and sepsis at 2 and 12 months post-TX respectively. By 1 year, 34.7% were on mTORi-based immunosuppression (37.5% and 42.8% of Single and Dual Implants respectively). There were no differences in renal function for Single vs Dual Implants upto 1 year post-TX (median eGFR at 1, 3, 6 and 12 mths of 38, 40, 44 and 42 mL/min/1.73 m^2 vs. 43, 47, 41, 39 mL/min/1.73 m^2 for Single vs Dual Implants respectively; p=NS).
Conclusions: This strategy of allocating ECD kidneys based on mRemuzzi scoring of explant biopsies increased the DD rate by 29.8% in Singapore. 52% of "ECD" kidneys could be transplanted to standard recipients as Single Implants with excellent outcomes. As 1 year-eGFR for Single and Dual Implants was excellent, allocation based on donor explant histology and mRemuzzi scoring permits optimal utilization of scarce DD kidneys.

552.8

50 years of transplantation in Poland

Roman Danielewicz, Piotr Malanowski, Malgorzata Dudkiewicz, Dorota Lewandowska, Jarosław Czerwiński.
Polish Transplant Coordination Center, Poltransplant, Warsaw, Poland.

On the 26 of January 1966 first successful kidney transplantation was performed in Poland, about number 600 kidney transplant in the world at this time. From 1966 to the end of 2015 there were 28204 organ transplantation in Poland. Our country made large progress in area of legislation, organization and coordination, working towards and accommodating best practices in the field (including "Spanish model" for transplant coordinators). It worth to underline that to the best of our knowledge there were no cases of organ trafficking in our country. Currently all transplant programs are active in Poland with exception of small bowel transplantation. Donation rate is at 13,6 pmp in 2015, which is obviously not satisfactory, with nearly 1600 recipients on the active waiting list. Despite the number of transplant programs, donation rate in last years reached around 31 pmp only in most active regions of Poland, which is our great concern.
Currently in Poland there is a number of transplant programs - 21 for kidney, 6 for liver, 6 for heart, 5 for pancreas, 6 for lungs, 2 for forearm and 1 for face and trachea. In 2015 there were 1021 kidneys transplants (plus 60 from LD), 332 livers (plus 22 from LD), 98 hearts, 41 pancreas and 24 lungs transplantation. Besides, there are 11 bone marrow (BMTx) transplant programs performing in 2014 BMTx from 175 related donors and 397 from unrelated donors. There were also over 1150 BM donation in 2015 from polish donors, due to large number of potential BM donor in our register which reached over 900 thousands in the end of 2015 (7th register of BM donors in the world). The results of organ transplantation in Poland are well above the average, with 10 years recipients/graft survival for kidneys at the level of 76%/58%, for livers at 80%/76% and heart at 47%/46% in 2014
In Poland there is nearly 400 hospitals with donation potential, while in 2014 only 147 (36,75%) were active in organ donors referral to Poltransplant (national transplant organization). Overall 64% of procedures were multiorgan procurement. In the same time 276 organ retrievals in 2014 (46,5%) took place in only 18 of most active donors' hospitals.
For number of years Poland was actively participating in European programs devoted to increase organ donation (inter alia EU Action Plan, ETPOD, Donor Action, COORENOR, ACCORD etc.). There is also National Program of Development of Transplantation medicine for years 2011-2010, including social promotion of organ donation. The last is aiming at increasing the number of deceased and living donation in our country to meet the demand of waiting list. Despite this numerous efforts the self-sufficiency of our country in term of organ procurement and transplantation, is far from satisfactory. Further efforts and continuation of promotional programs of organ donation together with professional activities in the field of transplantation medicine are necessary to meet the demand for organ transplantation in Poland.

552.9

Risky organs: trends in comorbidities among potential and actual Australian organ donors in New South Wales

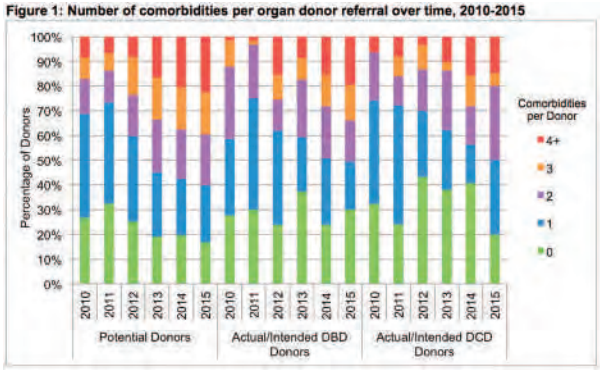
Imogen Thomson[1], Rebecca Hancock[2], Kate Wyburn[1,3], Michael O'Leary[1,4,5], Angela Webster[1,2,6,7].
[1]Sydney Medical School, University of Sydney, Sydney, Australia; [2]School of Public Health, University of Sydney, Sydney, Australia; [3]Renal Unit, Royal Prince Alfred Hospital, Sydney, Australia; [4]NSW Organ and Tissue Donation Service, Sydney, Australia; [5]Intensive Care Service, Royal Prince Alfred Hospital, Sydney, Australia; [6]Centre for Transplant and Renal Research, Westmead Hospital, Sydney, Australia; [7]Centre for Kidney Research, The Children's Hospital at Westmead, Sydney, Australia.

Introduction: The increase in organ donors in New South Wales (NSW) has been exceeded by a rise in the number of potential donors who do not proceed to donation. The overall burden of disease is an increasingly significant consideration when assessing the medical suitability of donor referrals. We sought to characterise the distribution of comorbidities among NSW organ donor referrals, and to explore changes over the past six years.

Materials and Methods: We undertook a cohort study of NSW Organ and Tissue Donation Service (OTDS) referral logs from 2010-2015 for the presence of donor comorbidities. We considered three groups of donors: potential donors (who were referred but did not proceed to donation), actual/intended donors after brain death (DBD), and actual/intended donors after circulatory death (DCD). Intended donors were those where donation was abandoned before organ retrieval, and actual donors those who proceeded to donate. We considered the presence or absence of ischaemic heart disease, non-ischaemic cardiovascular disease (e.g. cardiomyopathy, cardiac failure), cerebrovascular disease, chronic respiratory disease, chronic kidney disease, chronic liver disease, hypertension, diabetes and hyperlipidaemia. The overall burden of disease was estimated by summing the total number of comorbidities per referral. Non-parametric trend tests, logistic regression and a Kruskal-Wallis test were used to determine the significance of results between groups and over time.

Results: A total of 3005 referrals (2337 potential, 455 actual/intended DBD, 213 actual/intended DCD) between 2010-2015 were reviewed for the presence of comorbidities. The increase of donors with >2 comorbidities over this time was significant ($p<0.01$) for all groups of donors (figure 1). Potential donors had a greater comorbidity burden than both actual/intended donor groups ($p<0.01$). The comorbidities most strongly associated with not proceeding to donation were chronic kidney disease and non-ischaemic cardiovascular disease (figure 2). Conversely, hyperlipidaemia was associated with an increased likelihood of a donor referral proceeding to donation. However, this may reflect the more detailed reporting of the medical histories in actual and intended donor referrals, compared to those for potential donors. Our study was limited by an inability to easily differentiate comorbidity disease severity.

Figure 1: Number of comorbidities per organ donor referral over time, 2010-2015



Conclusion: Our results demonstrate that the distribution of comorbidities among NSW organ donors is changing. Of the comorbidities we considered, referrals with chronic kidney disease and non-ischaemic cardiovascular disease appeared least likely to proceed to donation. Overall, while donors with a greater burden of disease are increasingly being used for organ donation, an increasing proportion of donor referrals do not proceed for reasons of comorbidity.

Figure 2: Overall prevalence and time-trend of comorbidities among potential, actual/intended DBD, and actual/intended DCD organ donors

	Donor Type	N (of total 3005)	Odds Ratio (95%CI) *	P for change over time
Chronic Respiratory Disease	Potential	316	1.03 (0.78-1.36)	<0.001
	Actual/Intended DBD	46		0.95
	Actual/Intended DCD	29		0.88
Ischaemic Heart Disease	Potential	590	1.54 (1.21-1.96)	0.02
	Actual/Intended DBD	65		0.002
	Actual/Intended DCD	42		0.31
Non-Ischaemic Cardiovascular Disease	Potential	316	1.91 (1.47 - 2.47)	<0.001
	Actual/Intended DBD	55		0.001
	Actual/Intended DCD	30		0.005
Cerebrovascular Disease	Potential	959	0.91 (0.75-1.09)	0.005
	Actual/Intended DBD	226		0.24
	Actual/Intended DCD	74		0.37
Diabetes	Potential	400	1.34 (1.00-1.77)	<0.001
	Actual/Intended DBD	53		0.26
	Actual/Intended DCD	23		0.89
Hypertension	Potential	761	1.10 (0.87-1.38)	<0.001
	Actual/Intended DBD	143		<0.001
	Actual/Intended DCD	761		0.01
Hyperlipidaemia	Potential	296	0.54 (0.41-0.71)	<0.001
	Actual/Intended DBD	79		0.003
	Actual/Intended DCD	30		0.04
Chronic Liver Disease	Potential	121	1.69 (1.00-2.85)	<0.001
	Actual/Intended DBD	9		0.09
	Actual/Intended DCD	8		0.94
Chronic Kidney Disease	Potential	203	2.77 (1.60-4.79)	<0.001
	Actual/Intended DBD	11		0.21
	Actual/Intended DCD	4		0.63

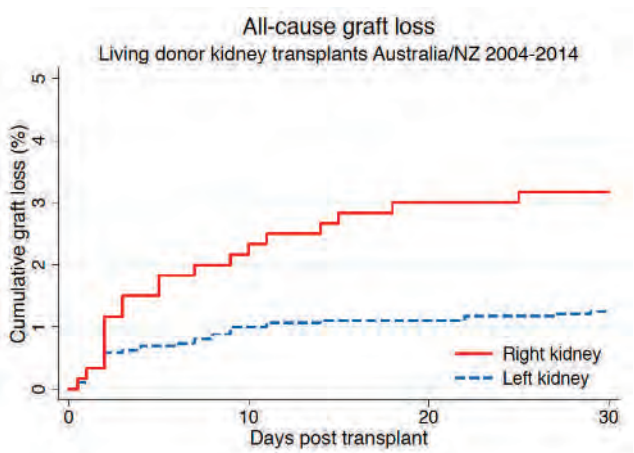
* Odds ratio was calculated as the likelihood of a donor referral not proceeding to donation given the presence of a particular comorbidity.

553.1

Adverse early outcomes for right-sided living donor kidney transplants

Deepak Chhabra[1,2], Philip Clayton[3], Arthur Vasilaras[1], Steven J.Chadban[1,2,3], Stephen McDonald[3], Henry Pleass[1,2], Richard Allen[1,2]. [1]Transplantation Services, Royal Prince Alfred Hospital, Sydney, Australia; [2]Sydney Medical School, University of Sydney, Sydney, Australia; [3]Australia and New Zealand Dialysis Transplant Registry, University of Adelaide, Adelaide, Australia.

Background: Kidney transplant surgeons prefer to transplant left rather than right-sided living donor (LD) kidneys because of the longer and more favourable left renal vein. Centre-specific data suggest that the results of right-sided LD kidney transplants may be inferior. We aimed to determine whether this is true for a large, multi-national cohort.
Methods: A retrospective analysis of all adult LD kidney transplants performed in Australia and New Zealand in the eleven-year period from 2004 to 2014 was undertaken to assess outcomes of left versus right LD kidneys. The data was reported prospectively to the Australia and New Zealand Dialysis and Transplant (ANZDATA) Registry. Cox proportional hazards models were used to examine the associations between kidney side and patient, graft and death-censored graft survival, adjusting for other donor, recipient and transplant characteristics.
Results: 23 kidney transplant centres performed 3315 living donor kidney transplants in adult recipients between 2004 and 2014. Of these, 600 (18.1%) involved use of a right-sided donor kidney. Higher volume transplant centres were more likely to undertake right-sided LD transplant procedures and 4 centres performed none. Donor nephrectomy was performed most commonly by pure laparoscopic surgery (54.1%), followed by hand-assisted laparoscopic surgery (33.8%) and open surgery (12.1%). Use of open nephrectomy was more common and laparoscopic nephrectomy less common for right-sided kidneys (p<0.001). Compared to recipients of left-sided LD kidneys, there were no significant differences in donor age or eGFR, or for recipient, race, primary renal disease, and degree of HLA mismatch or PRA for recipients of right-sided donor kidneys. All cause graft loss at 30 days was 3.2% (95%CI 2.0-4.9) versus 1.3% (0.9-1.8) for right and left LD kidneys respectively (adjusted HR=2.37, 95%CI 1.31-4.30, p=0.004) (Figure 1). Death-censored graft failure was also significantly greater in recipients of right-sided LD kidneys at 30 days after transplantation (HR 2.52, CI 1.34-4.76, p=0.004). After 30 days, there were no significant differences in all cause graft loss (HR=1.05, CI 0.81-1.36, p=0.72) or death-censored graft failure (HR=1.10, 95% CI 0.79-1.55, p=0.56). The overall uncensored graft survival for the 3315 LD kidneys at 5 years was 86.3% (CI 82.9-89.1) and 89.1% (CI 90.4-87.7) for right and left kidneys respectively.



Conclusions: This registry-based study demonstrates a small but significant inferior outcome for the transplantation of right living donor kidneys (p=0.004). This difference occurs only in the first 30 days after transplantation. Interpretation is limited by absence of data about reasons for transplant centres choosing right rather than left LD donor kidney, and, the presumed caution of others to limit or not use right-sided LD donor kidneys. Nevertheless, it is recommended that:
1. The patient consent process should include discussion about the increased risk of early adverse outcome for recipients of right-sided LD kidneys. There is a 1 in 50 greater chance of graft loss in the first 30 days.
2. When comparing outcomes LD kidney transplantation between different transplant centres, acknowledgement of the use of right-sided kidneys, or otherwise, should be made.

553.2

Global kidney exchange

M.A. Rees[1,3], T. Dunn[2], S. E.Rees[3], J. Rogers[4], L. J.Reece[3], A. E.Roth[5], C. Kuhr[6], O. Ekwenna[3], D. E.Fumo[1], K. Krawiec[7], S. Jain[1], C. Marsh[8], M. Tan[9], S. Paloyo[10].
[1]University of Toledo, Toledo, OH, United States; [2]University of Minnesota, Minneapolis, MN, United States; [3]Alliance for Paired Kidney Donation, Perrysburg, OH, United States; [4]Wake Forest, Winston-Salem, NC, United States; [5]Stanford University, Stanford, CA, United States; [6]Virginia Mason, Seattle, WA, United States; [7]Duke University, Durham, NC, United States; [8]Scripps Green Hosp, La Jolla, CA, United States; [9]Piedmont Hosp, Atlanta, GA, United States; [10]Phillipine Gen Hosp, Manila, Philippines.

Introduction: Instead of thinking of the developing world as a place where there are desperate people who will sell their kidneys for money, we propose a new approach where the developing world can be seen as a place where there are desperate patients with kidney failure who need kidney transplants and who have willing, living kidney donors, but insufficient financial resources to pay for their transplant and subsequent immunosuppression.
Methods: Kidney paired donation (KPD) is utilized to overcome barriers to transplantation due to immunological barriers such as blood type (BT) or donor specific antibodies, and also to optimize characteristics such as age or HLA-matching. We propose that the financial barrier faced by potential patient/donor pairs in developing countries could be another such barrier solvable by participation in KPD, when one considers the cost differential between dialysis and transplantation in the First World. By extending First World quality healthcare to impoverished patients in the developing world, we reverse the practice of transplant tourism and shed light and transparency on the black market organ trade by acknowledging that a kidney has financial value, while simultaneously protecting the fact that exchanging a kidney for a kidney transplantation for a desired patient is an altruistic gift and not a commercial exchange.
Results: A blood type (BT) O donor and a BT A, PRA 0% ESRD patient from the Philippines were unable to pay for dialysis or transplantation. A United States (US) non-profit paid for their evaluation and some dialysis in the Philippines. A nonsimultaneous extended altruistic donor (NEAD) chain was identified starting with a US BT A non-directed donor (NDD) with no match in the US KPD pool. The US NDD donated to the Filipino recipient, resulting in a BT O Filipino donor who simultaneously donated to continue the chain. To date the chain has resulted in eleven kidney transplants and an active bridge donor. Five recipients had the US government as their health insurance payer and five recipients had Commercial insurance. The transplant cost (including NDD nephrectomy and donor complication insurance) for the Filipino recipient was paid for by a US non-profit organization. An additional $50,000 was reserved for subsequent immunosuppression and donor/recipient follow-up in the Philippines. One year post-transplantation, the Filipino donor/recipient are doing well and $45,000 remains reserved for continuing care in the Philippines. The savings from transplanting 10 US patients compared with the cost of dialysis will exceed $3M over the next 5 years.
Conclusion: Global kidney exchange provides a unique solution to the lack of available donor kidneys. A very small fraction of the cost savings experienced by First World payers could be gifted to establish renewable funds for future efforts, in turn simultaneously enhancing further cost savings and the health of kidney donors and recipients in our global community.

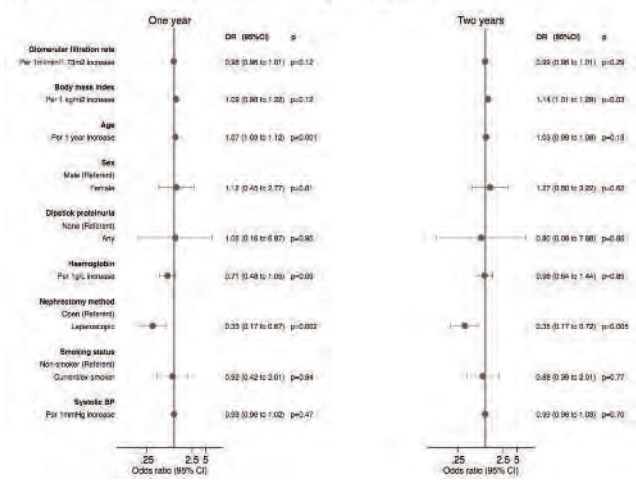
553.3

Chronic kidney disease among living kidney donors: a cohort study

Philip Masson, Lorna KHenderson, Alex Walker, Gabriel Oniscu.
Department of Renal Medicine and Renal Transplantation, Royal Infirmary of Edinburgh, Edinburgh, United Kingdom.

Background and Aims: How living kidney donation affects glomerular filtration rate (GFR) and proteinuria after donation is unclear. We aimed to identify risk factors for chronic kidney disease (CKD) among living kidney donors.
Methods: We performed a retrospective cohort study of all living kidney donors at the Royal Infirmary of Edinburgh. Demographic and clinical information at the time of kidney donation were recorded, along with GFR and urinary dipstick findings at one and two years following kidney donation. We calculated the risks of developing either CKD stage ≥3 or new dipstick proteinuria associated with specific baseline characteristics using multivariate-adjusted logistic regression models. Results were expressed as odds ratios (OR) with their 95% confidence intervals (CI).
Results: 376 people (52% female and 48% male) donated a kidney between 1993 and 2015. Mean age at donation was 49.5 years (SD, standard deviation 11.1) and mean GFR was 96.1ml/min/1.73m2 (SD 15.1). Only 12 donors (3.2%) had dipstick proteinuria at the time of kidney donation. By one year after donation 126 (34%) of donors had CKD ≥3. With each year of advancing age at the time of donation, people were 7% more likely to have CKD ≥3 (OR 1.07, CI 1.03 to 1.12). Donors undergoing laparoscopic nephrectomy were 67% less likely to have CKD ≥3 than donors undergoing an open nephrectomy (OR 0.33, CI 0.17 to 0.87) (**Figure 1**). By two years after donation, people with a higher body mass index (BMI) at the time of donation were more likely to have CKD ≥3, each 1kg/m2 increase in BMI being associated with a 14% increased risk (OR 1.14, CI 1.01 to 1.29). Donors undergoing a laparoscopic nephrectomy still had a 65% lower risk of CKD ≥3 than donors undergoing an open nephrectomy (OR 0.35, CI 0.17 to 0.72). Considering proteinuria, by one year after donation 36 (10%) of donors had dipstick proteinuria ≥ trace. People with a higher BMI at donation were more likely to have proteinuria, with each 1kg/m2 increase in BMI being associated with a 28% increased risk of proteinuria (OR 1.28, CI 1.09 to 1.53). Neither GFR nor systolic blood pressure at donation were associated with the risk of developing either CKD ≥3 or proteinuria at any time during follow-up.

Figure 1. Risk factors for living kidney donors having CKD stage ≥ 3



Conclusions: Our main novel finding was that living kidney donors undergoing laparoscopic nephrectomy were less likely to develop CKD

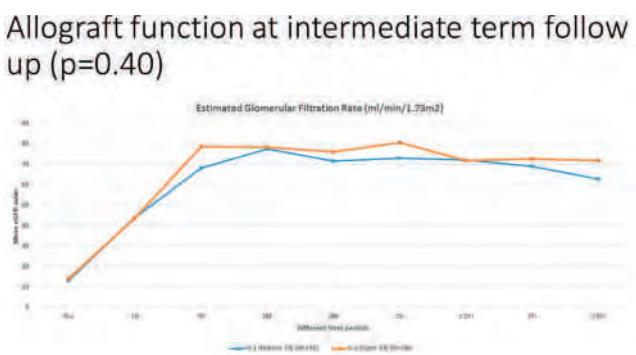
≥3 during follow-up than people undergoing open nephrectomy. This study should serve to generate hypotheses about possible mechanisms by which surgical technique might affect long term kidney outcomes for living kidney donors.

553.4

Technique and outcome of robotic kidney transplantation in 161 adult recipients

Pranjal Modi[1,2], Bipin Pal[1,2], Suresh Kumar[1,2], Yusuf Saifee[1,2], Ramya Nagarajan[1,2], Rohit Chauhan[1,2], Vivek Kute[1,2], Himanshu Patel[1,2], Manisha Modi[1,2], Veena Shah[1,2], Hargovind L.Trivedi[1,2].
[1]Transplantation Surgery and Urology, Smt G R Doshi and Smt K M Mehta Institute of Kidney Diseases and Research Centre, Ahmedabad, India; [2]Transplantation Surgery and Urology, Dr. H.L. Trivedi Institute of Transplantation Sciences, Ahmedabad, India.

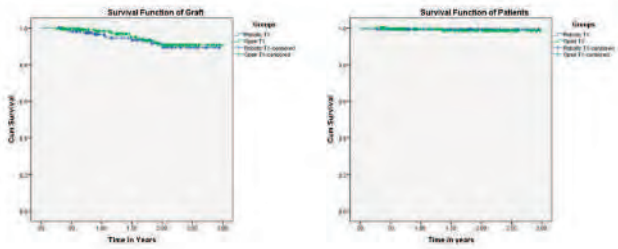
Introduction: Robotic kidney transplantation (RKT) is emerging new modality of treatment for kidney recipient with intention to minimize the wound related morbidity in recipients[1],[2]. Here we present technique and outcome of RKT at a single surgical unit.
Materials and Methods: With permission of Internal Review Board, total 180 RKT and 243 open kidney transplants (OKT) in adult patients were performed between January 2013 and October 2015 at single surgical unit. In 19 female recipients different method of RKT was used and hence excluded from analysis. Deceased donor kidney was used in 21 and 47 cases of RKT and OKT respectively. For RKT, a 6 cm Pfannenstiel incision was placed to insert the kidney in the abdomen and da vinci(R) surgical platform was used for vascular anastomosis and ureteric reimplantation. Regional hypothermia was used in initial 68 cases of RKT. Primary end point was to evaluate estimated glomerular filtration rate (eGFR), graft and patient survival at 12 months. Tacrolimus based triple immunosuppressant regime was used in all patients.
Results: There was no difference in demography in either group. All but four patients in RKT group were transplanted successfully. Intra-operative bleeding from vein and thrombosis of artery in two cases each required conversion to open surgery. Anastomosis time and total operative time were 34.92±8.28 min. and 218.09±48.61 min. for RKT and, 31.52±13.54 and 199.52±69.38 min. for OKT respectively ($p<0.01$). Ureteric reimplantation time (33.30±8.85 min for RKT and 40.63±10.79 min. in OKT, $p<0.01$) and estimated blood loss (58.56±89.36 ml for RKT, 112.23±69.38 ml for OKT, $p<0.001$) were lower in RKT. Mean analgesic requirement was 2.5 mg and 5.6 mg morphine equivalent ($p<0.01$) in RKT and OKT group respectively. There was no difference in time to resume orally, time to ambulation, time to catheter removal and total hospitalization in either group. No lymphocele vs. 14 lymphocele were observed in RKT and OKT group respectively; one required laparoscopic defoofing. Four cases in OKT required re-exploration for perigraft hematoma. One case in RKT group required laparoscopy for renal allograft biopsy. There was no statistically significant difference in eGFR value and graft and patient survival in RKT and OKT group. Fig. 1 and 2 shows eGFR, graft and patient survival in RKT and OKT group having living donor.



On subgroup analysis, eGFR, graft and patient survival were not statistically different in patients with and without regional hypothermia.

Conclusion: RKT is having advantage over OKT in terms of less pain, early ambulation, no lymphocele or peri-graft collection. eGFR, graft and patient survival at one year are similar in both groups.
Sarita Tapodhan; Anal Mehta

Graft and patient survival



References:
[1] Oberholzer J, Giulianotti P, Danielson KK, Spaggiari M, Bejarano-Pineda L, Bianco F, Tzvetanov I, Ayloo S, Jeon H, Garcia-Roca R, Thielke J, Tang I, Akkina S, Becker B, Kinzer K, Patel A, Benedetti E. Minimally invasive robotic kidney transplantation for obese patients previously denied access to transplantation. Am J Transplant. 2013 Mar;13(3):721-8. doi: 10.1111/ajt.12078
[2] Menon M, Sood A, Bhandari M, Kher V, Ghosh P, Abaza R, Jeong W, Ghani KR, Kumar RK, Modi P, Ahlawat R. Robotic kidney transplantation with regional hypothermia: a step-by-step description of the Vattikuti Urology Institute-Medanta technique (IDEAL phase 2a). Eur Urol. 2014 May;65(5):991-1000. doi: 10.1016/j.eururo.2013.12.006.

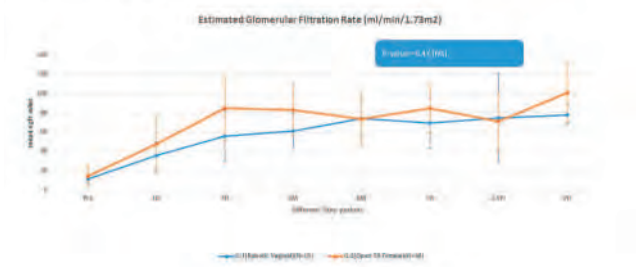
553.5

Transvaginal insertion of kidney and robotic kidney transplantation: First 19 cases

Pranjal Modi[1,2], Bipin Pal[1,2], Suresh Kumar[1,2], Yusuf Saifee[1,2], Ramya Nagarajan[1,2], Rohit Chauhan[1,2], Manisha Modi[1,2], Veena Shah[1,2], Vivek Kute[1,2], Himanshu Patel[1,2], Hargovind L.Trivedi[1,2].
[1]Transplantation Surgery and Urology, Smt G R Doshi and Smt K M Mehta Institute of Kidney Diseases and Research Centre, Ahmedabad, India; [2]Transplantation Surgery and Urology, Dr. H.L. Trivedi Institute of Transplantation Sciences, Ahmedabad, India.

Introduction: Robotic kidney transplantation (RKT) is emerging new modality of treatment for kidney recipient with intention to minimize the wound related morbidity in recipients. Feasibility and safety of kidney insertion through vagina and carrying out laparoscopic kidney transplantation has been demonstrated by our group[1]. We and others have demonstrated feasibility of transvaginal insertion of kidney and robotic kidney transplantation (V-RKT)[2],[3]. Here we present technical modifications and outcome of V-RKT.
Materials and Methods: With permission of Internal Review Board, 19 female recipients underwent V-RKT and 39 females had open kidney transplantation (OKT) between July 2013 and October 2015. Patient inclusion criteria were history of at least one normal vaginal delivery, capacious vagina on physical examination and consent for episiotomy/or insertion of kidney through abdomen. Exclusion criteria were nulliparous woman, atrophic vagina and previous hysterectomy associated with extensive intra-abdominal adhesions. Antibiotic prophylaxis (Inj Cefazolin 1.0 gm and Inj. Metronidazole 500 mg iv) was given for three days in all recipients along with povidone iodine vaginal pessary. For V-RKT, patient was placed in 300 Trendelenburg positon with both legs spreaded. Da Vinci Si(R) surgical platform was used. Following preparation of external iliac vessels for transplant, an incision is placed on the posterior vaginal wall. The assistant surgeon inserts the kidney through the vagina and close the vagina. The console transplant the kidney with external iliac vessels. Ureteroneocystostomy was done by Lich's method. The primary end point was to evaluate feasibility and safety of the procedure. The secondary end point was to evaluate estimated GFR and one year graft and patient survival.
Results: There was no difference in demography. Vascular anastomosis time, ureteric reimplantation time and total operative time were 32.74±5.67 min. and 32.38±12.08 min., 34.47±8.24 min. and 43.36±9.81 min., and, 237.37±64.88 min. ($p<0.01$) and 205.51±33.63 min. respective in V-RKT and OKT groups respectively. Mean analgesic requirement was 0.8 mg Vs. 3.2 mg of morphine equivalent ($p<0.01$) and Mean time to resume fluid orally was 9.2 hours and 14.12 hours ($p<0.01$) in V-RKT and OKT group respectively. There was no statistically significant difference between time for ambulation, time for catheter removal and total hospital stay. Sexual function was resumed after 30 days of transplantation by all recipients. No difference in eGFR ($p= 0.47$) in either group (Figure-1) with 100% graft and patient survival till the last follow up.

Estimated Glomerular Filtration Rate



Conclusion: V-RKT is feasible and safe in selected group of female recipients. It is associated with less pain. eGFR, graft and patient survival are similar in V-RKT and OKT group.

Sarita Tapodhan; Anal Mehta

References:

[1] Modi P, Pal B, Kumar S, Modi J, Saifee Y, Nagaraj R, Qadri J, Sharmah A, Agrawal R, Modi M, Shah V, Kute V, Trivedi H. Laparoscopic transplantation following transvaginal insertion of the kidney: description of technique and outcome. Am J Transplant 2015;15(7):1915-22

[2] Modi P, Pal B, Modi J et al. Vaginal Insertion of Kidney and Robotic Assisted Laparoscopic Kidney Transplantation: The First World Experience. Am J Transplant. 2014;14 Suppl 3: 15

[3] Doumerc N, Roumiguie M, Rischmann P, Sallusto F. Totally robotic approach with transvaginal insertion for kidney transplantation. Eur Urol 2015;68(6):1103-4

553.6

Preliminary results from a pilot study utilizing ERAS protocol in living donor nephrectomy

Aparna Rege[1], Jayanth Reddy[1], Deepak Vikraman[1], William Irish[2], Debra Sudan[1], Ahmad Zaroura[1], Todd Brennan[1], Kadiyala Ravindra[1]. [1]Surgery, Div of Abdominal Transplant, Duke University medical center, Durham, United States; [2]Health Outcomes Research & Biostatistics, CTI Clinical Trial and Consulting, Durham, United States.

Background and Introduction: Gastrointestinal (GI) recovery after major abdominal surgery can be delayed from ongoing need for narcotic analgesia thereby prolonging hospitalization. Enhanced recovery after surgery (ERAS) is a multimodal perioperative care pathway designed to facilitate early recovery after major surgery while showing equivalent morbidity, mortality and readmission rates in comparison to the traditional standard of care[1]-[3]. Laparoscopic living donor nephrectomy has significantly transformed the outlook for individuals considering kidney donation[4]. However, a 30% rate of Emergency Room visits / readmission was recorded at our center in 2014 largely from delayed GI recovery. Thus, a pilot trial to utilize ERAS protocols in living kidney donors was initiated.

Methods: This is a single-center retrospective analysis comparing the outcomes of the first 14 live kidney donors subjected to laparoscopic nephrectomy with ERAS protocol to 18 donors operated prior to ERAS with traditional standard of care. Both groups were matched by patient demographics. Our ERP includes reduced duration of fasting with preoperative carbohydrate loading, intraoperative fluid restriction to 3ml/kg/hr, target urine output of 0.5 ml/kg/hr, use of sub fascial Exparel injection(Bupivacaine liposome suspension) and postoperative narcotic free pain regimen with Acetaminophen, ketorolac, tramadol.

Results: ERAS protocol reduced postoperative median length of stay decreased from 2.0 to 1.0 days (P 0.001). Overall pain scores were significantly lower in the ERAS group (peak pain score 6.50 vs 9.00 - p 0.001, morning after surgery pain score 3.00 vs 7.00 - p 0.012) despite absence of narcotics in the postoperative period. Average duration of surgery was shorter with the ERP as compared to the standard protocol (241 vs 277 min - p 0.019). Average amount of intraoperative fluid used was significantly lower in the ERAS group in comparison to standard of care protocol (2000ml vs 3000ml, p 0.002), without affecting the donor urine output intraoperatively or the percent change in donor serum creatinine on postop day 1(70 vs 77, p 1.000). Incidence of delayed graft function was similar in the two groups (2 vs 1, p 0.597). A trend towards lower readmission was noted with the ERAS protocol. (2 vs 4, p 0.656). GI dysfunction was the most common reason for readmission.

Conclusion: Application of ERAS protocol in laparoscopic living donor nephrectomy was associated with reduced length of hospitalization. Improved pain scores resulted from intraoperative use of sub fascial Exparel and shorter duration of ileus. Optimizing intraoperative fluids minimized excessive third spacing & bowel edema reducing operative times and facilitating gut recovery without adversely impacting recipient graft function. This study suggests that ERAS has the potential to enhance the advantages of laparoscopic surgery for live kidney donation through optimizing donor outcomes and perioperative patient satisfaction and may further incentivize population for undergoing laparoscopic live kidney donation.

References:

[1] 1. Wind J, Polle SW, Fung Kon Jin PH, et al; Laparoscopy and/or Fast Track Multimodal Management Versus Standard Care (LAFA) Study Group; Enhanced Recovery after Surgery (ERAS) Group. Systematic review of enhanced recovery programmes in colonic surgery. *Br J Surg. 2006.* ; 93:800-809.

[2] Lassen K, Soop M, Nygren J, et al. Consensus review of optimal perioperative care in colorectal surgery: enhanced recovery after surgery (ERAS) Group recommendations. *Arch Surg 2009*; 144:961–9.

[3] Greco M, Capretti G, Beretta L, Gemma M, Pecorelli N, Braga M. Enhanced recovery program in colorectal surgery: a meta-analysis of randomized controlled trials. *World J Surg 2014*;38:1531.
[4] Ratner LE, Hiller J, Sroka M, et al. Laparoscopic live donor nephrectomy removes disincentives to live donation. *Transplant Proc 1997*;29:3402.

553.7

Obesity is associated with increased risk of ESRD among living kidney donors

JaymeE. Locke[1], Cora E.Lewis[1], Rhiannon D.Reed[1], Vineeta Kumar[1], Deirdre Sawinski[2], Allan Massie[3], Paul A.MacLennan[1], Roslyn B.Mannon[1], Robert Gaston[1], Dorry L.Segev[3].
[1]University of Alabama at Birmingham, Birmingham, AL, United States; [2]University of Pennsylvania, Philadelphia, PA, United States; [3]Johns Hopkins University, Baltimore, MD, United States.

Background: Living kidney donor (LKD) demographics have changed, and transplant centers have relaxed selection criteria to include LKDs who are obese. Prior studies of LKDs have failed to stratify donors by the presence of obesity. The goal of this study was to assess the risk of ESRD among LKDs with obesity at the time of donation.
Methods: 119,769 LKDs in the US (1988-2013) were studied. Obese LKDs were defined as donors with body mass index (BMI) ≥ 30kg/m2 at the time of donation. Donors were followed until the earliest of ESRD development, death, or administrative end of study. Multiple imputation using chained equations were used to impute BMI, systolic and diastolic blood pressure, relationship to recipient, eGFR, and smoking and insurance status when missing. Maximum follow-up was 20 years. Main outcome measures included cumulative incidence and adjusted risk of ESRD (adjusted for age, race, blood pressure, gender, eGFR, and relationship to recipient).
Results: Compared to non-obese LKDs, obese LKDs were more likely male, African American, and to have higher blood pressure (Table). Estimated risk of ESRD at 20 years after donation was 93.9 per 10,000 for obese LKDs and 39.7 per 10,000 in their non-obese LKD counterparts.

Table 1. Patient characteristics by obesity status at donation (N= 119,769)

	BMI ≥ 30 (N=29,575)	BMI < 30 (N=90,194)
	%	%
Age at donation, Mean (SD)	40.2 (10.8)	39.9 (11.3)
Female	55.1	60.0
Race		
African American	16.8	11.5
Non-African American	83.2	88.5
History of smoking	20.7	20.2
Insurance at donation	81.8	82.2
Systolic BP, Mean (SD)	124.9 (13.2)	120.8 (13.4)
Diastolic BP, Mean (SD)	76.3 (9.4)	73.7 (9.3)
BMI, Mean (SD)	33.7 (2.6)	24.8 (3.1)
eGFR, Mean (SD)	98.2 (19.5)	99.5 (19.4)
Related to recipient	81.4	81.9

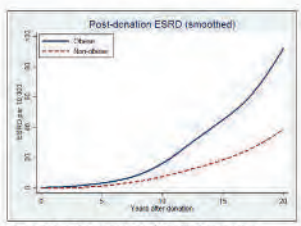


Figure 1. Cumulative incidence of ESRD by obesity status

On unadjusted analyses, LKDs with obesity had a 2.5-fold increased risk of ESRD compared to their non-obese LKD counterparts. After adjustment, the risk of ESRD among LKDs with obesity was 1.95-fold higher than non-obese LKDs (adjusted hazard ratio (aHR): 1.95, 95%CI: 1.37-2.78, $p < 0.001$). For each one unit increase in BMI there was an associated 8% increase in the risk of ESRD (aHR: 1.08, 95% CI: 1.05-1.12, $p < 0.001$).
Conclusions: Compared with non-obese LKDs, LKDs who are obese at the time of donation had an increased risk of ESRD within 20 years of donation. These findings may help to inform selection criteria and discussions with persons considering live kidney donation.

553.8

What distinguishes donors from non-donors? Analysis of 2306 potential living kidney donors

Hillary Braun[1], John Feiner[2], Garrett R.Roll[3].
[1]School of Medicine, University of California, San Francisco, San Francisco, CA, United States; [2]Department of Anesthesia, University of California, San Francisco, San Francisco, CA, United States; [3]Department of Surgery, University of California, San Francisco, San Francisco, CA, United States.

Introduction: UCSF implemented an online donor screening system to streamline the initial evaluation process for potential living donors in 2013 and make it easier for patients to successfully interact with us. A validated[1],[2] web-based software platform (BREEZE TRANSPLANT , MedSleuth, Inc.) performs automated pre-donation screening and post-donation monitoring of living donors. This software allows for a rapid and robust data collection, including comparison of characteristics between patients that eventually went on to become donors and those that did not. Here we present data collected during the first three years of this online system.
Methods: Potential living kidney donors initiated the process by completing an online questionnaire using the web-based system, Breeze (MedSleuth, San Francisco, CA). The questionnaire gathered basic demographics including age, gender, ethnicity, and relationship to intended recipient as well as components of the respondent's medical history that might affect donation including BMI, activity level, medical comorbidities, and substance use. Descriptive data was calculated as mean ± SD and n (%). Continuous data was compared with Wilcoxon rank sum test and proportions were compared with Chi square or Fisher's exact test.
Results: 2306 potential living kidney donors completed the screening form between 2013-2015, of which 11.9% (275/2306) went on to successfully donate. The average age was significantly greater among donors compared with non-donors (42.9 ± 12.2 vs. 40.8 ± 13.7, p=0.008) and donors were more likely to be married compared with non-donors (62.8% vs. 50.0%, p=0.001). Relationship to the recipient was also significant; 71.2% of donors were blood relatives with the recipient compared with 53.9% of non-donors (p<0.001). A greater majority of donors denied any history of tobacco use compared with non-donors (82.3% vs. 68%, p<0.001). Age, blood relationship, and amount of calculated exercise were significantly associated with successful donation (OR 1.01, 2.03, 1.14, respectively, p-values <0.05).
Discussion: Screening potential donors with a validated online system has enabled robust data collection and analysis, especially with regard to understanding what donor characteristics predict successful donation. Age, marital status, tobacco use, and activity level were the significant differences we observed between patients who went on to be selected as donors and those that were not. Interestingly, these variables have not historically been shown to correlate with differences in graft and recipient outcome.
Conclusion: Age, marital status, tobacco use, and activity level help predict who is likely to be selected as kidney donor and who is not. These variables can be weighted for use in web-based software algorithms to improve our donor selection efficiency.
References:
[1] A851, ASA 2011, USA
[2] S104, IARS 2011, Canada

553.9

Establishing a learning curve for laparoscopic living donor nephrectomy

Zubir Ahmed, Pankaj Chandak, Raphael Uwechue, Nicos Kessaris, NIzam Mamode.
Transplantation, Guy's Hospital, London, United Kingdom.

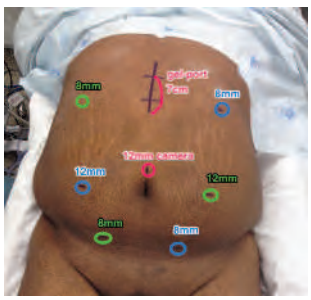
Introduction: Despite over 5000 hand assisted laparoscopic donor nephrectomies (HALDNs) having been performed in the UK a paucity of data still exists in establishing how many procedures are required in order to safely ascend the learning curve. The production of such parameters is essential to the development of appraisal, safety and training programmes in this expanding surgical programme within transplantation. Recent data from other equivalent surgical procedures suggests both intraoperative and postoperative outcome data parameters can be used to establish the learning curve.
Methods: The caseload of two surgeons who had individually performed over 180 HALDNS each was interrogated. Using cumulative sum analysis (CUSUM), operating time, hospital stay, the occurrence of major and minor complications and the need for readmission or reoperation were assessed. The learning curve was analysed using graphical representations to detect an inflexion point which would represent a stability of process. The number of procedures required to arrive at this point was assumed to represent successful ascent of the learning curve. Statistical analysis using the Pr > zL statistic was also used to quantify whether such a stability of process had been achieved.
Results: Surgeons 1 and 2 performed 189 and 183 cases over an 8 year period. All were intraperitoneal using a standardised technique. Patient demographics between the 2 surgical caseloads were similar (% female: 51 v 52%, mean age 44.1 v 44.7 yrs, mean BMI 26.5 v 27.2, % left sided cases 80 v 85%). CUSUM analysis revealed no discernible inflexion points for hospital stay (zL = 0.3 p=0.07), occurrence of clavien 2 and above complications (zL =0.84, p=0.337), readmission (zL=0.696 p=0.243) or reoperation (zL= -0.366 p=0.643). Operating time however demonstrated a visible stability of process initially at case 25 but this was more sustained by case 40 to 45 for both surgeons.
Discussion: A learning curve can be reproducibly established for HALDN using operating time as a surrogate marker. Contrary to popular belief true ascent of the learning curve may mean the performance of up to 50 procedures rather than 20 – 25. In our series the occurrence of stability in operating times at case 25 was likely biased by careful patient selection and hence by the stability occurring by case 50 is representative of normal surgical casemix. We anticipate this data will better inform the development of surgical training programmes in HALDN.

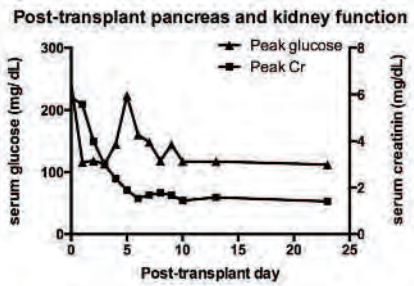
554.1

Robotic simultaneous pancreas kidney transplantation in obese recipient

Chun Chieh Yeh[1,2], Mario Spaggiari[2], Jose Oberholzer[2].
[1]Department of Surgery, China Medical University Hospital, Taichung, Taiwan; [2]Division of Transplant, University of Illinois at Chicago, Chicago, United States.

Introduction: Robotic simultaneous pancreas kidney transplantation in obese recipients has never been reported before.
Materials and Methods: Herein, we report a laparoscopic robot-assisted simultaneous pancreas kidney transplantation in a patient with class I obesity and diabetic nephropathy. This 49-year-old, insulin demanding diabetes complicated with end stage renal failure, African American male patient with a BMI of 34(kg/m2) was listed for simultaneous pancreas kidney transplantation. The pancreas and kidney graft were procured and benched in standard way. Methylene blue mixed in University of Wisconsin (UW) solution was used to search for any potential vascular leaks on the back table. The laparoscopic, robot-assisted pancreas transplantation was completed via two 12mm ports (one for laparoscopic bed-side assistance and the other for the camera), two 8mm ports for the robotic arms, and a hand port on a 7cm epigastric midline incision (Fig.1, blue marking).
Results: The arterial Y graft and portal vein of the pancreas were anastomosed to the recipient's left external iliac artery and vein, respectively. Enteric drainage was performed between the duodenum of graft and recipient's distal jejunum with a circular stapler (EEA 21/4.8mm). Duration of warm and cold ischemia in pancreas transplantation was 54min and 8.7hr, respectively. The robotic kidney transplantation was performed in similar approaching but with reversed port site positioning (Fig.1, green marking). The kidney graft was implanted to right external iliac artery and vein. Duration of warm and cold ischemia in kidney transplantation was 31min and 12hr, respectively. The whole procedure was recorded (surgical video clip). The patient was discharged uneventfully on day 11 without any wound related complications. Appropriate short-term metabolic control was achieved with achieving euglycemia without insulin supplement before discharge (Fig.2).
Conclusion: Robotic simultaneous pancreas kidney transplantation can be successfully applied in patients with obesity without compromising safety and short-term metabolic outcome.





Dr. Enrico Benedetti

554.2

Tips for a successful normothermic liver perfusion

David Nasralla[1], Hynek Mergental[2], Thamara Perera[2], Wayel Jassem[3], Annemarie Weissenbacher[1], Carlo Ceresa[1], Chris Morris[5], Les Russell[5], Constantin Coussios[4], Peter Friend[1].
[1]Nuffield Department of Surgical Sciences, University of Oxford, Oxford Transplant Centre, Oxford, United Kingdom; [2]Liver Transplant Surgery, Queen Elizabeth Hospital, Birmingham, United Kingdom; [3]Liver Transplant Surgery, King's College Hospital, London, United Kingdom; [4]Institute of Biomedical Engineering, University of Oxford, Oxford, United Kingdom; [5]OrganOx Ltd, OrganOx Ltd, Oxford, United Kingdom.

Normothermic Machine Perfusion (NMP) of the liver offers a potential solution to the global organ shortage crisis. Our group in Oxford have now perfused over 150 human livers that have gone on to be transplanted.
Meticulous surgical technique is required during preparation and cannulation of the liver if NMP is to be performed successfully.
We illustrate the process of connecting the liver to the OrganOx *metra*, achieving haemostasis during NMP, and give examples of common challenges encountered and how they are managed.

554.3

Contrast-enhanced intraoperative ultrasonic cholangiography in living donor hepatectomy

Takeshi Urade[1,2], Takumi Fukumoto[1], Masahiro Kido[1], Atsushi Takebe[1], Motofumi Tanaka[1], Kaori Kuramitsu[1], Hisoka Kinoshita[1], Shinichi So[1], Toshihiko Yoshida[1], Keisuke Arai[1], Daisuke Kuroda[2], Yonson Ku[1].
[1]Hepato-Biliary-Pancreatic Surgery, Kobe University, Kobe, Japan; [2]Surgery, Kita-harima Medical Center, Ono, Japan.

Introduction: Radiographic intraoperative cholangiography (IOC) has been widely used to reduce the biliary complications in living donor hepatectomy (LDH), but it has radiation exposure and difficulty with handling a C-arm machine, generating 3D images and delineating thin caudate branches. Recently, we have established a novel IOC technique, contrast-enhanced intraoperative ultrasonic cholangiography (CE-IOUSC) as a tool for real-time biliary navigation in hepatobiliary surgery[1],[2]. In this video, we demonstrate the usefulness of CE-IOUSC compared with radiographic IOC in LDH.
Materials and Methods: CE-IOUSC was performed using an AplioXG ultrasound imaging system with a 4D probe, a T-shaped linear probe and a micro-convex probe (Toshiba Medical Systems). After temporary clamping of the common bile duct, diluted ultrasound contrast agent Sonazoid (perfluorobutane) was injected via a 4Fr transcystic catheter. CE-IOUSC was performed followed by radiographic IOC before hepatectomy, before bile duct division, and after purocurement of the graft. We describe the case of a 29-year-old woman who was scheduled to undergo extended left hepatectomy as donor using CE-IOUSC and radiographic IOC.
Results: 3D CE-IOUSC could detect accurate biliary configuration as well as radiographic IOC. 2D CE-IOUSC could help us to determine an optimal cutting point of the hepatic duct at the hepatic hilum. In addition, 2D CE-IOUSC on the cut surface showed the patency of the hepatic duct in the remnant liver without biliary stricture. The postoperative course was uneventful in the donor and the recipient.
Discussion: CE-IOUSC can facilitate the surgeon's understanding of the biliary configurations with 3D mapping and 2D regional anatomy of biliary tree to reduce the potential risk of bile duct injury. In particular, 2D-CEIOUSC can facilitate understanding the spatial relationships between bile ducts and liver parenchyma simultaneously as opposed to radiographic IOC. CE-IOUSC is relatively safe without radiation exposure.
Conclusions: CE-IOUSC is a novel IOC technique that provides excellent visualization of the biliary tree for LDH. This biliary navigation technique facilitates identification of the biliary system, guidance for bile duct division and confirmation of the remnant biliary system.
References:
[1] Urade T, et al. Contrast-enhanced intraoperative ultrasonic cholangiography for real-time biliary navigation in hepatobiliary surgery. *J Am Coll Surg*. 2014;218(2):e43-50.
[2] Fukumoto, et al. A Novel Technique for the Intraoperative Identification of Biliary Drainage Areas in the Liver after Hepatobiliary Resection for Perihilar Cholangiocarcinoma. *J Am Coll Surg*. 2016 in press.

554.4

Total artificial heart bridge to combined heart-liver transplantation for transthyretin amyloidosis

Amit Sharma, Adrian Cotterell, Daniel Tang, Chandra S.Bhati, Keyur Shah, Richard Stravitz, Marlon Levy, Vigneshwar Kasirajan.
Surgery, Virginia Commonwealth University, Richmond, VA, United States.

Introduction: Transthyretin amyloidosis (ATTR) associated cardiomyopathy is due to cardiac deposition of mutant transthyretin protein produced by the liver. In patients with severe cardiomyopathy and limited other organ involvement, combined heart-liver transplantation (HLT) is the preferred treatment. Due to limitations in available donor organs, many patients in need of a heart transplant require bridging with mechanical circulatory support. The vast majority is supported with left ventricular assist devices (LVAD). However, the restrictive cardiomyopathy associated with ATTR is anatomically suboptimal for LVAD support. We share our experience of four cases of combined HLT for ATTR. This video demonstrates one of the recipients' who was bridged with a total artificial heart (TAH) before under going HLT.
Materials and Methods: We retrospectively analyzed our center's databank for patients who received a combined heart-liver transplantation (2013-present). Primary outcomes included patient and graft survival. Secondary outcomes included allograft rejections and post-operative complications. Informed consent was obtained from all recipients as per institutional guidelines.
Results: Four patients (Table 1) received a combined heart-liver transplantation for ATTR associated cardiomyopathy. Two patients were bridged with TAH. The most common mutation in our patients was Ile122 (75% patients). One patient experienced renal failure requiring hemodialysis post-transplant. All four patients are alive with excellent cardiac and liver allograft function. None of the patients experienced any rejection episodes.
Heart-Liver Transplantation for ATTR Cardiomyopathy: Single center experience (2013-present)
Age/SexMutationPre-Tx Mechn. SupportDomino Liver TxFollow up (mo)ComplicationsRejection147 MAsp18AsnNoneYes63Ventral herniaNone257 FIle122NoneYes48Renal failureNone354 MIle122Total artificial heartNo38NoneNone461 MIle122Total artificial heartNo3Groin abscessNone
Discussion of Case Video Summary: 61 M (patient #4) with transthyretin amyloidosis (Ile122 mutation) presented with symptomatic cardiomyopathy. He underwent multidisciplinary evaluation and was listed for combined heartliver transplantation. He progressed to severe decompensated heart failure despite inotropes and underwent TAH implant as a bridge to transplant. He was discharged home with the TAH on a portable driver. After 109 days of TAH support, he matched to a brain dead deceased donor and successfully underwent combined heart-liver transplantation.
Conclusions: Combined heart-liver transplantation is the preferred treatment for ATTR associated cardiomyopathy. These patients have unique considerations that require multidisciplinary planning and coordination in the pre-, intra-, and post-operative phases for successful outcomes.
Joe Morris for video editing
References:
[1] Nelson LM, et al. Long-term outcome in patients treated with combined heart and liver transplantation for familial amyloidotic cardiomyopathy. *Clin Transplant*. 2013;27(2):203-9.
[2] Reich HJ, et al. Combined Heart and Liver Transplantation: The Cedars-Sinai Experience. *Transplant Proc*. 2015 Nov;47(9):2722-6.
[3] Raichlin E, et al. Combined heart and liver transplantation: a single-center experience.*Transplantation*. 2009 Jul 27;88(2):219-25.

554.5

Robotic assisted kidney transplantation

Volkan V.Tugcu[1], SelcukS. Sahin[1], Abdullah Yavuzsan[1], Fatih Gokhan F.G.Akbay[2], Serdar S.Karadag[1], Ahmet Faysal A.F.Guler[1], Suheyla S.Apaydin[2].
[1]Urology Clinic, Bakirkoy Dr. Sadi Konuk Training and Research Hospital, Istanbul, Turkey; [2]Nephrology Clinic, Bakirkoy Dr. Sadi Konuk Training and Research Hospital, Istanbul, Turkey.

Purpose: Kidney transplantation is the best treatment of patients with end-stage renal disease. While open surgery remains the gold standard, minimally invasive surgery has recently been introduced for the recipient undergoing kidney transplantation. We present in this video kidney transplantation using da Vinci-Xi robotic system in our clinic.
Material and Methods: A left live donor kidney was transplanted into a 43-year-old male patient who had underwent hemodialysis for 2 months. Surgery was done with the help of the da Vinci-Xi robotic system. A GelPOINT access port was placed within a 4 cm periumblikal incision for delivery of ice-slush and introduction of the graft kidney. Vascular dissection and anastomosis as well as ureterovesical anastomosis was performed completely using robotic system.
Results: Operation time (incision to closure) was 230 minutes. Total blood loss was 170cc. There wasn't any perioperative or postoperative complications. The uretral catheter and 2 drains withdrawed at 3th day of the operation and the patient was discharged at 5th day of the operation.
Conclusion: Robotic assistance made anastomosis possible by its unique ability of stereoscopic magnification and ultra-precise suturing techniques due to the flexibility of the robotic wristed instruments. Robotic kidney transplantation(RKT) is a safe and feasible surgical alternative to the standard open approach of kidney transplantation. RKT is associated with reduced postoperative pain, analgesic requirement, and better cosmesis.

554.6

Reno- and splenoportal anastomosis for a retransplant patient with situs inversus

Hajime Uchida, Akinari Fukuda, Takanobu Shigeta, Kengo Sasaki, Mureo Kasahara.
Transplant Center, National Center for Child Health and Development, Tokyo, Japan.

Portal vein (PV) thrombosis following pediatric liver transplantation is a serious complication that may lead to graft loss. Living donor liver transplantation (LDLT) poses limitations with regards to the availability of vein grafts for complex PV reconstructions. We herein show a unique reconstruction of the portal vein inflow in a 1-year-old boy with situs inversus undergoing re-LDLT. The inflow was derived from the splenic vein and the right renal vein. A common channel was created utilizing a donor inferior mesenteric vein and the recipient explant left hepatic vein as vascular conduits. With the application of innovative surgical reconstructions, pre-existing portomesenteric thrombosis may be amenable to re-LDLT in the pediatric population.
References:
[1] Neto JS, Fonseca EA, Feier FH, Pugliese R, Candido HL, Benavides MR, et al. Analysis of factors associated with portal vein thrombosis in pediatric living donor liver transplant recipients. *Liver Transpl* 2014;20:1157-67
[2] Englesbe MJ, Mathur AK, Waits SA, Magee JC. Wow, that portal vein is small: preventing portal vein thrombosis in liver transplantation for small children. *Liver Transpl* 2014;20:742-3.
[3] Ueda M, Oike F, Kasahara M, Ogura Y, Ogawa K, Haga H, et al. Portal vein complications in pediatric living donor liver transplantation using left-side grafts. *Am J Transplant* 2008;8:2097-105.

554.7

A case of deceased donor liver transplantation using renoportal anastomosis in patients with diffuse portomesenteric thrombosis

Tae-Seok Kim, Keun Soo Ahn, Yong Hoon Kim, Hyun Ji Lee, Hyoung Tae Kim, Koo Jeong Kang.
Surgery, Dongsan Medical Center, Keimyung University School of Medicine, Daegu, Korea.

Introduction: Adequate portal inflow is essential for successful liver transplantation. However, portal vein thrombosis (PVT) is not uncommon in end stage liver disease and PVT was considered as relative contraindication of liver transplantation in past because of technical difficulties that induced an increased intraoperative bleeding and an inability to establish adequate portal inflow. However, with technical improvement such as venous eversion thrombectomy, vascular interposition grafts, and use of portal vein collaterals, PVT has not been accepted as contraindication of liver transplantation any more. Here, we describe a successful case of deceased donor liver transplantation (DDLT) with renoportal anastomosis in patient with diffuse portomesenteric thrombosis.
Case: A 67-year-old man was diagnosed with alcoholic liver cirrhosis 17 years ago and had been followed-up regularly. Segmental PVT was developed 8 years ago and had been extended to superior mesenteric vein and splenic vein. The patient had undergone endoscopic esophageal variceal ligation twice for hematemesis and paracentesis frequently for ascites control. Preoperative CT scan revealed features of diffuse mesoportal thrombosis, splenic vein thrombosis with splenomegaly, cavernous transformation of portal vein, and large splenorenal shunt. This patient underwent deceased donor liver transplantation. Inferior vena cava was anastomosed with piggy-back technique and portal flow was obtained from left renal vein (eno-to-end anastomosis) using iliac vein graft from . The anastomosis of hepatic artery and bile duct was performed rountine manner. Introperative and postoperative Doppler sonography showed adequate portal flow and liver function was improved gradually. CT which performed at postoperative 10 days and 1 month showed good portal inflow from left renal vein.
Conclusion: In case of diffuse portomesenteric thrombosis with splenorenal shunt, renal vein can be a good source for reconstruction of portal inflow.

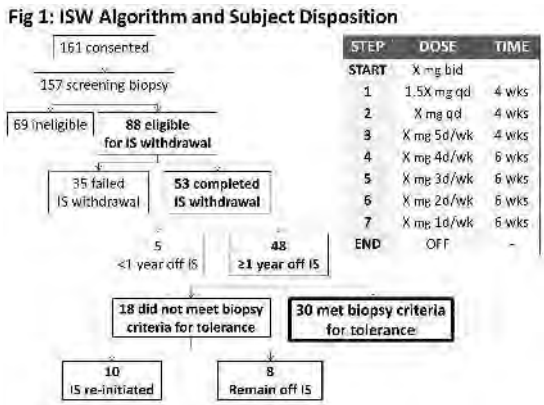
555.1

Primary outcome of iWITH: A 12 center North American clinical trial of immunosuppression withdrawal in stable pediatric liver transplant recipients

S. Feng[1], J. Bucuvalas[2], A.J. Demetris[3], K. Spain[4], J. Magee[5], G. Mazariegos[3], S. Kanaparthi[6], E. Doo[7], N. Bridges[8].
[1]UCSF, San Francisco, CA, United States; [2]Cincinnati Childrens Hosp, Cincinnati, OH, United States; [3]University of Pittsburgh, Pittsburgh, PA, United States; [4]RHO, Chapel Hill, NC, United States; [5]University of Michigan, Ann Arbor, MI, United States; [6]ITN, Seattle, WA, United States; [7]NIDDK, Bethesda, MD, United States; [8]NIAID, Bethesda, MD, United States.

Background and Aim: Considering the chronic and cumulative disease burden imposed by conventional immunosuppression (IS), it is intuitive that IS discontinuation would improve patient outcomes. A pilot study of pediatric parental living donor liver transplant (LT) recipients undergoing IS withdrawal (ISW) with 60% (12 of 20 subjects) success has been reported [1]. iWITH, a multi-center clinical trial funded by NIAID, NIDDK, and ITN, was designed to extend these preliminary results by:1) including deceased donor LT recipients 2) determining prevalence of operational tolerance (OT) within a clinically useful confidence interval for the specified population, 3) confirming the safety of ISW and 4) elucidating biomarkers and/or mechanisms of OT.
Methods: 88 highly selected pediatric LT recipients (39 boys/49 girls; 31 living/57 deceased grafts; Fig 2a) at 12 North American centers met clinical, biochemical, and histological eligibility criteria. ISW occurred in 8 steps over 36-48 weeks (Fig 1). ALT or GGT >100 IU/L mandated for cause liver biopsy. The 1° endpoint of OT was assessed 1 year after the last IS dose and was defined per protocol as stable ALT, GGT and histology compared to study entry 2 years earlier. Logistic regression analyses assessed for associations between completed ISW and baseline clinical, immunologic [Class II donor specific antibody (DSA)] and histological (including C4d score) factors.
Results: 35/88 (40%) subjects failed ISW prior to OT assessment, all 2° to rejection [31 biopsy-proven (24/6/1 Banff mild/moderate/inadequate per central pathologist) and 4 clinical; Fig 1]. No baseline clinical, immunological or histological factors, including living/deceased donor, Class II DSA presence/absence, or C4d score predicted completed (n=53) vs failed (n=35) ISW (Fig 2b). Five subjects are off IS <1 year and await OT assessment (last subject due before 4/1/2016). The remaining 48 subjects have been assessed for OT. All subjects met laboratory OT criteria; 30/48 (62.5%) subjects have also met histological OT criteria [Fig 1]. Of the 18 (37.5%) subjects who did not meet histological OT criteria,10 have re-initiated while 8 remain off IS. 5 subjects experienced 5 study-related serious adverse events: 3 related to liver biopsy (bile leak; cholangitis; cellulitis) and 2 related to ISW (in-patient rejection treatment with intravenous corticosteroids).

Fig 1: ISW Algorithm and Subject Disposition

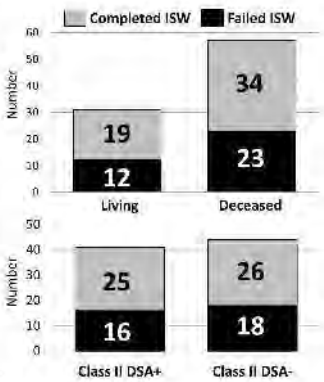


Conclusions: Selected pediatric LT recipients from living or deceased donors can safely attempt ISW and may achieve OT. Notably, a significant proportion met biochemical but not histological criteria for OT, suggesting a pivotal role for biopsy to assess for OT and long-term safety. Although baseline clinical, immunological and histological features did not predict the outcomes of ISW, planned mechanistic assays including immunophenotyping and gene expression profiling of tissue and peripheral blood may identify an OT biomarker and/or elucidate OT mechanisms.

Fig 2a: Subject Demographics

Factor	N = 88
Age (yrs) mean ± SD Transplant	1.7 ± 1.7
Age (yrs) mean ± SD Enrollment	10.4 ± 3.4
Male gender	39 (44%)
Biliary atresia	51 (58%)
Living donor	31 (35%)
Deceased whole	41 (47%)
Deceased partial	16 (18%)
ALT (IU/L; mean ± SD)	25.4 ± 7.4
GGT (IU/L; mean ± SD)	16.4 ± 7.2
Class II DSA positive (n=85)	41 (48%)

Fig 2b: Completed vs Failed ISW Living/Deceased Donor Class II DSA +/-



NIAID; NIDDK; ITN

References:

[1] Feng S et al. JAMA 2012 307:283-93

555.2

AT1R antibodies at 1-year negatively impact 5-year native renal function in liver transplant recipients

Duska Dragun[1,4], Aurelie Philippe[1], Harald Heidecke[3], Robert Freeman[2], Linda W.Jennings[2], Rusan A.Catar[1,4], Goran B.Klintmalm[2], Jacqueline G.O'Leary[2].
[1]Nephrology and Critical Care Medicine, Charité University Berlin, Berlin, Germany; [2]Annette C. & Harold C. Simmons Transplant Institute, Baylor University Medical Center, Dallas, TX, United States; [3]CellTrend GmbH, Luckenwalde, Germany; [4]Berlin Institute of Health, Berlin, Germany.

Angiotensin II Type-1 Receptor (AT_1R) antibodies have been associated with pulmonary hypertension, renal allograft loss, and fibrosis progression in liver allograft recipients especially if combined with HLA donor-specific alloantibodies (DSA). However, long-term outcome in liver allograft recipients is not only impacted by allograft fibrosis but also renal function. We therefore sought to determine if native renal function was impacted in liver allograft recipients by the presence of AT_1R antibodies.

Methods: Primary liver allograft recipients at Baylor University Medical Center from 1/00 to 4/09 had their prospectively collected pre-transplant (1269 patients) and year-1 post-transplant (795 patients) serum tested retrospectively for AT_1R (>10) antibodies. AT_1R-antibody testing was accomplished with standardized solid phase assay. Since AT_1R antibodies have been associated with hypertension this factor was not controlled for in multivariable modeling.

Results: Pretransplant AT_1R did not change the median delta creatinine from pretransplant to 3-months post-transplant. In patients with vs. without AT_1R at 1-year post-transplant a median unadjusted change in MDRD6 of -5.4. vs. -1.1mL/min (p=0.01) was found. In multivariable analysis when controlling for diabetes (DM) and calcineurin inhibitor (CNI) use at 1-year AT_1R-Ab at 1-year remained statistically significantly associated with a decline in GFR (calculated by MDRD6) from year 1 to 5 post-transplant (p=0.018, Table 1). This decline may have been more pronounced (p=0.06) in patients on a CNI; however, the decline was most pronounced in diabetic patients with AT1R at year-1 (p=0.004).

Conclusion: AT_1R antibodies post-liver transplant are associated in multivariable analysis with an increased risk of native renal function decline especially in diabetic patients. AT_1R positive patients may benefit from CNI free immunosuppression.

Multivariable at 1-year to predict decline in renal function at 5 years			
AT1R >10		**P=0.018**	
DM		P=0.021	
CNI		P=0.798	
Change in MDRD6 from year 1-5			
DM	**AT1R**	**-10.89 mL/min**	
	None	-3.53 mL/min	
No DM	AT1R	-3.60 mL/min	
	None	-0.35 mL/min	P=0.004
CNI	**AT1R**	**-5.64 mL/min**	
	None	-0.97 mL/min	
No CNI	AT1R	-0.27 mL/min	
	None	-3.33 mL/min	P=0.06

555.3

The impact of preformed donor-specific antibodies in living donor liver transplantation depending on graft volume

Ryoichi Goto[1], Yasuyuki Koshizuka[1], Norio Kawamura[1], Masaaki Zaitsu[1], Minoru Ota[2], Toshiya Kamiyama[1], Kenichiro Yamashita[3], Akinobu Taketomi[1], Tsuyoshi Shimamura[3].
[1]Department of Gastroenterological Surgery I, Hokkaido University, Sapporo, Japan; [2]Division of Organ Transplantation, Hokkaido University Hospital, Sapporo, Japan; [3]Department of Transplant Surgery, Hokkaido University, Sapporo, Japan.

Background: Preformed donor specific antibodies (DSAs) have been reportedly associated with poorer outcome in heart and kidney transplantation. On the contrary, the correlation between DSA and the liver graft outcome remains controversial because large size of organ, liver graft may be able to absorb antibodies. It is likely that smaller graft size has a relatively higher risk of preformed DSAs on graft survival.
Aim: To identify the prognostic factor of the recipients who developing the preformed DSAs in living donor liver transplantation (LDLT).
Patients and Methods: Adult recipients who underwent LDLT (n=175) in our institute from Sep. 1997 to Jan. 2016 were studied. To assess the preformed DSAs, T (T cell CM) and B (B cell CM) lymphocyte cytotoxicity test (LCT) and T cell IgG flow cytometric cross match (FCXM) test were examined. The cut-off value in LCT and FCXM was 40% and 20% respectively. Results. Of adult LDLT recipients, 28 (16.6%) were pre-transplant FCXM positive alone (i.e. LCT negative). Sixteen (9.1%) were detected as LCT positive. Of 16 LCT (+) patients, 15 were T cell CM positive. There was a trend toward worse graft outcome of FCXM (+) alone (Fig. 1, 5 y graft survival rate; 71.1%) as compared to that of control (79.0%). Notably 7 of 15 T cell CM (+) were died with graft failure (median survival time was 135 days) and the graft outcome was significantly poorer (Fig. 1). Four of 7 graft loss cases with T cell CM (+) were correlated with severe graft rejection. All of cases with T cell CM (+) were performed LDLT before 2006 so that the pre-transplant treatments has not been appropriately evaluated. To identify the risk factors of poorer graft survival, we compared the pre-transplant status and donor factors between graft loss and alive in T cell CM (+) patients. Indeed, 3 of 7 graft loss cases were critically ill in intensive care unit although 5 of 8 were outpatients in alive group. Also MELD score tends to be higher in graft loss group (24±4.7, P=0.12, vs. 15.5±3.4 in alive, medain±SEM). In terms of living donor graft, 6 of 7 graft loss cases (85.7%) were transplanted with a left hepatic lobe. In contrast, 6 of 8 patients (75%) were used a right lobe of living graft in alive patients. Actually Gv/Sv ratio (36.2±2.1%) and graft volume (368.0±23.6g) of graft loss cases were significantly lower than those of alive cases (Fig. 2, *p<0.03, 52.8±6.7% and 563.5±63.4g respectively, median ± SEM).

Fig.1

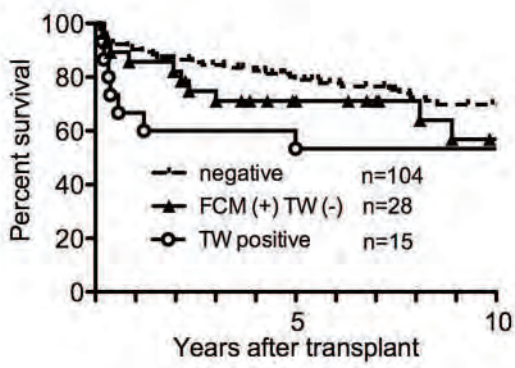
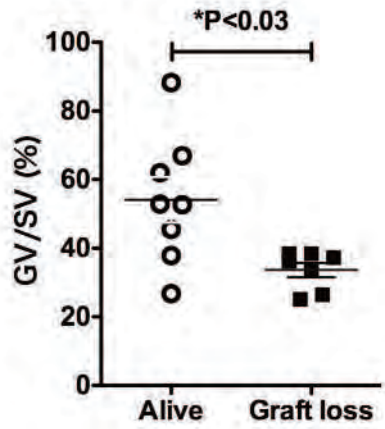


Fig. 2



Conclusion: With recent acvances in immunotherapy, pre-transplant desensitization may reduce the risk of graft loss in preformed DSA patients. However our data suggests that smaller graft volume such as left partial grafts in the presence of preformed DSAs can predict the graft failure. Under the preformed DSAs, the living donor selection should be consider the graft volume.

555.4

The strategy for living donor liver transplantation in the recipients with preformed donor specific antibodies

Atsushi Yoshizawa[1], Eri Ogawa[1], Shintaro Yagi[1], Koichiro Hata[1], Aya Miyagawa[2], Hideaki Okajima[1], Toshimi Kaido[1], Hironori Haga[2], Shinji Uemoto[1].
[1]Department of Surgery, Kyoto University Hospital, Kyoto, Japan; [2]Department of Diagnostic Pathology , Kyoto University Hospital, Kyoto, Japan.

Introduction: The impact of preformed donor-specific anti-HLA antibodies (DSA) on the survival rate of liver transplantation is controversial. We previously reported that the one-year survival rate of those with preformed DSA was 60%, significantly lower than those without preformed DSA, and that the quantity of DSA influenced on the prognosis[1]. We also reported the cases with perioperative fatal antibody mediated rejection (AMR)[2]. We have adopted the strategy to prevent fatal AMR; the sera are preoperatively examined in various ways and if DSA are detected strongly, we change the donors or performed preoperative desensitization by Rituximab.
Methods: 315 recipients underwent liver transplant between 2009 and 2014, including 289 cases of living-donor liver transplantation (LDLT), 26 cases of deceased-donor liver transplantation (DDLT). The sera were examined to detect preformed DSA by complement-dependent cytotoxicity (CDC), flow cytometry crossmatch (FCXM), and Luminex method using Labscreen single antigen beads. The current strategy for DSA-positive patients is as follows. For LDLT cases, we change the CDC-positive donors, if possible. Otherwise, we administer Rituximab prophylactically. When DSA are detected by FCXM or Luminex, but negative in CDC, we will examine DSA successively after operation and we will perform liver biopsy and diagnose AMR as soon as possible when liver function tests are abnormal. For DSA-positive DDLT cases, we closely examined HLA antibodies by Luminex, and we diagnosed AMR as soon as possible after DDLT. We diagnosed AMR by the pathological findings, the positive C4d staining, and the existence of DSA in the serum. We treated AMR by steroid pulse therapy and intravenous immunoglobulin.
Results: As for LDLT cases, the positive cases of examination of DSA were 10 in CDC, 17 in FCXM, 25 in Luminex method, respectively. In CDC-positive cases, the donors of 5 cases were changed, and Rituximab was preoperatively administered in 6 cases. One year survival rate was 78.8% in DSA-positive recipients, while 82.4% in DSA-negative patients. (p=0.638) Five cases with preformed DSA were diagnosed as antibody mediated rejection and successfully treated by intravenous immunoglobulin, steroid pulse therapy, and plasma exchange. Acute cellular rejection (ACR) was diagnosed in 27% of DSA-negative cases and in 43.8% of DSA-positive cases within one year after operation. (p=0.242) On the other hand, as for DDLT, five cases were positive in the examination of DSA by CDC and Luminex method. One case died one month after DDLT due to infection. And the other two cases were diagnosed as AMR by liver biopsy and treated successfully by steroid pulse therapy and intravenous immunoglobulin. The other two cases were free from AMR, but they were diagnosed as ACR three and seven months after operation and treated by steroid pulse therapy. All cases with preformed DSA turned to be negative DSA one year after operation.
Conclusion: The current strategy for prevention from AMR significantly has improved the survival rate of the patients with preformed DSA. The preoperative examination of DSA is essential to avoid fatal AMR and to diagnose and treat AMR immediately after operation.
References:
[1] Yoshizawa A. Significance of semiquantitative assessment of preformed donor-specific antibody using luminex single bead assay in living related liver transplantation. *Clin Dev Immunol*. 2013; 972705.
[2] Hori T. Does a positive lymphocyte cross-match contraindicate living-donor liver transplantation? *Surgery*. 2010; 147, 840-844.

555.5

Outcomes of sirolimus conversion for calcineurin inhibitor induced neurotoxicity in liver transplantation

Greg McKenna, Peter TKim, Giuliano Testa, Richard Ruiz, Nicholas Onaca, Tiffany Anthony, James Trotter, Robert MGoldstein, Goran BKlintmalm.
Simmons Transplant Institute, Baylor University Medical Center, Dallas, TX, United States.

Introduction: Calcineurin Inhibitor (CNI) neurotoxicity is a complication affecting up to 15% of liver recipients and can be treated with conversion to sirolimus (SRL) along with CNI discontinuation. Symptoms include headache, confusion, dysarthria, seizures, and coma. Concerns of rejection (ACR) and other mTOR inhibitor complications often deter clinicians from making this conversion. We report outcomes of the largest experience of SRL conversion for CNI neurotoxicity.
Materials and Methods: All 3910 pts from 1988-2015 were retrospectively reviewed using a prospectively obtained database, to identify a cohort where CNI was discontinued and SRL was initiated for neurotoxicity symptoms. This was compared to a control group where SRL conversion was done for non-neurotoxicity causes. This cohort was evaluated for ACR, other complications and post-conversion survival.
Results: From 11/1999–12/2014, there were 679 pts converted to CNI-free SRL immunsuppression, with 58 pts (8.5%) converted for CNI neurotoxicity. Median time to SRL conversion was 25 d post OLT (range 6 d–10 yrs) vs 736 d in those converted for non-neuro reasons. 56/58 pts (96.6%) improved/resolved neurotoxicity symptoms following CNI discontinuation.

Complications Post Conversion to SRL-Based CNI Free Regimen

Complication	SRL for Neurotoxicity (n=58)	SRL for Non-Neuro (n=621)	p value
ACR	13 (22.4%)	48 (7.7%)	<0.001
CMV	3 (5.1%)	22 (3.5%)	NS
TA-I	0 (0%)	5 (0.8%)	NS
BD Stricture/Leak	2 (3.4%)	15 (2.4%)	NS
Incisional Hernia	11 (15.5%)	58 (9.3%)	0.04

All 13 ACR episodes in those converted for neurotoxicity occurred when converted in the first 47d post OLT; 53% occurred in conversions in the first 2 weks. Median time to ACR development post SRL conversion, was 13 d vs 79 d for neuro vs non-neuro based conversion.

ACR and Hernia Incidence of ACR by Timing from OLT to SRL Conversion

Timing of Conversion Post OLT	# of Pts	ACR Incidence	Incisional Hernia Incidence
< 30 d	30	12 (40.0%)	6 (20.0%)
> 30 d	28	1 (3.6%)	5 (17.9%)

Comparing SRL conversion for neuro vs non-neuro reasons, the 1 yr pt survival was 92.0% vs 90.1% (p=NS) respectively, while 55% vs 77% remained on SRL for >1 yr (median duration 658 d vs 1721 d). The reason for eventual SRL discontinuation in neuro vs non-neuro pts: pending surgery (13.6% vs 12.8%), ACR (18.9% vs 4.7%), infection (5.2% vs 3.7%) and patient death (1.7% vs 8.5%).
Conclusion: SRL conversion for CNI neurotoxicity can be successful but with a higher ACR and incisional hernia rates compared to other SRL conversion pts. Delaying the conversion after the first 30d post OLT may reduce this risk.

555.6

Comparison of the effects of brand name versus generic immunosuppressants on long-term graft failure risk among U.S. liver transplant recipients: analysis of SRTR and Medicare claims data

Jarcy Zee[1], Ghalib A.Bello[1], Murewa Oguntimein[2], Charlotte A.Beil[1], Qian Liu[1], Jeong Park[3], Adam R.Saulles[4], Sangeeta Goel[4], Rajesh Balkrishnan[5], Melissa Fava[1], Robert M.Merion[1], Alan B.Leichtman[1], Pratima Sharma[6].
[1]Arbor Research Collaborative for Health, Ann Arbor, MI, United States; [2]Food and Drug Administration, Silver Spring, MD, United States; [3]Clinical Pharmacy, University of Michigan, Ann Arbor, MI, United States; [4]Specialty Pharmacy Services, University of Michigan Health System, Ann Arbor, MI, United States; [5]Public Health Sciences, University of Virginia, Charlottesville, VA, United States; [6]Medicine, University of Michigan, Ann Arbor, MI, United States.

Introduction: The use of generic immunosuppressants for liver transplantation (LT) has increased in some countries following expiration of brand name patents. However, the reported risks and benefits of generic substitution have been inconsistent, particularly because bioequivalence requirements differ across countries and only small, short-term studies have examined the effects of substitution on transplant outcomes. Our aim was to compare the effects of brand name and generic tacrolimus on the long-term risk of graft failure in a large, national cohort of U.S. LT recipients.
Methods: Data from the Scientific Registry of Transplant Recipients (SRTR) were used to identify LT recipients who were transplanted from 2008-2013 and Medicare Part D claims data were used to identify patterns of generic and brand name tacrolimus use. LT recipients were followed from the time of transplant until the earliest of repeat transplant, death, loss to follow-up, or end of the study period. Generic or brand name tacrolimus use was recorded at each month of follow-up. Analysis was limited to recipients who had Medicare Part D claims for tacrolimus at any time after transplant and who had graft function at 30 days after transplant. We used a joint model to test the association between brand/generic tacrolimus use and a composite graft failure outcome defined as the earlier of repeat transplant or death. The model was constructed by linking a longitudinal model for the pattern of long-term brand name or generic tacrolimus use with a Cox proportional hazards model for graft failure, adjusted for recipient, donor, and transplant program characteristics.
Results: Table 1 shows descriptive summary statistics on the study cohort (N=4,421). The cohort was predominantly male, white, and aged 50-64 years. The most common primary cause of end-stage liver disease was hepatitis C.

Table 1: Descriptive Statistics on Study Cohort

Age, years, Mean (SD)	54.8 (10.28)
Male, % (N)	63.6 (2811)
Previous Liver Transplant, % (N)	5.4 (237)
Race/Ethnicity, % (N)	
White	67.8 (2996)
Black	10.3 (457)
Asian/Other	5.5 (241)
Hispanic/Latino	16.4 (727)
Primary Cause of Liver Disease, % (N)	
Acute Hepatic Necrosis	3.7 (163)
Alcoholic Liver Disease	12.8 (567)
Cholestatic Disease	7.6 (334)
Hepatitis C	30.9 (1365)
Malignancy	19.3 (852)
Other	25.8 (1140)
Follow-up Time, years, Median (IQR)	3.6 (2.4-4.9)

SD: Standard Deviation, IQR: Interquartile Range (25th 75th percentiles)

There was no significant difference in the long-term risk of graft failure when comparing the use of generic and brand name tacrolimus in LT recipients (Hazard Ratio=1.002, 95% Credible Interval=(0.998-1.006), p-value=0.381).
Conclusion: Our study of a large, national cohort of U.S. LT recipients shows that generic substitution of tacrolimus does not result in a significant difference in the long-term risk of graft failure. Generalizability of the study results beyond the U.S. may be limited by differences between generic tacrolimus formulations used in the U.S. compared with those used in other countries.
Funding for this research was made possible by the Food and Drug Administration through grant 1U01FD005274-01. Views expressed in written materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Food and Drug Administration or the Department of Health and Human Services; nor does any mention of trade names, commercial practices, or organization imply endorsement by the United States Government

555.7

Normal liver enzymes do not exclude significant pathology in liver transplant recipients

Richard Gilroy[1], Czarina Sanchez[1], Phillip Johnson[2], Atta Nawabi[1], Bart Kane[1], Shelbi Olson[1], Sean Kumer[1], Timothy Schmitt[1].
[1]Center for Transplantation, University of Kansas Medical Center, Kansas City, KS, United States; [2]Department of Radiology, University of Kansas Medical Center, Kansas City, KS, United States.

Background: Liver biopsies serve a variety of roles in post-transplant analysis and most often are used to evaluate abnormal liver enzymes (LE); however biopsies are not without risk. In the post-transplant setting protocol biopsies (PB) are employed by some centers however their role remains controversial. This study evaluates whether PBs serve any purpose in those with normal LEs at the time biopsy by assessing for the frequency of abnormal pathologies, the significance of these pathologies and for what factors might predict a significant pathology being present with normal LEs.
Methods: A 3 year analysis of a prospectively maintained medical record was queried in all patients enrolled in a PB pathway. All patients included in th analysis were post-liver transplant with normal LEs on 2 occasions immediately prior to PB being performed. Normal LEs were defined as AST < 40, ALT <45 and Alkaline Phosphatase <100 and imaging with Doppler ultrasound also had to be normal. All demographic data on the cases and all complications, histology, and clinic actions generated from the histology identified were collected. A statistical analysis using SPSS was then used to evaluate for both predictors of abnormal histology and clinical action.
Results: 400 PBs were performed in 178 patients of which 84 PBs were associated with normal LEs. Of these patients the most common liver diseases were HCV, alcohol, NASH, AIH, and PSC and the average time from transplant to PB was 2 years (range 6 months to 8 years). A total of 2 episodes of cholangitis occurred with PBs and there were no bleeding episodes requiring hospitalization or transfusion. 79% of the PB had abnormal histology findings including varying grades of steatosis, inflammation, hepatic fibrosis and in 6 cases cirrhosis were identified. The presence of abnormal LEs at 3 months post-transplant (n=29) was associated with higher inflammatory (p=0.027) and fibrosis (p=0.003) scores at the time of any PB when compared to those with normal LEs at 3 months. In those with normal LEs and abnormal PBs, interventions including treatment of HCV, evaluation for biliary stricture disease, treatment of rejection or changes to immunosuppression management occurred in over 50% of patients. in performing the PBs these were equally likely to be performed by hepatology and interventional radiology and no difference in complications seen.
Conclusions: The early clinical course, donor characterists, and the presence of abnormal LEs at 3 months influence the probability of a PB being abnormal. PBs are infrequently associated with complications and provide useful clinical information that does impacts clinical care pathways in those with normal LEs. A terminology change to management biopsies rather than PBs might be considered in our era of immunosuppression minimization strategies and the greater use of extended criteria donors as a role for PBs appears evident.

555.8

Preferential accumulation of T helper cells but not cytotoxic T cells characterizes benign subclinical rejection of human liver allografts

Anna K.Baumann[1], Fatih Noyan[1,2], Matthias Hardtke-Wolenski[1], Jerome Schlue[3], Frank Lehner[2,4], Juergen Klempnauer[4], Michael P.Manns[1], Elmar Jaeckel[1,2], Richard Taubert[1,2].
[1]Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany; [2]Integrated Research and Treatment Center Transplantation (IFB-Tx), Hannover Medical School, Hannover, Germany; [3]Pathology, Hannover Medical School, Hannover, Germany; [4]General, Abdominal and Transplant Surgery, Hannover Medical School, Hannover, Germany.

Introduction: Subclinical rejection (SCR) is a common event in protocol biopsies after liver transplantation. So far the understanding of its underlying histological changes, immune regulation and clinical significance is very limited. Previous studies were restricted to SCR manifestations within the first weeks after transplantation and showed no consequences on clinical outcome even when SCR was left untreated.
Materials and Methods: We included all 94 patients from our prospective protocol biopsy program from 2008 until 02/2013 who received at least one liver biopsy and who had no viral hepatitis (HBsAg and HCV-RNA negativity). To allow for a longer follow-up these patients we then observed for another 30 months. SCR was defined as histological rejection with liver enzymes (AST, ALT, AP) below the two times upper limit of normal, while acute cellular rejection (ACR) was defined as rejection with liver enzymes above this threshold. Patients with late SCR were compared to late ACR regarding the intrahepatic and peripheral blood T cell compartment (CD4+FOXP3+ regulatory T cells, CD4+ and CD8+ cells).
Results and Discussion: Late SCR, at least 3 months after transplantation, was a common event (41/94 patients) in our program. SCR manifested with in mean 27 (4-83) months after liver transplantation much later than ACR. In the second year after transplantation the SCR incidence in protocol biopsies reached a plateau of about 25% and remained there until the latest observed manifestations more than 5 years after transplantation. During a mean follow-up of 36 (12-73) months after SCR no acute or chronic rejection, no progressive graft fibrosis, graft loss or liver related death occurred even without specific therapy for SCR. Immunophenotyping of liver biopsies during SCR showed that similar to ACR, the composition of intrahepatic T cells was depended on the severity of histological rejection. However, SCR showed a different pattern of infiltrating T cells with a stronger accumulation of CD4+ cells, an increasing CD4+/CD8+ ratio, and an increasing CD4+FOXP3+ regulatory T cell/CD8+ ratio with the severity of histological rejection, which was not seen in ACR. These intrahepatic T cell patterns were not reflected in the peripheral blood.
Conclusion: Late SCR after liver transplantation has a good prognosis and it seems save to leave it untreated. This benign clinical course compared to ACR is associated with T cell infiltration patterns in the graft that point to a stricter regulation of the cytotoxic T cell compartment.

555.9

Experience with mTOR inhibitors in pediatric liver transplantation

Figen Özçay[1], Zeren Barış[1], Meltem Gülşan[1], Gökhan Moray[2], Mehmet Haberal[2].
[1]Department of Pediatric Gastroenterology, Baskent University Hospital, Ankara, Turkey; [2]Department of General Surgery, Baskent University Hospital, Ankara, Turkey.

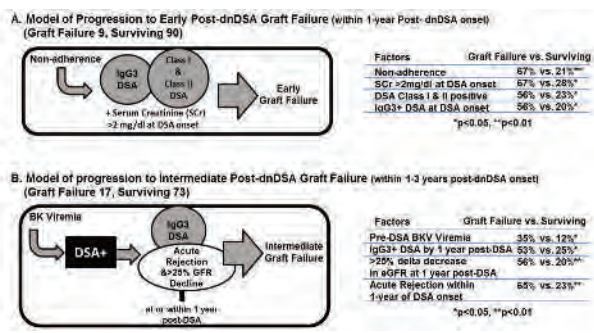
Aim: We evaluated the use of mTOR inhibitors in pediatric liver transplanted patients.
Methods: We retrospectively collected the data of children who underwent liver transplantation between 2003-2015 in Baskent University Hospital and used mTOR inhibitors as a part of immune suppressive treatment. We evaluated the demographic features of the patients, indications for use and complications of mTOR inhibitors and changes in leucocyte, thrombocyte counts and glomerular filtration rates (GFR) of patients during treatment course.
Results: There were a total of 21 patients (male/female ratio:11/10) who used mTOR inhibitors during course of immune suppressive treatment. Patients were between 147,57±72,17 months old. The mean age at transplantation was 81,23±71,46 months.
Fourteen patients used sirolimus, while 7 patients used everolimus. The reasons to switch to sirolimus was posttransplant lymphoproliferative disease (PTLD) in five patients (35,7%), chronic rejection and decrease in graft functions in two patients (14,3%), malignancy in two (14,3%) (relaps of Langerhans cell histiocytosis and hepatocellular carcinoma), inflammatory bowel disease in two (14,3%), neuropathy due to calcineurin inhibitors (CNI) use in two (14,3%) and recurrent acute rejection in one (7,1%) patient. The reasons to switch to everolimus was nephrotoxicity of CNI in three patients (42,8%), recurrent acute rejection in two (28,6%), chronic rejection in one (14,3%) and sepsis in one (14,3%) patient.
Sirolimus was used as the only immune suppressive drug in 11 patients and combined with tacrolimus in three patients, and everolimus was combined with low dose tacrolimus in all patients. Postoperative times to introduce sirolimus and everolimus were 26,46±24,49 months (min-max: 3-78 mo) and 14,78±28,97 months (min-max: 1-80 mo) respectively.
The mean trough levels of sirolimus were 8,67±3,37 ng/ml. The mean trough levels of everolimus were 4,49±1,58 ng/ml. Postoperative starting time was significantly shorter (p=0,031), mean period of time of use was significantly shorter (p=0,006) and the mean GFRs at the beginning of the treatment were significantly lower (p=0,015) in everolimus group when compared to sirolimus group.
Complications of sirolimus treatment were noted as acute rejection, infection and aphtous stomatitis, while infection, aphtous stomatitis and growth failure were recorded as complications in patients using everolimus. We did not observe any complications in 9/14 patients who used sirolimus and 5/7 patients who used everolimus.
Sirolimus was discontinued in three patients because of health insurance problems (one patient) and rejection (two patients) after 6,5 years, 5 months and 8 months respectively. Everolimus was discontinued in one patient due to severe candida esophagitis after 5 months.
Conclusion: Use of mTOR inhibitors in pediatric liver transplantation is safe and efficacious and offers promising results.

556.1

Models of allograft failure in primary renal transplant recipients with anti-HLA alloantibodies (DSA)

Matthew Everly[1], Lorita M.Rebellato[2], Carl E.Haisch[2], Kimberly P.Briley[2], Paul Bolin[2], Scott A.Kendrick[3], Claire Morgan[2], Angela Q.Maldonado[4], Anh Nguyen[1].
[1]Terasaki Foundation Laboratory, Los Angeles, CA, United States; [2]East Carolina University, Greenville, NC, United States; [3]Eastern Nephrology Associates, Greenville, NC, United States; [4]Vidant Medical Center, Greenville, NC, United States.

A better understanding of which patient and alloantibody characteristics correlated to timing of graft failure (early, intermediate, and late post-dnDSA) is needed to help in making an informed decision regarding treatment. In our study we aimed to descibe the most common model of post-dnDSA graft failure (and non-failure).
Methods: We performed a single center retrospective analysis of 99 dnDSA positive patients receiving a primary transplant between 3/99 to 12/10 that had 3 years of post-dnDSA follow-up. All patients had HLA antibody monitoring by single antigen beads pre-txp, post-txp at 1,3,6,9,12 months, and annually, thereafter. IgG subclass testing was performed on samples following dnDSA. All patients were pre-txp DSA (-).
Results: Of the cohort, 9% of patients developed early post-dnDSA graft failure (<1 year post-dnDSA). This was triggered by non-adherence ($p<0.01$ vs grafts surviving beyond 1 year post-dnDSA, Fig A). The early graft failure group's profile at dnDSA onset had higher rates of IgG3 dnDSA, HLA Class I + II dnDSA, and serum creatinine above 2 mg/dL.
Of the cohort, 17% of patients developed intermediate post-dnDSA graft failure (1-3 year post-dnDSA). The intermediate graft failure group (n=17) was compared to the group with good graft function at least 1-3 years post-dnDSA (n=70) (Fig B). The intermediate graft failure patients had a higher rate of BK viremia (p=0.02). The development of IgG3 dnDSA by 1 year post-dnDSA occurred in two times as many intermediate graft failure patients [hazard ratio (HR) 3.4, 95%CI 1.2-9.0]. Additionally, acute rejection by 1 year post-dnDSA (HR 5.0, 95% CI 1.9-13.7) and >25% eGFR decline at 1-year post-dnDSA (compared to eGFR at dnDSA onset, HR 6.5, 95% CI 2.4-17.5) predicted graft failure between 1 - 3 years post-dnDSA.
Finally, 15% of dnDSA positive cases developed late graft failure >3 yeats post-dnDSA). This group (n=15) was compared to dnDSA + grafts functioning > 3 years post-dnDSA (n=58). Acute rejection by 1 year post-dnDSA and IgG3 dnDSA by 1 year post-dnDSA were good predictors of late graft failure. In late graft failure, 87% eventually developed IgG3 (vs 21% in. Long-term function grafts).



Conclusions: Identifying BK virus viremia, non-adherence, IgG3 DSA, and post-DSA acture rejection or >25% decrease in eGFR may be useful when trying to prevent early and intermediate allograft failure after the appearance of dnDSA.

556.2

Tubulointerstitial expression of connective tissue growth factor in renal allograft protocol biopsies at 3 months predicts interstitial fibrosis at 5 years

Thomas Vanhove[1], Hiroshi Kinashi[3], Tri Q.Nguyen[2], Evelyne Lerut[4], Roel Goldschmeding[2], Dirk R.J.Kuypers[1].
[1]Nephrology and Renal Transplantation, University Hospitals Leuven, Leuven, Belgium; [2]Pathology, University Medical Center Utrecht, Utrecht, Netherlands; [3]Nephrology, Nagoya University Hospital, Nagoya, Japan; [4]Pathology, University Hospitals Leuven, Leuven, Belgium.

Introduction: Connective tissue growth factor (CTGF) is an important mediator of renal allograft fibrosis and urinary CTGF levels correlate with the development of human allograft interstitial fibrosis. We set out to evaluate the added predictive value of CTGF protein expression in early protocol biopsies for the subsequent development of allograft fibrosis.
Methods: Single center observational cohort study of 171 kidney transplant recipients with paired protocol biopsies at 3 months as well as 5 years after transplantation. Biopsies were scored according to the revised 1997 Banff criteria. Three month biopsies were immunohistochemically stained for CTGF. Tubulointerstitial CTGF positive surface area was scored visually and categorized as absent (<1%), minimal (1-10%), moderate (10-25%) or extensive (>25%). Urinary CTGF at 3 months after transplantation was measured using a sandwich ELISA (Fibrogen, South San Francisco, CA).
Results: In multivariate multinomial regression analysis, the only predictors of interstitial fibrosis at 5 years were donor age (OR 1.04 [1.01-1.07], OR 1.06 [1.03-1.11], OR 1.05 [1.01-1.08] for ci score 1, 2 and 3 vs. 0, respectively; $p<0.01$ for all) and moderate to extensive CTGF positivity (OR 2.48 [1.09-5.61], p=0.03; OR 4.57 [1.33-16.63], p=0.016; OR 2.12 [0.77-5.81], p=0.15 for ci score 1, 2 and 3 vs. 0, respectively). In the subgroup of patients (n=126) with no interstitial fibrosis at 3 months (ci score 0), independent predictors of progression to any degree of interstitial fibrosis (ci score ≥ 1) at 5 years were donor age (OR 1.04 [1.02-1.07], p=0.001), moderate to extensive CTGF positivity (OR 2.68 [1.12-6.42], p=0.027) and urinary CTGF concentration (OR 5.51 [1.52-19.99] per 10-fold increase, p=0.009). Independent predictors of renal function at 5 years were donor age ($p<0.001$), renal function at 3 months ($p<0.001$) and occurrence of biopsy-proven acute rejection in the first 3 months (p=0.019).
Conclusion: CTGF expression in protocol biopsies 3 months after transplantation predicts interstitial fibrosis in protocol biopsies at 5 years, independent of donor age. CTGF staining might contribute to early detection of patients at high risk for chronic allograft injury.

556.3

Effect of epigenetic modifications in graft kidneys progressing to chronic allograft dysfunction

Sai Vineela Bontha[1], Daniel G.Maluf[1], Erika Rhone[2], Kenneth Brayman[1], Valeria Mas[1].
[1]Transplantation, UVA, Charlottesville, VA, United States; [2]Pediatrics / Nephrology, UVA, Charlottesville, VA, United States.

Background: Interstitial fibrosis with tubular atrophy (IF/TA) is a final event leading to graft loss post-kidney transplantation (KT) and represent the result of continuous immunological and non-immunological insults to the graft. DNA methylation is an epigenetic factor that regulates downstream gene expression in response to environmental/metabolic stimuli and may provide important insight into the gene expression pattern associated with allograft dysfunction. Herein, the effect of methylation patterns on gene expression in renal allograft with IF/TA when compared to grafts with normal function and histology and pre-implantation biopsies.
Methods: 62 Illumina 450K methylation and 22 Gene expression (n=22) (Affymetrix HG-U133Av2 Gene Chips) array analyses were performed on samples obtained from allograft nephrectomies/biopsies from KT recipients. 40 pre-implantation tissue samples were classified based on the consequent development of IFTA post-transplantation (n=20 IFTA, n= 20 normal histology). Using 1-Pearson's correlation supervised cluster analysis, the methylation pattern in pre-implantation biopsies was compared to a separate cohort of 22 biopsy samples obtained at 24 months post-transplantation (n=11 IFTA, n=11 normal graft function (NFA)). Gene expression was also donein same biopsies Differentially methylated CpG regions were identified using Minfi Bioconductor package and Subset within array normalization method was used. For gene expression analysis, the two groups were compared using a moderated t-test using the limma Bioconductor package and probe sets were considered significant when the false discovery rate (FDR) was <0.01. DNA methylation and gene expression data integration a probeset analysis were conducted using IPA.
Results: Cluster analysis of differentially methylated CpGs showed all 24 month IF/TA biopsies in one cluster. All of the pre-transplant biopsies and 24 month NFA biopsies formed a separate cluster. This suggests that the change in methylation pattern of allograft may drive progression towards IF/TA. The role of differential allograft methylation on gene expression was addressed by paired methylation and gene expression analysis in biopsies from IF/TA and NFA. A total of 21,351 CpG sites associated with 6235 genes were differentially methylated in renal allografts with IF/TA in comparison to NFA (FDR < 0.01). 48.6% of the CpG sites were hypermethylated and involved genes like NPNT, LAMC1, ENaC, SLC9A1 that are important for repair and functioning of the kidney. The 51.3% that were hypomethylated involved immune response, apoptosis and antigen presentation related genes. Using gene expression analysis 2412 genes were differentially expressed between IF/TA and NFA allografts of which 648 genes had associated CpG sites differentially methylated. Integration analysis resulted in 31 genes that mapped to >10 significant CpG sites. These include genes like TAP1, PSMB8, HLA-DPA1 associated with Antigen presentation pathway (pvalue = 2.66 E-07), PTPN6, PIK3CD associated with cytokine signaling and upstream regulator like SP1 involved in differentiation or activation of B cells and macrophages.
Conclusion: DNA methylation modifications of allografts associate withprogression to IF/TA possibly by regulating genes related to inflammatory response, kidney tissue atrophy and apoptosis.

556.4

Inflammation in scarring on 1 year surveillance biopsies does not change the 5 year prognostic of kidney transplantation

Grégoire Couvrat-Desvergnes[1,2,3], Karine Renaudin [4], Aurélie Le Thuaut[5], Jacques Dantal [2,3], Anne Devys[7], Stéphanie Castagnet[7], Richard Danger[3], Sophie Brouard[3], Yohann Foucher[6], Magali Giral[2,3].
[1]Nephrology, CHD La Roche sur Yon, La Roche sur Yon, France; [2]ITUN, CHU Nantes, Nantes, France; [3]INSERM UMR 1064, University Nantes, Nantes, France; [4]Anatomopathologie, CHU Nantes, Nantes, France; [5]Unité de Recherche Clinique, CHD La Roche sur Yon, La Roche sur Yon , France; [6]EA-SPHERE Laboratory EA 4275 – Biostatistics, Clinical Research and Pharmaco-Epidemiology, University Nantes, Nantes, France; [7]Laboratoire HLA, Etablissement Francais du Sang, Nantes, France.

Introduction: Interstitial Fibrosis and Tubular Atrophy (IFTA) is a common histologic lesion present on one year surveillance biopsies in kidney transplantation. Occurrence of inflammation in the scarred compartment is not a rare situation on this type of biopsy without any other lesion of subclinical rejection or borderline changes. After identifying clinical features associated with IFTA with inflammation in scarring, without superimposed lesions of subclinical rejection, on one-year surveillance kidney biopsies, we studied allograft survival and renal function at 5 years follow-up.
Patients and Methods: We enrolled recipients between 2004 and 2011 with a functionnal kidney and having a one year surveillance biopsies. Biopsies were classified as isolated IFTA with inflammation (infl-isoIFTA) or without inflammation (IFTA) according to the Banff 2011 classification. Normal histology and lesions classically related to alloimmunity (including humoral and/or cellular acute or chronic rejection and borderline changes) were excluded. "infl-isoIFTA" was defined presence of IFTA and inflammation according to the ti-score previously defined in Banff 2007 classification with including the low"i-banff" score (i=0 or 1) without a sufficient scoring to retained borderline changes or TCMR diagnosis.
Results and Discussion: Finally 155 patients were included (76 in infl-isoIFTA group and 79 in IFTA group). We showed that patients who presented infl-isoIFTA shared similar clinico-biological profiles with those with non inflammatory IFTA except a higher one year daily proteinuria (p=0.02). The allograft survival seemed decrease in infl-isoIFTA compared with IFTA alone, but not significantly in multivariate analysis (HR=2.02 [0.82-4.94]; p=0.12). Patients with infl-isoIFTA did present a similar decrese of renal function evolution of e-GFR (coeff= -0.18) throughout the first 5 years of follow-up than patients with IFTA (coeff= -0.14) (p=0.216).
This is the first study that evaluates graft outcome of patients with infl-isoIFTA in systematic biopsies, contrary to previous publicions concerning the impact of the "classical immunologic inflammation" i-banff[1],[2]. Moreover all studies concerning inflammation in scarring, on biopsies for clinical indications, analyzed infiltrates disclosing other damage (glomerular, TCMR, ABMR…) but never excluding the lesions due to allo-immune processes[3],[4].
Conclusion: Thus occurrence of inflammatory infiltrates in the scarred compartment observed for one-year surveillance biopsies seems to be insufficient by itself to justify immunosuppressive therapeutic changes.
References:
[1] Cosio FG. Am. J. Transplant. 2005 Oct; 5(10): 2464–72.
[2] Park WD. J. Am. Soc. Nephrol. 2010 Nov; 21(11): 1987–97
[3] Mengel M. Am. J. Transplant. 2009 Aug; 9(8): 1859–67.
[4] Mannon RB. Am. J. Transplant. 2010 Sep; 10(9): 2066–73.

556.5

The One year Renal Biopsy Index (1-RBI): a clinical marker to screen patients for one year surveillance biopsy indication after the transplantation

Magali Giral[1], Karine Renaudin[2], Christophe Legendre[3], Henri Kreis[3], Emmanuel Morelon[4], Fanny Buron[4], Maartens Naesens[9], Redmer Luning[9], Alexandre Huneau[5], Chloe Paul[6], Sophie Brouard[6], Grégoire Couvrat-Desvergnes[17], Yohann Foucher[8], Etienne Dantan[8].
[1]Kidney Transplantation , Nantes University Hospital, Nantes, France; [2]Anatomo Pathology, Nantes University Hospital, Nantes, France; [3]Kidney Transplantation, Necker University Hospital, Paris, France; [4]Kidney Transplantation, Lyon University Hospital, Lyon, France; [5]Biostatistics, Nantes University , Nantes, France; [6]Kidney Transplantation, Inserm 1064, Nantes, France; [7]Nephrology, La Roche sur Yon Hospital, La Roche sur Yon, France; [8]Biostatistics, Nantes University, Nantes, France; [9]Kidney Transplantation , Leuven University Hospital, Leuven, Belgium.

Introduction: Surveillance biopsy remains debated after renal transplantation since the histological diagnostic do not lead to clear therapeutic recommendations. The objective of our study was to propose a medical decision signature to predict histological lesions on one year surveillance biopsy for which therapeutic intervention could be considered.
Patients and Methods: From the French multicenter DIVAT cohort, we studied 467 adult recipients of kidney, transplanted between 2006 and 2012 from deceased donor. From one year surveillance biopsy, patients displaying major histological lesions of "allo-immunity" (i.e. Borderline, acute or chronic humoral and/or cellular rejection), IFTA grade 2 or 3 and glomerulonephritis composed the Interventional Group (IG) that was compared to the Non-Interventional Group (NIG) including minor lesions (i.e. normal histology or IFTA grade 1). The predictive signature was identified using a lasso penalized logistic regression and internally validated by ROC 0.632+ bootstrap. A discriminating threshold was proposed from predictive values and externally validated from an independent cohort of 645 French and Belgian kidney recipients.
Results: 229 patients (49%) were considered in the interventional group among the 467 recipients. The predictive signature, named 1-Year Renal Biopsy Index (1-RBI), included 5 variables. Women recipient (OR=2.27), patients with current pre-transplantation anti-class I immunization (OR= 1.26), those receiving transplant from a male donor (OR=1.20), those who displayed high serum creatinine level at 6 month (OR=1.34) and 12 months (OR=1.53) have a higher probability to belong to the IG, than to be classified NIG. Area under the associated ROC curve was estimated at 0.69. In a medical decision making perspective, we assumed a minimal positive predictive value at 80% leading to a discriminating threshold at 0.44. This medical decision rules was internally and externally validated.
Conclusion: The use of the 1-RBI could be proposed for medical teams that do not perform routine surveillance 1 year biopsy in their follow-up policy. It may help physicians to recommend 1-year surveillance biopsy with a good confidence to patient presenting a 1-RBI higher than the discriminating threshold since they are considered at high risk of abnormal histological lesions. The 1-RBI calculation is possible from online calculator (www.divat.fr/en/online-calculators/1-RBI).

556.6

Angiotensin system blockade reduce proteinuria in kidney transplant recipients: A meta-analysis

Guodong Chen, Longhui Qiu, Changxi Wang, Jiang Qiu, Lizhong Chen.
Organ Transplant Center, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, People's Republic of China.

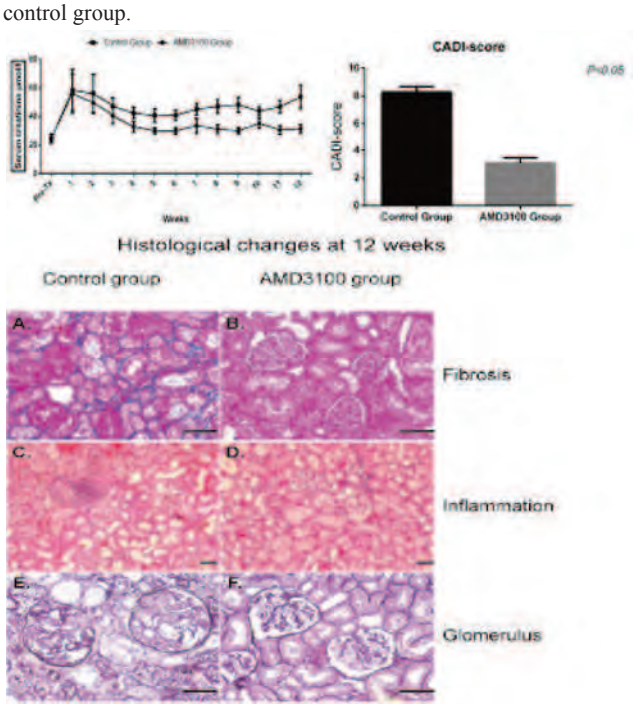
Background: Proteinuria is a common complication after kidney transplantation. Angiotensin system blockadeincluding Angiotensin-Converting Enzyme Inhibitors (ACEI) or Angiotensin Receptor Blockers (ARB), have been reported to be effective on reducing proteinuria in chronic kidney diseases. We carried out this meta-analysis to evaluate theanti-proteinuric effect of angiotensin system blockade on kidney transplant recipients.
Methods: Databases including Pubmed, Embase, Scopus, Cochrane Library, and Web of Knowledge were comprehensively retrieved. 17 randomized controlled trials (RCTs) involving 1299 kidney transplant recipients were contained.Both Review Manager 5.2 and Stata Version 18.0 were utilized in trial synthesis.
Results: Our results showed that comparing with other hypertensive agents or placebo, either short-term or long-term treatment with ACEI or ARB significantly decreased proteinuria(mean difference [MD] -0.30 g/24h, 95% confidence intervals [CI] -0.41 to -0.20, $\rho<0.00001$, $I2 = 44\%$),with no significantchange in serum creatinine (Scr) levels. However, we found no significant difference in graft failure or patient mortality between ACEI/ARB and control group. Hemoglobinand glomerular filtration rate (GFR) level were decreased in ACEI/ARB group, while serum potassium level was significantly higher in ACEI/ARB group.
Conclusion: ACEI or ARB caneffectively reduce proteinuriain kidney transplant recipients.However, they can not improve long-term graft and patient survival.

556.7

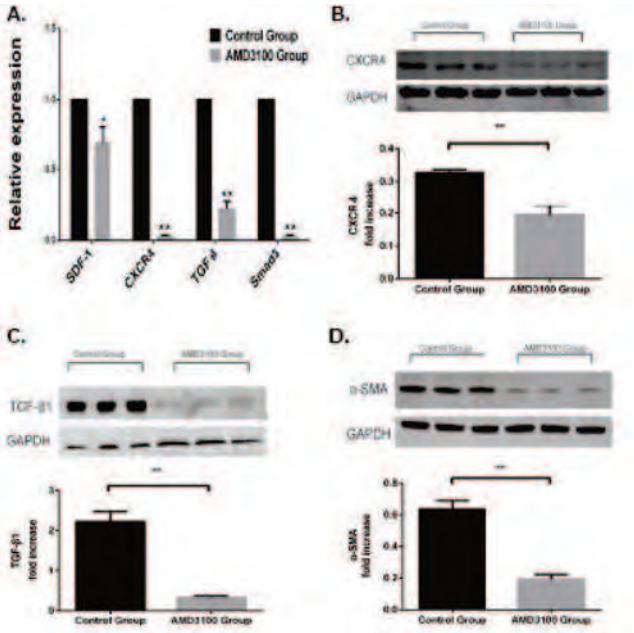
Chemokine receptor CXCR4 antagonist, a novel pathway to prevent chronic allograft nephropathy.

Yue Xu, Qiang Zhang, Wenrui Xue, Zijian Zhang, Song Zeng, Xiaopeng Hu.
Urology, Beijing Chao-yang hospital, Beijing, People's Republic of China.

Background: Chronic allograft nephropathy (CAN) remains a major problem for long term graft survival and different pathways participate in its development. CXC chemokine receptor 4 (CXCR4) is significantly upregulated following renal injury and fibrotic response. We investigated the effect of AMD3100, a CXCR4 antagonist, on the development of CAN in rat models.
Methods: CAN rat models (n=20) were established using male Fisher 344 to Lewis rats. Rats in experimental group (n=10) were treated by AMD3100 (1mg/kg/day subcutaneously, 0-12 weeks), rats in control group (n=10) were treated by saline. The serum creatinine levels were monitored every week. Kidney grafts were harvested 12 weeks after modeling for histological analysis. We used chronic allograft damage index (CADI) scores to evaluate each group. QPCR and western blotting were used to measure SDF-1/CXCR4 axis, TGF-β1/Smad3 signaling pathway and α-smooth muscle actin (α-SMA) expression in renal allograft tissue.
Results: CXCR4 expression was increased significantly in control group which developed intense chronic changes after 12 weeks. Histological changes of CAN in experimental group were ameliorated by AMD3100 which also made better graft function compare to the control group.



AMD3100 significantly blunted the increase in the mRNA expression level of SDF-1/CXCR4 axis, TGF-β1 and Smad3. A significant reduction in TGF-β1 and α-SMA protein content was observed only in experimental group as shown in a representative western blot.
Conclusion: Based on these findings, CXCR4 expression may mediate in part the development of CAN. AMD3100 may ameliorate histological changes of CAN and maintain better allograft function. Its treatment blunts downstream effects of TGF-β1 signaling and fibroblast activation. Therefore, antagonism of CXCR4 may provide a novel way to prevent the development of CAN.



This work was supported by the National Natural Science Foundation of China (No.81270837), the Beijing Natural Science Foundation (No. 7132107), and Beijing Municipal Administration of Hospitals Clinical Medicine Development of Special Funding Support (ZYLX201408)

556.8

Peripheral NK-cell repertoire distribution in chronic allograft dysfunction after renal transplantation

Sailaja Kesiraju[1,3], Uma Maheswara Rao Ch[2], Purna Paritala[2], Shreedhar Reddy[2], V.S. Reddy[2], Sahariah Sarbeshwar[2].
[1]Dept of Immunology , Bhagwan Mahavir Medical Centre, Hyderabad, India; [2]Transplantation Immunology& Research Centre, Transimmun, Hyderabad, India; [3]Department of Nephrology and Transplantation, Krishna Institute of Medical Sciences, Hyderabad, India.

Introduction: Recent studies demonstrated that NK-cells play a major role in acute and chronic rejection /tolerance of allograft. Nkcells have a potent cytolytic function and can produce pro-inflammatory cytokines. It has been noted that NK-cells become activated early after transplantation and can contribute in the development of cell mediated allo response by secreting pro-inflammatory cytokines thereby causing acute rejection. Antibody mediated rejection is associated with microvascular damage, and NK-cell antibody dependent cell mediated cyto-toxicity (ADCC) could be one of the contributing factors. Recent work has clearly recognized donor specific antibodies (DSA) as the main cause of chronic rejection.
Aim: Assessing distribution of NK and NKT cells and the possible relationship between peripheral NKcell subsets and circulating donor-specific and non-donor-specific anti HLA class I&II antibodies in the development of chronic allograft dysfunction.
Methods: Prospective observational study of 61 kidney transplant recipients who have been transplanted more than one year and whose graft biopsy was performed. Of them, 18 patients were with DSA class II anti-HLA, 21 were with DSA class I anti-HLA and 22 were without HLA antibodies: all of the former were compared with 18 healthy donors. Analysis of anti HLA antibodies was performed by ELISA. The percentages and absolute counts of Tcell (CD3+), natural killer (CD3-CD16CD56+), NKT(CD3+/CD56+) lymphocytes in erythrocyte lysed whole blood NKG2A in peripheral blood mononuclear cells(PBMC) were performed using multi-test CD3FITC/CD16+CD56PE/ with a four colour direct immunofluorescent reagent. Cytokine levels of IFNγ, TNFα TGFβ and IL10 were estimated by ELISA method.
Results and Discussion: Nk-cell phenotypes differ significantly between healthy donors and kidney transplant recipients. There was no significant difference in the percentage of NK-cells in the lymphocyte population among these patients. However patients with DSA had significantly lower NK-cell numbers compared to patients without any HLA antibodies. Lower number of NKcells and presence of donor alloantigens promotes the adaptive immune responses. The number of NKG2A was increased while their percentage decreased in both the groups containing anti HLA antibodies. Cytokine levels were low when compared to healthy donors: especially IL-10 levels, which were significantly low (p=>0.05)
Conclusion: Decreased number of NKcell repertoire supports the hypothesis that there is a functional link between DSA and NKcells, and that DSA are strongly associated with chronic rejection. Identifying the molecular mechanisms that trigger the rejection process and tissue injury could facilitate individualized adjustment of immunosuppression.
References:
[1] Christine Neudoerfl, Bernadett J. Mueller, et al. **The peripheral NK cell repertoire after kidney transplantation is modulated by different immunosuppresive drugs.** *Front in Immunol* , 2013 Feb.|Vol 4|Article 46

556.9

Progressive allograft dysfunction is associated with an early (12 months) increase in levels of VEGF-C, PLGF and follistatin after renal transplantation

G.J. Dreyer[1], J.W. de Fijter[1], DM Briscoe[2], K.P. Daly[2], M.E.J. Reinders[1].
[1]Nephrology, LUMC, Leiden, Netherlands; [2]Transplant Research Program, Boston Children's Hospital, Boston, MA, United States.

Introduction: The integrity of the microvasculature is critical for long term survival after solid organ transplantation and biomarkers of endothelial injury and repair have been reported to be associated with chronic rejection after cardiac allograft vasculopathy. Here, we investigate if early angiogenic biomarkers are predictive of later allograft dysfunction in a large cohort of renal transplant recipients.
Methods: Levels of 17 angiogenic proteins and donor specific HLA-antibodies (DSA) were measured by multi analyte profiling 12 months after renal transplantation in sera of 152 recipients. Forty five patients had progressive renal dysfunction, defined as at least 20 ml/min/1.73m2 eGFR loss between year 1 and 5 (mean baseline eGFR 54±22 ml/min/1.73m2) post transplant;107 control patients had stable function (mean baseline eGFR 48±15 ml/min/1.73m2) during this follow up period. All patients had standard immunological risk and started on triple therapy with calcineurin inhibitors. Six months post-transplant 33 patients switched to dual therapy, including 13 with mTOR inhibitors.
Results: 5-year death-censored graft survival was 100% in the control group and 79.2% in the progressors. The angiogenic and pro-inflammatory factors VEGF-C (median(IQR) = 276(372) vs 183(274) resp, p=0.029) and PLGF (median(IQR) = 16(37) vs 10(26) resp, p=0.019) were increased in the progressors, independent of treatment regimen. Also Follistatin, a promoter of tubular regeneration, was significantly increased in recipients with progressive renal dysfunction (median(IQR) = 1146(1060) vs 826(1341) in controls, p=0.033). Remaining biomarkers including endothelin1, FGF1/2 and VEGF-A showed no association with loss of renal function. In total 46 patients had allograft rejection. Late, but not early rejection, was associated with progressive renal decline at 5 years (OR 3.15, 95%CI 1.27–7.81, p=0.013). De novo DSAs were found in 22 patients (14.5%), however no association with biomarkers was found.
Conclusion: Increased levels of VEGF-C, PLGF and Follistatin 12 months post-transplant were associated with progressive renal dysfunction at 5 years. These specific biomarkers have been reported to play important roles in endothelial and lymphatic angiogenesis and inflammation and have the potential to identify patients with progressive renal dysfunction at early times post-transplant.

557.1

Quantification of lymphatic flow using SPECT/CT lymphoscintigraphy reveals increased lymph flow during chronic rejection

Lindsey A.Edwards, Anna K. Nowocin, Nazila V.Jafari, Lucy L.Meader, Kathryn Brown, Aurélien Sarde , Carolyn Lam, Alex Murray, Wilson Wong.
MRC Centre for Transplantation, Division of Transplantation Immunology & Mucosal Biology, King's College London, School of Medicine at Guy's, King's and St. Thomas' Hospitals, London, United Kingdom.

Background: Chronic rejection leading to cardiac allograft vasculopathy (CAV) remains a major cause of cardiac graft loss[1]-[3]. The lymphatic system plays a crucial role in the alloimmune response by facilitating trafficking of antigen presenting cells to draining lymph nodes, where they encounter T lymphocytes and initiate alloimmunity[4]. Donor lymphatic vessels are not anastomosed to that of the recipient during transplantation. The pathophysiology of lymphatic disruption is unknown and whether disruption enhances or hinders the alloimmune responses is unclear[5]. Although histological analysis can yield structural information of the lymphatics, the function following cardiac transplantation is completely unknown.
Methods: Female C57BL/6 $EYFP^+$ recipient mice received either wild-type gender mismatched male or female heart grafts. The male antigen HY evokes a chronic rejection response, resulting in CAV[6]. Using Single photon emission computed tomography/CT (SPECT/CT) lymphoscintigraphy we quantified the lymphatic flow index following cardiac transplantation[7]. Subsequently, we quantified the number of male donor cells trafficking to the draining mediastinal lymph nodes using the Y-chromosome specific gene, Zfy1, amplified by QPCR[8]. Immunohistochemistry was performed to determine the origin of lymphatic vessels (donor or recipient), number of infiltrating inflammatory cells and allograft vasculopathy.
Results and Discussion: Following transplantation of HY-mismatched heart grafts, the lymphatic flow index was significantly increased compared with gender-matched controls. Furthermore, the enhanced lymphatic flow index strongly correlated with an increased in male donor cells in the draining mediastinal lymph nodes (p<0.0001). Lymphatic density by LYVE-1 expression was higher in HY-mismatched grafts indicative of lymphangiogenesis and the lymphatic flow index correlated with vessel area. However, no correlation was observed between vessel number and donor cells trafficking to the draining mediastinal lymph nodes; the lymphatic flow index; or the number of inflammatory cells infiltrating the graft. The increased percentage of male donor-derived cells correlates with enhanced numbers of $CD8^+$ T cells within the donor graft (p=0.0081); In turn, the number or $CD8^+$ T cells strongly correlates with the lymphatic flow index (p<0.0001) and the level of CAV, determined by vessel occlusion (p<0.0001). Therefore, in our model the lymphatic flow index provided a good predictor of CAV.
Conclusions: SPECT/CT lymphoscintigraphy allows quantitative measurement of lymphatic function following transplantation. Increased lymphangiogenesis is observed during chronic rejection and this is associated with increased lymph flow towards draining lymph nodes. Lymph flow from donor grafts correlates with donor passenger cells trafficking to lymph nodes as well as graft inflammatory infiltration, suggesting a cause and effect relationship.
This work was funded by the British Heart Foundation grant number: PG/13/29/30121
References:
[1] Yin N, Zhang N, Xu J, Shi Q, Ding Y, Bromberg JS (2011) Targeting lymphangiogenesis after islet transplantation prolongs islet allograft survival. Transplantation 92(1):25–30
[2] Lund LH *et al.* The Registry of the International Society for Heart and Lung Transplantation: Thirty-first Official Adult Heart Transplant

Report 2014; Focus Theme: Retransplantation. The Journal of Heart and Lung Transplantation 33(10):996-1008
[3] Nykanen AI *et al.* Targeting lymphatic vessel activation and CCL21 production by vascular endothelial growth factor receptor-3 inhibition has novel immunomodulatory and antiarteriosclerotic effects in cardiac allografts. Circulation 2010 March 30;121(12):1413-22.
[4] Ochando JC *et al.* Alloantigen-presenting plasmacytoid dendritic cells mediate tolerance to vascularized grafts. Nat Immunol 2006 June;7(6):652-62.
[5] Kathryn Brown, Lindsey A. Edwards, Wilson Wong Imaging the Lymphatic System in Heart Transplantation and Its Immunological Implications. Chapter 7, The Cardiac Lymphatic System. Springer ISBN 978-1-4614-6774-8
[6] Hasegawa T *et al.* Heterotopic vascularized murine cardiac transplantation to study graft arteriopathy. Nat Protoc 2007;2(3):471-80.
[7] Brown K *et al.* SPECT/CT lymphoscintigraphy of heterotopic cardiac grafts reveals novel sites of lymphatic drainage and T cell priming. Am J Transplant 2011 February;11(2):225-34.
[8] An N, Kang Y. Using quantitative real-time PCR to determine donor cell engraftment in a competitive murine bone marrow transplantation model. J Vis Exp 2013;(73):e50193.

557.2

Receptor tyrosine kinase mer mediates expansion of MDSCs critical for transplantation tolerance

Lei Zhang[1], Mathew DeBerge[2], Xiaomin Zhang[3], Jiaojin Wang[3], Zheng Zhang[3], Edward Thorp[2], Xunrong Luo[1,2,3].
[1]Division of Nephrology and Hypertension, Northwestern University Feinberg School of Medicine, Chicago, IL, United States; [2]Department of Microbiology and Immunology, Northwestern University Feinberg School of Medicine, Chicago, IL, United States; [3]Comprehensive Transplant Center, Northwestern University Feinberg School of Medicine, Chicago, IL, United States.

Introduction: The TAM (Tyro3, Axl and Mer) receptor tyrosine kinases (RTKs) mediate homeostatic phagocytosis of apoptotic cells, and transmit regulatory signals that modulate immune response. Currently, their role in transplant tolerance is unknown.
Methods: In this study, we address the role of Mer in transplant tolerance in a BALB/c à B6 heart transplant model in which tolerance is induced by recipient treatment with donor splenocytes (SP) treated with 1-ehtyl-3-(3-dimethylaminopropyl) carbodiimide (ECDI).
Results: While baseline expression of Mer was primarily only detected on the red pulp macrophages, recipient mice treated with donor ECDI-SP significantly up-regulated Mer expression on both the CD68+SIGN-R1+ marginal zone macrophages and CD68+CD169+ metalophilic macrophages. This up-regulation was most prominent in macrophages that had phagocytosed the injected donor ECDI-SP. To determine the role of Mer in mediating transplant tolerance by donor ECDI-SP, we utilized Mer-/- mice. Bone marrow derived macrophages (BMDM) from Mer-/- and Mer+/+ were cultured with allogeneic ECDI-SP. While Mer+/+ BMDM exhibited marked up-regulation of M-CSF and essentially completely inhibited expressions of IFN-g and TNF-a following culturing with allogeneic ECDI-SP, Mer-/- BMDM exhibited an opposite pattern, i.e. inhibited up-regulation of M-CSF but marked up-regulation of IFN-g. This altered pattern of expressions of M-CSF and inflammatory cytokines correlated with an absence of the expansion of myeloid derived suppressor cells (MDSCs) that were otherwise induced in recipients treated by donor ECDI-SP. Consistent with a critical role of Mer signaling in the induction of transplant tolerance, recipient Mer deficiency completely abolished allograft protection provided by donor ECDI-SP.
Conclusion: The receptor tyrosine kinase Mer plays a critical role in tolerogenic signaling in splenic macrophages, MDSC expansion, and consequently in the induction of transplantation tolerance.

557.3

The role of PI3kγ and PI3kδ in alloimmunity

Mayuko Uehara[1], Azzi Jamil[1], Shunsuke Ohori[1], Zhabiz Solhjou[1], Martina McGrath[1], Naima M.Banouni[1], Catherine A.Evans[2], Jonathan P.Dinitto[2], David G.Winkler[2], Reza Abdi[1].
[1]Transplantation Research Center, Renal Division, Brigham and Women's Hospital/Harvard Medical School, Boston, MA, United States; [2]Infinity Pharmaceuticals, Inc, Boston, MA, United States.

We studied the role of phosphatidylinositol-3-kinases (PI3k) γ and δ pathway in alloimmunity. Generated Foxp3-GFP-PI3kγ and -PI3kδ knockout mice (C57BL/6 background) were used in heart transplant models and GVHD models. Multiple experiments were done using FACS sorting, flow and phospho-flow, ELISpot, Luminex and Western Blot assays. While PI3kγ$^{-/-}$ and PI3kδ$^{-/-}$ recipients of BALB/c hearts exhibited significant heart allograft survival prolongation compared to WT (MST: 14 vs. 14 vs. 7 days, respectively), the administration of single dose CTLA4Ig (sCTLA4Ig: 250μg on day 2) induced indefinite allograft survival of the PI3kγ$^{-/-}$ recipients compared to WT (MST: >100 vs. 41 days, respectively) with a marked increase in Tregs and a reduced % of CD4^{+} and CD8^{+} Teff along with significant suppression of Th1/Th17 cytokines in the spleen and draining lymph nodes (DLN). Surprisingly, the absence of PI3kδ abrogated the effect of CTLA4Ig treatment with a marked decrease in Tregs with significant increase in CD4^{+} and CD8^{+} Teff in the spleen and DLN of the PI3kδ$^{-/-}$ recipients along with significant upregulation of Th1/Th17 cytokines. Adoptive transfer of Foxp3-GFP-PI3kδ$^{-/-}$ and Foxp3-GFP-PI3kγ$^{-/-}$ Tregs into a GVHD model showed that PI3kδ$^{-/-}$ Tregs went more into apoptosis compared to PI3kγ$^{-/-}$ and WT Tregs. We also examine the effect of pharmacologic inhibition of PI3k isoforms using IPI-1828 (PI3kδ selective inhibitor) and INK-055 (PI3kγ and PI3kδ inhibitor). BALB/c heart allografts were transplanted into C57BL/6 and PI3kγ$^{-/-}$ mice respectively and each recipient was treated with IPI-1828 or INK-055 with or without sCTLA4Ig respectively. WT and PI3kγ$^{-/-}$ recipients treated with IPI-1828 and PI3kγ$^{-/-}$ recipients treated with IPI-1828 plus sCTLA4Ig showed similar allograft survivals as previous model (MST: 13.5 vs. 20 vs. >100 days, respectively). Furthermore, the allograft survival of WT recipients treated with INK-055 plus sCTLA4Ig confirmed that PI3kδ abrogates the effect of CTLA4Ig treatment. Using double knockout recipients (CD28$^{-/-}$PI3kγ$^{-/-}$ and CD28$^{-/-}$PI3kδ$^{-/-}$ mice), allograft histology was observed and showed less lymphocyte infiltration in CD28$^{-/-}$PI3kγ$^{-/-}$ recipient compared with CD28$^{-/-}$PI3kδ$^{-/-}$. Allograft survival data also reflected the histology data of CD28$^{-/-}$ PI3kγ$^{-/-}$ and CD28$^{-/-}$PI3kδ$^{-/-}$ (MST: >30 vs. 27.5 days, respectively). Our data shows a differential role of PI3kγ and PI3Kδ in Tregs homeostasis and function with significant application in the future of PI3k based therapies in solid organ transplantation.

557.4

Regulatory T cells with HLA-A*02-specific chimeric antigen receptors can control strong allogeneic immune responses and induce operational tolerance

Fatih Noyan[1], Katharina Zimmermann[1], Matthias Hardtke-Wolenski[1], Ann-Kathrin Knoefl[2], Elvira Schulde[1], Robert Geffers[3], Michael Hust[4], Jochen Huehn[5], Melanie Galla[6], Michael Morgan[6], Andreas Jokuszies[7], Michael PManns[1], Elmar Jaeckel[1].
[1]Dept. Gastroenterology, Hepatology & Endocrinology, Hannover Medical School, Hannover, Germany; [2]Dept. Cardiothoracic, Transplantation and Vascular Surgery, Hannover Medical School, Hannover, Germany; [3]Dept. of Genome Analytic, Helmholtz Center for Infetious Research, Braunschweig, Germany; [4]Dept. Biotechnology, Technical University Braunschweig, Braunschweig, Germany; [5]Dept. Experimental Immunology, Helmholtz Center for Infectious Research, Braunschweig, Germany; [6]Dept. Experimental Haematology, Hannover Medical School, Hannover, Germany; [7]Dept. of Plastic Surgery, Hannover Medical School, Hannover, Germany.

Introduction: CD4+CD25high FOXP3+ regulatory T cells (Tregs) are involved in graft-specific tolerance after solid organ transplantation. Recent data from Todo et al. has proven the clinical feasibility of Tregs-supported weaning, by achieving operational tolerance in 7/10 patients after liver transplantation. However, the adoptive transfer of polyspecific Tregs alone was so far unable to prevent graft rejection even in rodent models. Instead graft-specific Tregs will be needed. This poses the question how to obtain large numbers of stable, donor-reactive Tregs with long-term survival after adoptive transfer. We have developed a highly specific chimeric antigen receptor recognizing the HLA molecule A*02 (referred as A2-CAR).
Methods: The A*02-specific single chain variable fragment (scFv) was obtained from a phage display library generated from an A*02 sensitized patient. It was highly specific for A*02 alleles. The scFv was cloned into a new 2nd generation CAR cassette consisting of a modified CD8 hinge and CD28/CD3 intracellular activation motifs.
Results: We could demonstrate surface expression and strong signal transduction of the A2-CAR. Transduction into nTregs changed the specificity of the Tregs without changing the regulatory phenotype of the cells as assessed by transcriptome analysis. nTreg activation via the A2-CAR caused strong activation, proliferation and suppressor function of modified nTregs. A2-CAR Tregs were superior to nTregs in controlling strong allospecific immune responses in vitro and in humanized mouse models. They completely prevented the rejection of allogeneic targets in immune reconstituted humanized mice in the absence of any immunosuppression (operational tolerance). They could be generated with a short in vitro transduction protocol without the need for prolonged in vitro expansion.
Conclusion: A2-CAR Tregs therefore offer the possibility to induce graft-specific tolerance without the perturbation of the general immune competence of the recipient. They might have a huge potential in clinical trials of Treg supported weaning in HLA-A*02- recipients receiving an HLA-A*02+ graft. The findings could be further extended to other HLA-mismatch transplantations.

557.5

miRNA-181 promotes graft prolongation by plasmacytoid dendritic cells by decreasing marginal zone B cells and plasmablasts

Audrey H.Lau[1,2], Matthew Vitalone[2], Xiumei Qu[2], Todd Shawler[2], OliviaM. Martinez[2], Carlos O.Esquivel[2], Sheri M.Krams[2].
[1]Department of Pediatrics, Division of Gastroenterology, Hematology and Nutrition, Stanford University, Stanford, CA, United States; [2]Department of Surgery, Division of Abdominal Transplantation, Stanford University, Stanford, CA, United States.

Introduction: Liver allografts are well tolerated and other solid organ allografts, when transplanted concurrently with livers, show improved outcomes. However, the mechanisms underlying "hepatic tolerance" have yet to be elucidated. Previous data show that hepatic dendritic cells (DC) have diminished antigen presentation and immune stimulatory function compared with DC in lymphoid tissue. Immature plasmacytoid (p)DC have been shown to induce graft prolongation and we have previously demonstrated that the microRNA, miR-181 is increased in pDC ($CD11c^+CD11b^-CD317^+$) as compared to conventional DC $CD11c^+CD11b^+CD317^-$).
Materials and Methods: BALB/c mice were pre-treated with hepatic C57Bl/6J (wild-type;WT) pDC (n=5) or hepatic C57Bl/6J miR-181a1b1$^{-/-}$ (KO) pDC (n=3) seven days before performing allogeneic (C57BL/6) vascularized heterotopic heart transplantation and graft survival followed. To determine the mechanism of graft prolongation, splenocytes were obtained at Day 7 post-transplantation and cytometry by time-of-flight (CyTOF, mass cytometry) was utilized to comprehensively characterize the immune response (on the basis of the expression of 17 surface and three intracellular markers). Correlation analyses of molecular features derived from mass cytometry data were performed using Citrus, a method for unsupervised identification of significant cellular populations.
Results: Allograft recipients pre-treated with hepatic pDC (n=5) had significantly prolonged allograft survival to day 17.6 ± 1.4 days as compared to the control group (n=4) which were not treated with pDC and rejected their allografts by day 7 (Mantel-Cox log rank for 2 groups, $p \leq 0.005$). However, recipients pre-treated with hepatic KO pDC (n=3), which lack expression of miR-181a1b1 rejected their allografts by day 8 ± 0.6 days (Mantel-Cox log rank for 3 groups, $p \leq 0.005$). Splenocytes were analyzed by mass cytometry and it was determined that a B cell population was elevated in the rejecting KO group (WT 0.99±0.18% vs. KO 2.76±0.11%, $p \leq 0.0005$). Further analysis of B cell subsets revealed a significant increase in marginal zone B cells (2.18±0.14% vs. 3.31±0.56%; $p \leq 0.05$) and plasmablasts in (0.26±0.09% vs. 0.59±0.07%; $p \leq 0.05$) in the absence of miR-181a1b1 (KO group) and a significant ($p \leq 0.01$) upregulation in IgG1 subclass antibodies as compared to the recipients that received miR-181 expressing pDC and had prolonged graft survival.
Conclusions: Our findings suggest that miR-181 expressing pDC prolong graft survival by limiting B cell activation and antibody production. Further studies are necessary to determine if, pDC and miR-181 can prevent donor specific antibody formation. Further, our findings demonstrate that CyTOF is a powerful new technology to identify immune cell populations that alter outcomes post-transplant.

557.6

Molecular landscape of allograft rejection: definitive mapping of shared and selective molecules for T cell-mediated rejection and antibody-mediated rejection

Philip Halloran[1,2], Jeffery Venner[2], Konrad Famulski[1,2].
[1]University of Alberta, Edmonton, AB, Canada; [2]Alberta Transplant Applied Genomics Centre, Edmonton, AB, Canada.

While the molecules selective for T cell-mediated rejection (TCMR) and antibody-mediated rejection (ABMR) are important, understanding the complete molecular phenotype requires that we also define the molecules shared by ABMR and TCMR. The present analysis mapped the shared and selective features in microarray results from 703 kidney transplant indication biopsies (Clinicaltrials.gov NCT 01299168).
First we mapped "Rejection Landscape" in a comparison between two classes: all rejection (TCMR, ABMR, and mixed) and all other biopsies (Figure 1), plotting association strength (p value; x axis) vs. fold change (y axis). Of the top 30 rejection-associated transcripts, 27/30 were IFNG-inducible molecules (e.g. chemokines CXCL9,10,11; guanylate-binding proteins GBP1,4,5; enzymes IDO and WARS) and three were shared by NK cells and effector T cells (CCL4, KLRD1, and PRF1). Many of those were used by a Rejection classifier to assign a Rejection probability score to each biopsy. The Rejection score was high in biopsies with histologic ABMR and TCMR, and correlated with the defining histologic lesions of both. TCMR had high Rejection and TCMR scores, and ABMR had high Rejection and ABMR scores.

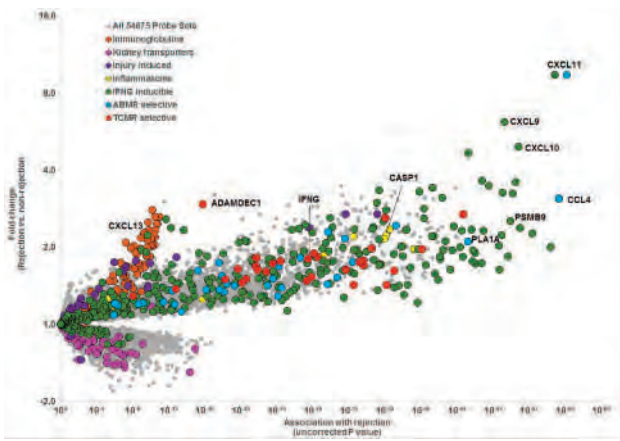


Table 1. "Top molecules"
Features most strongly associated (p value) with rejection syndromes

		Algorithm	Top genes	Examples
#1	All rejection vs. Others	ABMR vs. EE*	IFNG-induced; shared T/NK	CXCL9, 10, 11; IDO, WARS; CCL4, KLRD1
#2	ABMR vs. Other except TCMR	ABMR vs. EE, no TCMR	IFNG-induced; T/NK	Combined 1 and 3 features
#3	ABMR-selective vs. All others including TCMR	ABMR vs. EE; incl.* TCMR	NK and endothelial activation; selective IFNG effects	SH2D1B; ROBO4; PLA1A
#4	TCMR vs. Other except ABMR	TCMR vs. EE, no ABMR	T, DC, macrophage activation	Combined 1 and 5 features
#5	TCMR-selective vs. All others including ABMR	TCMR vs. EE, incl. ABMR	T, DC, macrophage activation; selective IFNG effects	CTLA4, SLAMF8; IFNG, ADAMDEC1, ANKRD22

*EE=everything else; incl. = including

Next, we assembled a complete list of Rejection, ABMR, and TCMR molecules using five algorithms - various combinations of ABMR,

TCMR, and all others ("everything else", EE) (#1-#5: Table 1). The selection of top transcripts was highly dependent on the details of the class comparison algorithm, and whether rejection-processes were included or excluded from the comparator. Many of the top shared molecules reflect elements conserved between the cognate recognition/signaling systems: for TCMR, T cell receptor-MHC-antigen presenting cells; and for ABMR, NK CD16a-IgG-MHC-endothelium, both triggering IFNG release.
The principle is that rejection mechanisms mapped by gene expression must use multiple algorithms to identify disease-selective and shared features to map the complete phenotype. "Top rejection gene" lists will always be some variation of the lists generated by these 5 algorithms, dependent on case mix in the positive and negative classes compared. These changes have been validated in new biopsy sets, and class comparisons in hearts transplants (clinicaltrials.gov NCT02670408) suggesting that the molecular phenotype of rejection will be similar in all organ transplants.

557.7

Exosomes released into the circulation following lung and heart transplantation: Composition and role in allograft rejection

Thalachallour Mohanakumar[1,2], Muthukumar Gunasekaran[1,2], Monal Sharma[1,2], Zhongping Xu[1,2], Deepak Nayak[1,2], Wei Liu[1,2], Nancy Steward[2], Nicholas Benshoff[2], Ramsey Hachem[3], Rajat Walia[1], Michael Smith[1], Ross Bremner[1].
[1]Norton Thoracic Institute, St. Joseph's Hospital and Medical Center, Phoenix, United States; [2]Surgery, Washington University School of Medicine, St. Louis, United States; [3]Medicine, Washington University School of Medicine, St. Louis, United States.

Introduction: Exosomes are nano-vesicles (40-100nm in diameter) often released into the circulation by stressed cells. The goals of this study are to determine whether exosomes are released into circulation following human lung and heart transplants, define the kinetics of release towards its use as a biomarker for rejection, and characterize the composition using both clinical specimens and murine models of allograft rejection.
Materials and Methods: Exosomes were isolated from sera and broncho-alveolar lavage (BAL) by ultra-centrifugation method from human lung transplant recipients (LTxR) and cardiac allograft recipients diagnosed with coronary artery vasculopathy (CAV). Purity of the isolated exosomes was determined by flow cytometry using CD63 and by western blot by Annexin-V. Expression of surface antigens were determined including HLA and tissue restricted self-antigens (Collagen-V and K-alpha 1 tubulin for lung, cardiac myosin and Vimentin for heart) by flow cytometry and electron microscopy. Micro-RNA (miRNA) content was determined by Affymetrix array and pathway analysis with DIANA-mirpath. Quantitative RT-PCR was performed for differentially regulated miRNA validated in independent cohorts. Murine models of chronic rejection of lungs and hearts were also analyzed for exosome formation and role in rejection.
Results and Discussion: Exosomes containing mismatched donor HLA and lung self-antigens were detected from sera and BAL from LTxR (10 patients each) diagnosed with acute rejection (AR) and chronic rejection (Brochiolitis Obliterans Syndrome (BOS)) 3 months before the diagnosis of AR and 6 months prior to BOS suggesting its use as a biomarker for rejection. Exosomes from 10 stable LTxR did not express donor HLA nor lung self-antigens. Five human cardiac transplant recipients with CAV had circulating antibodies to myosin and vimentin, and exosomes expressing the self-antigens were also detected. Exosomes isolated from both lung and cardiac transplant recipients were validated with independent cohorts contained several immune-regulatory miRNAs (miR-92a, 182, 155, 21 and 31). In a murine model of anti-MHC induced obliterative airway disease as well as anti-cardiac myosin induced cardiac rejection, exosomes were released into circulation prior to rejection expressing tissue restricted self-antigens and miRNAs.
Conclusions: We conclude that exosomes are released into the circulation both in human and animal models of acute and chronic rejection expressing donor mismatched HLA as well as tissue restricted self-antigens prior to clinical diagnosis of rejection suggesting that isolated exosomes with self-antigens can be a non-invasive biomarker for impending rejection. Further, exosomes may play an important role in the pathogenesis of rejection due to their surface antigenic and miRNA composition which may facilitate the spread of immune responses against donor MHC and self-antigens.
This work was supported by NIH HL056643 (TM)

557.8

NK cells are required in B-cell dependent antibody mediated islet transplant tolerance

Christian Schuetz, Kang Mi Lee, Lisa Kojima, Laura Washburn, Liye Liu, Wei-Hui Liu, James Kim, James F.Markmann.
Transplant Surgery, Massachusetts General Hospital, Boston, MA, United States.

Immunologic tolerance to solid organ and cell grafts has been achieved in various rodent models using antibodies directed at CD45RB and Tim-1. We have shown that this tolerance mechanism is B lymphocyte dependent, presumably requiring immune regulatory function. In contrast to other models such as autoimmunity, the regulatory B cells (Breg) in our model do not depend on IL-10 and may therefore represent a yet to be characterized subpopulation of Bregs. In an effort to elucidate the mechanism of B cell induced tolerance we investigated the requirement of NK and NKT cells in vitro and in vivo. Murine islets (200-300 Islets/recipient), isolated from male Balb/c mice, were transplanted under the kidney capsule of STZ treated (200 mg/kg) C57bl/6 (B6), CD1d-/- and Lystbg/J (beige) mice. Tolerance was induced by intraperitoneal injection of 100 µg anti-mouse CD45RB (Bio X cell) on days 0, 1, 3, 5, and 7 following transplantation and 500 µg anti-mouse TIM-1 (Bio X cell, RMT1–10) i.p. on the day before transplant, and 300 µg on days 0 and 5 post-transplant. Blood glucose levels were monitored for a minimum of 2 weeks up to the time of rejection or 100 days post-transplant. One group of B6 recipients received 3 doses of NK1.1 Antibody i.p. (200 µg, days -7, 1 and 8) to deplete NK and NKT cells. This NK/NKT cell depletion resulted in the near absolute abrogation of the tolerizing effect of dual antibody treatment (anti CD45RB/Tim-1) as only 3/14 islet recipients treated with Nk1.1 antibody remained normoglycemic after 100 days. In the control group, 10/11 B6 recipients remained normoglycemic 100 days post islet transplant. In comparison, recipients that are deficient in NKT cells (CD1d-/-) demonstrated comparable long term graft survival rates at 100 days (4/5 CD1d-/- vs. 4/4 wt B6). Islet grafts were rejected between post-transplant day 14 and 20 in 4/4 beige recipient mice, treated with dual Antibody.In summary, these result point towards a crucial role of NK cells but not NKT cells in the maintenance of immune transplant tolerance induced by combined anti-CD45RB/anti-Tim-1 Antibody treatment. The mechanistic role played by NK cells and the potential interaction with Bregs, is under current investigation.

557.9

Short-term vascularized bone marrow transplant is sufficient to induced and maintained skin allograft tolerance in a mouse model

Cheng-Hung Lin[1], Madonna Rica Anggelia[1], Hui-Yun Cheng[1], Yen-Ling Wang[1], Wen-Yu Chuang[1], Chih-Hung Lin[1], Gerald Brandacher[2].
[1]Center for VCA,Department of Plastic and Reconstructive Surgery, Chang Gung Memorial Hospital, Taoyuan, Taiwan; [2]Department of Plastic and Reconstructive Surgery, VCA Laboratory, Johns Hopkins University School of Medicine, Baltimore, MD, United States.

Background: Accumulating evidences indicate that vascularized bone marrow transplantation (VBMT) plays a critical role in inducing tolerance of vascularized composite allografts (VCA). Recipients with long-term surviving allografts show a higher degree of cell trafficking between donor and recipient. We hypothesize that short-term existence of VBMT may promote engraftment the donor cells and induce allograft tolerance.
Methods: Osteomyocutaneous (OMC) allografts from Balb/c were transplanted onto C57BL/6 mice and 1 mg anti-CD154 (POD 0), 0.5 mg CTLA4Ig (POD 2), and 3mg/kg/day for 7 days every other day for 3 weeks of rapamycin were administered. Transplanted mice combined with skin graft were divided into 4 groups: Group 1, OMC + skin graft (n=4); Group 2, OMC removed at POD 30 + skin graft (n=4); Group 3, OMC removed at POD 60 + skin graft (n=4); Group 4, OMC removed at POD 120 + skin graft (n=4).
Results: Sixteen of 20 mice receiving OMC allografts achieved long-term graft survival (>120 days). In animals with long-term allograft survival, peripheral blood analysis showed that cellular and humoral responses (IgG and IgM) as well as proinflammatory cytokines (IL-1β, IL-21, IL-22, IL-31, IL-33, IFNγ, TNFα, CD40L) were suppressed. Peripheral and central tolerance were showed by a significant deletion of $Vb5^+$ $CD4^+$ cells in peripheral blood and thymus in tolerated allograft animals, but not in rejected mice ($p<0.05$). Interestingly, transplanted animals which OMC allografts were removed at POD 30 were able to achieve long-term skin allograft survival with (>60 days). It was consistent with the data that showed animals before OMC removed versus after OMC removed showed no significantly elevated immune response. Additional group using $Rag2^{-/-}$ mice, skin paddle of OMC allograft was able to delay rejection after $5x10^6$ Teff cell donor specific injection in comparison with control skin graft (MST=47 vs 33.5) (Figure 1). The percentage of $CD8^+$ T cells in the animals was slightly lower than control skin graft at 30 days post injection.
Conclusions: Efficacy of VBMT to promote long-term allograft survival has been shown in this study. The data also suggest that intra- and extrathymic clonal deletion is one of mechanisms contribute to maintenance of tolerance.

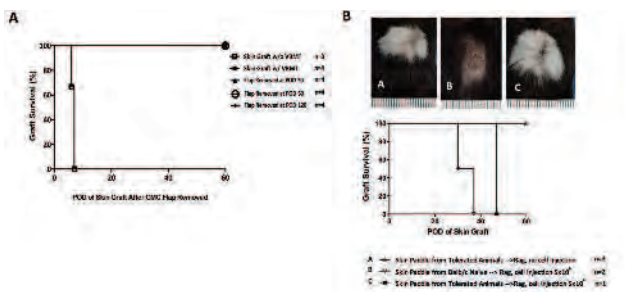


Figure 1. Skin allograft survival after VBMT removed in transplanted wild type animals (A) and skin paddle skin allograft survival in Rag 2 / animals treated with Teff adoptive transfer (B).

558.1

Human endothelial-like cells transdifferentiated from monocytes suppress allo-reactive T cells

Yuka Tanaka, Hideki Ohdan.
Gastroenterological and Transplant Surgery, Hiroshima University, Hiroshima, Japan.

Introduction: Although it is well known that liver allografts are often accepted by recipients, leading to donor-specific tolerance of further organ transplants, the underlying mechanisms remain unclear. We had previously showed that mouse liver sinusoidal endothelial cells (LSECs) had been proven capable of suppressing T-cells with cognate specificity for LSECs through programmed death (PD) 1/PD-L1 pathway in both of in vitro and in vivo models. Such immune-regulatory functions of LSECs have not been extensively investigated in human because of the limited availability of appropriate human samples. In the present study, we have determined immune-regulatory properties of human LSECs on allogeneic T cells. We have also proven that endothelial-like cells, phenotypically and functionally resembling LSECs, can be transdifferentiated from human peripheral blood monocytes.
Materials and Methods: The liver tissues were obtained from patients undergoing surgical resection of either hepatocellular carcinoma or colorectal liver metastasis. Human LSECs were isolated from the disaggregated liver tissues. Human CD14+ cells were isolated from peripheral mononuclear cells by using magnetic sorting and cultured in EGM2 medium supplemented with GM-CSF, IL-4, VEGF, hFGF-B, R3-IGF-1,and LPS for 14 days. The morphologic observations of the isolated/cultivated cells were carried out in a phase contrast microscope. The phenotype of those cells was analyzed by flow cytometry. In addition, we analyzed suppressive activity of those cells on allogeneic T cells by using a multiparameter MLR assay using CFSE labeling technique.
Results: Naïve human LSECs constitutively express molecules necessary for antigen presentation, i.e., freshly isolated CD105+ LSECs express MHC-class II, CD40, CD80, and CD86 together with PD-L1. The transdifferentiated cells from the CD14+ cells morphologically appeared endothelial-like cells and phenotypically resembled LSECs, i.e., they were MHC-class II+, CD40low, CD80low, CD86low, CD105+, PDL-1+. Allo-immune responses of both of CD4+ and CD8+ T cells in the MLR assays were significantly suppressed by subsequently adding the stimulator-type transdifferentiated endothelial-like cells. The proportion of CD25highCD4+ cells among the final products of the MLR assays with the endothelial-like cells was increased with propagated cells.
Conclusion: We have proven that human endothelial-like cells transdifferentiated from monocytes phenotypically resemble LSECs and suppress alloreactive T cells probably through PD-1/PD-L1 pathway. These findings raise a novel concept to induce hyporesponsiveness of alloreactive T cells by inoculating donor-derived those cells even in transplantation of organs other than liver.

558.2

Interaction between microchimerism-derived exosomes and host dendritic cells generates split tolerance.

William Bracamonte-Baran[1], Jonathan Flortentin[1], Ying Zhou[1], Ewa Jankowska-Gan [1], W. John Haynes[1], Todd Brennan[2], Arend Mulder[3], Frans Claas[3], Todd van Rood[3], WilliamJ. Burlingham[1].
[1]Surgery, University of Wisconsin-Madison, School of Medicine and Public Health, Madison, WI, United States; [2]Surgery, Duke University Medical Center, Durham, NC, United States; [3]Department of Immunohaematology and Blood Transfusion, Leiden University Medical Center, Leiden, Netherlands.

Maternal microchimerism (MMc) has been associated with development of allo-specific transplant tolerance, anti-tumor immunity, and cross-generational reproductive fitness, but its mode of action is unknown. We found in a murine model that MMc caused exposure to the non-inherited maternal antigens in all offspring, but in some, MMc caused membrane allo-antigen acquisition (mAAQ; "cross-dressing") of host dendritic cells (DC). Exosomes from serum of mAAQ+, but not mAAQ-, mice reproduced the DC cross-dressing phenomenon in vitro. In vivo, mAAQ was associated with increased expression of immune modulators PD-L1 and CD86 by myeloid (m)DC, and decreased presentation of allopeptide+self MHC complexes, along with increased PD-L1, on plasmacytoid (p)DC. Remarkably, membrane microdomains containing the acquired MHC alloantigens included CD86, but completely excluded PD-L1. In contrast, microdomains containing allopeptide+self MHC did not exclude PD-L1. Both findings were consistent with Western blot analysis of serum exosomes. Adoptive transfer of allo-specific transgenic CD4 T cells revealed a "split tolerance" status in mAAQ+ mice: T cells responding to acquired MHC alloantigens proliferated, while those responding to allopeptide+self MHC did not. Using isolated pDC and mDC for in vitro culture with allopeptide+ self MHC-specific CD4 T cells, we could replicate their normal activation in mAAQneg mice, and PD-L1-dependent anergy in mAAQ+ hosts. Finally, we found mAAQ+ mDC, and plasma exosomes capable of DC cross-dressing, in peripheral blood from a long-term microchimeric sibling kidney transplant recipient. We propose that exosomes provide a physiologic link between microchimerism and "split" tolerance, with implications for tumor immunity, transplantation, autoimmunity, and reproductive success.

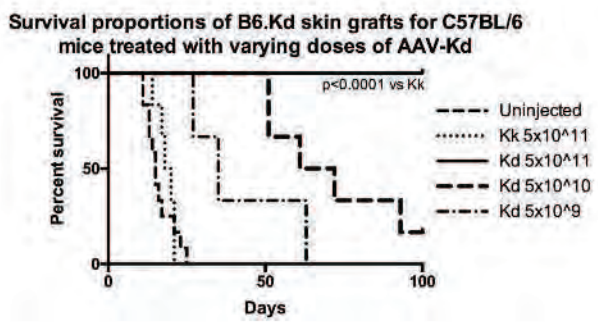
558.3

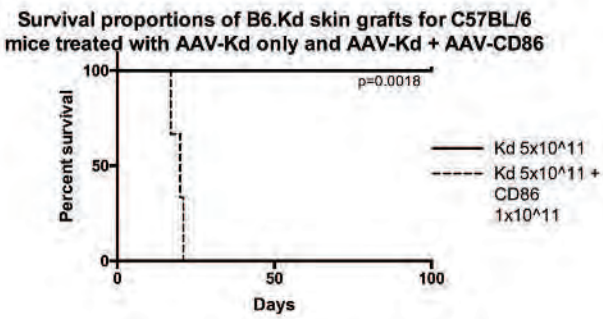
High-level co-expression of CD86 in recipient hepatocytes transduced with AAV-Kd breaks tolerance to Kd-bearing skin grafts

Mario Leong[1], Moumita Paul[1], Mamdoh Mouawadh[1], Eithne Cunningham[1], Szun Szun Tay[2], Chuanmin Wang[1], Patrick Bertolino[2], Alex Bishop[1], Ian Alexander[3], David Bowen[1,2], Alexandra Sharland[1]. [1]Collaborative Transplant Research Group, Central Clinical School, The University of Sydney, Sydney, Australia; [2]Liver Immunology Group, Centenary Institute, Sydney, Australia; [3]Gene Therapy Research Unit, Children's Medical Research Institute, Sydney, Australia.

Introduction: Allogeneic MHC class I expression by recipient hepatocytes following inoculation with liver specific AAV vectors results in tolerance to subsequent skin grafts expressing the same mismatched MHC allele[1]. Absence of CD86 expression by allo MHC-transduced hepatocytes may determine their ability to induce tolerance in alloreactive CD8+ T-cells.

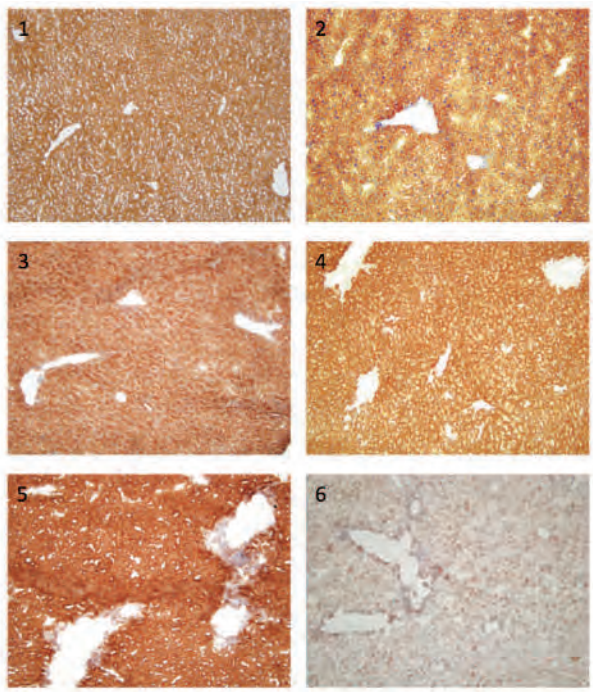
Methods: C57BL/6 mice were inoculated with AAV-Kd at doses $5x10^9$ to $5x10^{11}$vgc. A second cohort of mice were treated with AAV-CD86 at doses $1x10^{10}$ or $1x10^{11}$vgc in addition to AAV-Kd $5x10^{11}$vgc. AAV-Kk was the third-party control. Some mice received Kd-bearing skin grafts from B6.Kd mice at d7 post-inoculation. Graft survival, liver inflammation and Kd expression were monitored.





Results: Dose-dependent survival prolongation of Kd-bearing skin grafts in mice treated with AAV-Kd alone, but not control vector, culminated in indefinite survival of all grafts at $5x10^{11}$vgc ($p<0.0001$). Co-expression of high dose CD86 in conjunction with Kd produced significant liver inflammation, with transaminitis (ALT 1140-1570U/L), and heavy infiltration with CD8+ T cells (>2500 cells/hpf) and neutrophils (552 ± 21 cells/hpf) on d7. Livers transduced with Kd alone contained 5 CD8+ and 47 CD4+ T cells/hpf and ALTs of <50 U/L on d7. Rapid clearance of Kd-expressing hepatocytes was noted in mice receiving high-dose CD86. Whereas initial expression of Kd in these mice was strong and widespread (90% of the liver area expressed Kd on d7), by d14 post-inoculation, only 12.5% of the liver area expressed Kd, compared with 82% in mice inoculated with Kd alone. Concomitant expression of high-dose CD86 with Kd abrogates tolerance to B6.Kd skin grafts (MST 20 days vs >100 days) ($p=0.0018$). In contrast, co-administration of low dose CD86 with Kd resulted in mild elevation of ALT (88-120 U/L on d7), and a relatively sparse lymphocytic infiltrate where CD8+ T cells (46 ± 28 cells/hpf) were outnumbered by CD4+ T cells (117 ± 18 cells/hpf) in a ratio of 2.5:1. Kd expression in hepatocytes was maintained through d14 in mice also treated with low-dose CD86 (89-92% Kd-positive liver area, comparable to the AAV-Kd only group). Survival of B6.Kd skin grafts in mice inoculated with AAV-Kd and low dose AAV-CD86 is ongoing at the time of submission.

Discussion and Conclusions: Indefinite survival of Kd-mismatched skin grafts was achieved by administration of 5×10^{11} vgc AAV-Kd to C57BL/6 mice. High levels of CD86 co-expression in hepatocytes resulted in an intense inflammatory response within the liver, loss of expression of the Kd transgene, and abrogation of skin graft tolerance. Low dose CD86 produced mild inflammation and modest perturbation of liver function. CD4+ T cells predominate within the liver infiltrate of low-dose mice and may include Tregs; further characterization of these cells is in progress. Kd expression in hepatocytes was preserved in mice receiving low-dose CD86 at a time when it had been largely eliminated from mice treated with high-dose CD86. Absent or low expression of costimulatory molecules by hepatocytes is a requirement for tolerance induction by MHC I gene transfer.



IHC staining for Kd in: **1**-C57BL/6 mouse liver d7 post inoculation with AAV-Kd $5x10^{11}$ **2**-C57BL/6 mouse liver d14 post inoculation with AAV-Kd $5x10^{11}$ **3**-C57BL/6 mouse liver d7 post inoculation with AAV-Kd $5x10^{11}$ + AAV-CD86 $1x10^{10}$ **4**-C57BL/6 mouse liver d14 post inoculation with AAV-Kd $5x10^{11}$ + AAV-CD86 $1x10^{10}$ **5**-C57BL/6 mouse liver d7 post inoculation with AAV-Kd $5x10^{11}$ + AAV-CD86 $1x10^{11}$ **6**-C57BL/6 mouse liver d14 post inoculation with AAV-Kd $5x10^{11}$ + AAV-CD86 $1x10^{11}$

References:

[1] Cunningham E, Tay S, Wang C, Rtshiladze M, Wang Z, McGuffog C, Cubitt J, McCaughan G, Alexander I, Bertolino P, Sharland A, Bowen D, Bishop A. Gene Therapy for Tolerance: High-Level Expression of Donor Major Histocompatibility Complex in the Liver Overcomes Naive and Memory Alloresponses to Skin Grafts. *Transplantation*. 2013; Vol 95: Issue 1:70-77

558.4

A comparison of anti-CD28 (FR104) and anti-B7 (belatacept) immunotherapy in monkey cardiac allograft transplantation

Tianshu Zhang, Natalie A.O'Neill, Dawn Parsell, Siamak Dahi, Natalia Kubicki, Jhade Woodall, Mehmet C.Uluer, Lars Burdorf, Ivan Tatarov, Katherine Thomas, Elizabeth Kang, Alena Hershfeld, Agnes M.Azimzadeh, Richard NPierson.
Department of Surgery, University of Maryland, Baltimore, MD, United States.

Aims: Therapeutic targeting of the CD28/B7 costimulatory pathway with CD28-specific agents might offer advantage over B7 blockade by favoring B7-mediated immune regulatory signals to T cells through CTLA-4 and PD-L1. Here we compare the immunosuppressive effects between FR104 (a humanized pegylated anti-CD28 Fab fragment) and belatacept in a cynomolgus monkey heterotopic heart transplant model.
Methods: Cynomolgus monkey heterotopic cardiac allograft recipients received FR104 (5 mg/kg, n=4; 10 mg/kg, n=3) or belatacept (5mg/kg, n=2; 10 mg/kg n=2) on days 0, 4, 7, 14, 21, 28, 42, 56, 70 and 84. Postoperatively, graft function (electrocardiogram; left ventricular systolic and diastolic pressure; heart rate) and core temperature were monitored daily by implanted telemetry device. Grafts were explanted at graft failure. Protocol biopsies and graft explants were analyzed for ISHLT AR grade and mean CAV score (0, 0%; 1, 1-10%; 2, 11-50%; 3, >50% occlusion).
Results: Median graft survival time (MST) with FR104 (5 mg/kg), FR104 (10 mg/kg), and belatacept (10mg/kg) was 122, 167, and 123 days, respectively. With low dose belatacept (5 mg/kg), one allograft experienced acute rejection on day 8, and to date the other graft continues to function (>8 days). On post-operative days 90-150, ISHLT/CAV scores of protocol biopsy and explanted allografts were found to have ISHLT/CAV scores of 0.6±0.4 and 0.5±0.3 for FR104 (5 mg/kg, long-term surviving grafts), 0.5±0.4 and 0.7±0.3 for FR104 (10 mg/kg, long-term surviving grafts), and 1.8±1.4 and 1.3±1.0 for belatacept (10 mg/kg) treatment groups, respectively.
Conclusions: Anti-CD28 blockade by FR104 prolongs cardiac allograft survival and attenuates CAV compared to belatacept. This preliminary data suggest that FR104 may be superior in modulating alloimmunity in cynomologus monkeys.

558.5

Selective blockade of CD28 costimulation prevented human allo-skin graft rejection in a humanised mouse model

Masaaki Zaitsu[1,2], Fadi Issa[2], Joanna Hester[2], Kate Milward[2], Bernard Vanhove[3], Kathryn Wood[2].
[1]Department of Gastroenterological Surgery I, Hokkaido University Graduate School of Medicine, Sapporo, Japan; [2]Transplantation Research Immunology Group, Nuffield Department of Surgical Sciences, University of Oxford, Oxford, United Kingdom; [3]INSERM UMR643, University of Nantes, Nantes, France.

Background: T cells are central to the detrimental alloresponses that develop after transplantation. Targeting the CD28-CD80/86 pathway with CTLA-4Ig reagents is a promising alternative to current immunosuppressive treatments. However, the CD80/86 blocking strategies that use CTLA-4Ig also inhibit the co-inhibitory CTLA-4 signal that is crucial to the regulation of T cell activation. In order to preserve the regulatory function of the immune system, selective blockade of the CD28 signal is theoretically advantageous as it would preserve coinhibitory signals that are facilitative for the development of a tolerant state. Importantly, costimulatory signals affect regulatory T cell homeostatic proliferation and regulatory function independently of their effects on effector T cells. In the current study, we have investigated the effects of selective blockade of the CD28 signal by a non-activating monovalent antibody in humanised mouse model.
Methods: Human skin grafts were transplanted to immunodeficient BALB/c.Rag2$^{-/-}$.cϒ$^{-/-}$ mice and were allowed to heal for 35 days before adoptive transfer of 10x10^6 human peripheral blood mononuclear cells (PBMCs) intraperitoneally. Groups of mice were treated with selective CD28 antibody: FR104 (5mg/kg), CTLA-4Ig (10mg/kg) or saline control intravenously twice a week for three weeks. All mice were started on the treatment regimen three weeks post-adoptive transfer of allogeneic human PBMCs to ensure adequate levels of human leukocyte chimerism in recipient mice.
Results: In this human skin transplantation model, prolongation of skin graft survival was observed in FR104 treated group, but not in CTLA-4Ig or saline treated recipients (median survival time, MST=63 vs. CTLA-4Ig (MST=31) or Saline (MST=31), ($p<0.05$)). While FR104 treatment significantly affected the level of human leukocyte chimerism in the peripheral blood, it remained above the required reconstitution level known to induce skin rejection. FR104 treatment significantly reduces the number of graft infiltrating CD4$^+$ cells (47.8±24.8 vs 83.3±5.1 cells/HPF, $P<0.02$) and CD8$^+$ cells (50.0±11.9 vs 111±34.0 cells/HPF, $P<0.04$) as compared to CTLA-4Ig treatment. No differences were noted in the number of graft infiltrating FOXP3$^+$ cells between FR104 and CTLA-4Ig groups although FR104 significantly reduces CD4 infiltration into skin grafts.
Conclusion: Short term selective blockade of CD28 costimulation preserves regulatory T cells and inhibits alloresponses. Selective CD28 blocking strategies might offer a strategy for reversing on-going acute rejection.

558.6

Modulation of influenza-specific humoral responses by selective CD28 blockade

Elizabeth Kang, Siamak Dahi , Tianshu Zhang, Natalie O'Neill, Evelyn Sievert, Elana Rybak, Ivan Tatarov, Richard N. Pierson, Agnes M. Azimzadeh.
Department of Surgery, University of Maryland School of Medicine and Baltimore VAMC, Baltimore, MD, United States.

Background: Current methods of immunosuppression are broadly inhibitory and thus associated with an increased risk of opportunistic infections. Immunization against bacterial and viral pathogens is a key preventative therapy clinically however immunosuppressed individuals are known to have impaired responses to immunization. We have shown previously that of a novel humanized peggylated αCD28 Fab Ab fragment (FR104) induces long-term survival of cynomolgus monkey cardiac allografts in association with decreased chronic rejection. Here we evaluate antibody responses to influenza vaccinations in the same FR104-treated animals.
Methods: Seven monkeys received a cardiac allograft and immunomodulation with FR104 at 10mg/kg (hi) or 5mg/kg (lo) (D0,4,7,14,21,28,42,56,70,84). Influenza vaccination was administered at d42 and 14d post graft explantation, with an additional vaccination at d150 in long survivors. Eight control cynomologus monkeys were primary and secondarily immunized with influenza vaccine in the absence of immunosuppression. (Results from four additional monkeys primary immunized with vaccine in presence of calcineurin inhibitor (CsA) and boosted in absence of immunosuppression are in progress.) Antibody levels were measured by ELISA and expressed as fold increase on d7, 14, 21 and 28 vs. pre-immunization levels. A 1.4- (IgM) or 2- (IgG) fold or greater increase was defined as a "detectable" response; > 5(IgM)/10(IgG)-fold responses were considered "robust".
Results: Graft survival time was not significantly different between FR104 lo (50,167, 178d) and FR104 hi (57,80,164,178d) groups. All control non-transplanted animals receiving no immunosuppression mounted a detectable 1° IgM (8/8, 2 robust), 6/8 developed 2° IgM (1 robust) and 7/8 developed 1° IgG (2 robust) and 2° IgG (4 robust) IgG responses. In contrast, only 3/7 transplanted animals receiving FR104 showed detectable 1° IgM and none developed 1° IgG responses during therapy. Secondary IgM and IgG responses were inhibited in early rejectors still exposed to FR104 (0/2), whereas while 2° IgM (4/4 detectable, 1 robust) and some IgG (2/4) were detectable observed during weaning of theas drug FR104 levels became undetectable aroundat d150. No tertiary responses were detectable in long survivors despite absence of drug exposure by ELISA and CD28 receptor occupancy assay (0/4).
Conclusions: Relative to no immunosuppression, αCD28 strongly suppressed primary and secondary responses and class-switching during therapy. Absence of tertiary responses after removal of long surviving grafts suggests long-term modulation (regulation) of immunity to viral antigens (antigen-specific immunoregulation, or tolerance) is induced by FR104, as previously described for αCD154 and influenza viral antigens (Crowe J AJT 2003). Additional studies will determine if αCD28 induces a unique profile compared to conventional immunosuppression with calcineurin inhibitors or B7-targeting agents inhibition with respect to protective immunity against common pathogens, and tolerogenic potential.

558.7

Modulation of the immune system by secretome-deficient mesenchymal stem cells

Franka Luk[1], Samantha F.H.de Witte[1], Sander S.Korevaar[1], Marieke Roemeling-van Rhijn[3], Marcella Franquesa[1], Tanja Strini[1], Sandra van den Engel[2], Madhusudhana Gargesha[4], Debashish Roy[4], Frank J.M.F.Dor[2], Edwin M.Horwitz[5], Ron W.F.de Bruin[2], Michiel G.H.Betjes [1], Carla C.Baan[1], Martin J.Hoogduijn[1].
[1]Nephrology and Transplantation, Dept. of Internal Medicine, Erasmus MC-University Medical Center, Rotterdam, Netherlands; [2]Transplant Surgery, Dept. of Surgery, Erasmus MC-University Medical Center, Rotterdam, Netherlands; [3]Dept. of Internal Medicine, University Medical Center Groningen, Groningen, Netherlands; [4]BioInVision Inc., Mayfield Village, OH, United States; [5]Dept. of Hematology/Oncology/BMT, Nationwide Children's Hospital, Colombus, OH, United States.

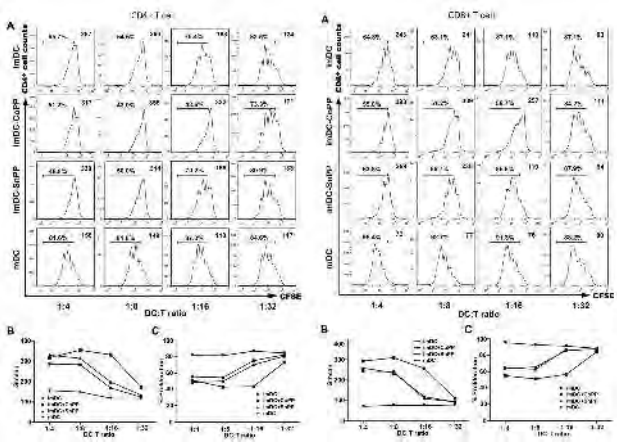
Mesenchymal stem cells (MSC) are widely studied as a cell therapeutic agent after solid organ transplantation. However, therapy with living culture-expanded cells comes with safety concerns. Furthermore, the development of effective MSC immune therapy is hampered by a lack of knowledge of the mechanisms of action and the therapeutic components of MSC. Such knowledge would allow better identification of diseases that are responsive to MSC treatment, appropriate timing of MSC therapy, optimization of the MSC product, and development of therapy based on functional components of MSC.
To close in on the components that carry the therapeutic immunomodulatory activity of MSC, we generated MSC that were unable to respond to inflammatory signals or secrete immunomodulatory factors but preserved their cellular integrity by heating the cells for 30 minutes at 50 °C. (HI-MSC). Secretome deficient HI-MSC and control MSC showed the same bio-distribution and persistence after infusion in mice with ischemic kidney injury. Both control and HI-MSC induced mild inflammatory responses in healthy mice, and, moreover, induced dramatic increases in IL-10 and reductions in IFN-γ levels in sepsis mice. In vitro experiments showed that in contrast to control MSC, HI-MSC lacked the capability to suppress T cell proliferation or induce regulatory B cell formation. However, both HI-MSC and control MSC modulated monocyte phenotype and their response to LPS.
The results of this study demonstrate that, in particular disease models, the immunomodulatory effect of MSC does not depend on their secretome or active cross-talk with immune cells but on the recognition of MSC by monocytic cells. These findings provide a new view on MSC-induced immunomodulation and help identify key components of the therapeutic effects of MSC.

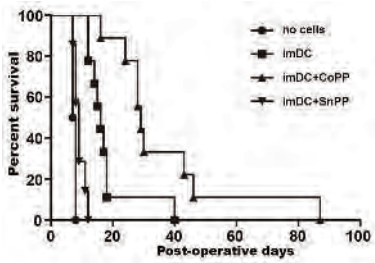
558.8

Upregulation of heme oxygenase-1 in immature dendritic cells improves their tolerogenic capacity in allotransplantation

Yu Jia, Gang Chen, Yue Zhao, Lu Wang, Song Chen , Xia Huang, Lan Zhu.
Institute of Organ Transplantation, Tongji Hospital, Huazhong University of Science and Technology, Wuhan, People's Republic of China.

Background: It has been reported that heme oxygenase-1 (HO-1) is critical for tolerogenic dendritic cells (tolDCs) to suppress T cell responses and tolDCs will lost their immunoregulatory effects when HO-1 is blocked. Therefore, significant upregulation of HO-1 may markedly improve the tolerogenic capacity of tolDCs.
Methods: Bone marrow-derived DCs (BMDCs) were generated from Balb/c mice with low doses of GM-CSF and IL-4. The adherent immature BMDCs were obtained as TolDCs. Upregulation of HO-1 in TolDCs (HO-1hi-TolDCs) was achieved by CoPP treatment. SnPP-treated TolDCs served as control cells. LPS was used to induce DC maturation. T cell proliferation was stimulated by anti-CD3/CD28 antibodies. Adoptive transfer of Balb/c donor-derived DCs (5×106) to C57BL/6 recipient mice was performed 7 days prior to cardiac transplantation. To study the distribution and location of adoptively transferred donor DCs in recipient mice, these cells were labeled with cellvue claret in advance.





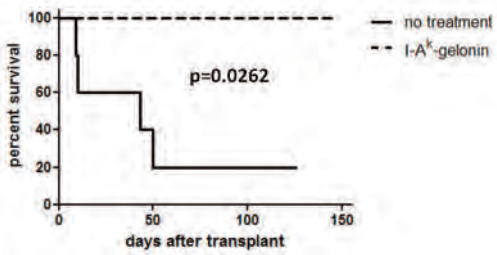
Results: CoPP treatment dramatically increased HO-1 expression in TolDCs, which rendered TolDCs refractory to LPS-induced maturation, enhanced their capability to suppress both CD4+ and CD8+ T cell proliferation stimulated by anti-CD3/CD28 antibodies (Fig.1), as well as induce more allogenic regulatory T cells (Tregs) in vitro after the coculture with naïve CD4+ T cells from C57BL/6 mice. Adoptive transfer of donor-derived untreated TolDCs significantly prolonged cardiac allograft survival compared to untreated control group (18.000±2.853 vs. 7.500±0.289 days). Interestingly, adoptive transfer of CoPP-treated TolDCs further extended the prolongation of allograft survival (36.778±6.974 days) (P<0.01, vs. untreated TolDCs group). In contrast, adoptive transfer of SnPP-treated TolDCs almost had no effects on the allograft survival (9.143±0.670 days) (Fig.2). Furthermore, adoptively transferred TolDCs were found to be mainly distributed in the recipient spleens. CoPP-treated donor TolDCs were found to be able to keep high levels of HO-1 expression and can survive longer than untreated TolDCs after adoptive transfer. In addition, alloreactive T cell response was significantly inhibited in CoPP-treated TolDCs group at day 7 after transplantation.
Conclusion: In vitro generated HO-1hi-TolDCs have enhanced tolerogenic capacity to modulate alloimmune responses both in vitro and in vivo, thus may provide an antigen-specific and cost-effective novel strategy to induce transplant tolerance.

558.9

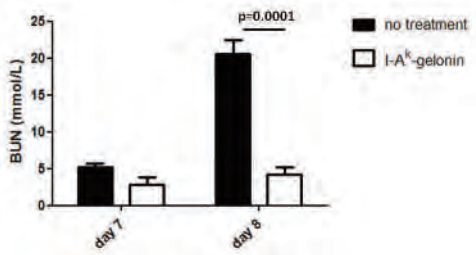
Indefinite survival of murine kidney allografts induced by depletion of donor passenger leukocytes

Kathryn Brown, Anna K.Nowocin, Lucy Meader, Lindsey A.Edwards, Richard A.Smith, Wilson Wong.
MRC Centre for Transplantation, King's College London, London, United Kingdom.

Introduction: While modern anti-rejection treatments have concentrated on modifying the immune system of the recipient, relatively less work has been done on modifying that of the donor. Rejection of donor organs depends on the trafficking of donor passenger leukocytes to the secondary lymphoid organs of the recipient to elicit an immune response. Therefore, the depletion of passenger leukocytes may be clinically applicable as a strategy to improve graft survival.
Materials and Methods: The F(ab')2 fragment of a monoclonal antibody against the donor MHC class II molecule I-Ak was conjugated with the plant-derived ribosomal inactivating protein gelonin to form an immunotoxin. After confirmation that the immunotoxin depleted target cells specifically *in vitro* and *in vivo*, it was administered to FVB recipients of target antigen-expressing, fully allogeneic C57BL/6 x CBA F1 kidneys.
Results: Treatment with immunotoxin resulted in indefinite survival of kidney allografts, compared with control allografts which had a median survival time of 43 days (p=0.0262).



Kidney function in the immunotoxin-treated group was also improved, with blood urea nitrogen at 4.2±1.03mmol/L at day 8 post transplant, compared with 20.64±1.77mmol/L in the control group (p=0.0001).



Immunotoxin-treated kidneys showed well preserved architecture and the presence of Foxp3+ cells, and recipients also displayed diminished donor-specific antibody formation and delayed rejection of subsequent donor type skin grafts.
Discussion: Our data suggest that depletion of donor passenger leukocytes, resulting in indefinite graft survival, can be achieved with a single treatment of an anti-donor MHC class II immunotoxin. The presence of an immune response against the graft, in the form of alloantibody generation and the rejection of challenge skin grafts, means that, if translated to the clinic, immunotoxin treatment would not induce indefinite graft survival, but may be a useful adjuvant to prevent rejection.
Conclusion: Current anti-rejection treatment is almost exclusively directed against the recipient's immune response. The approach described here, directed against donor cells, opens a new, simple and safe therapeutic avenue that can be used in conjunction with conventional therapy.

559.1

Immunoglobulin M therapy prevents development of type 1 diabetes by modulating the expression of dysregulated pancreatic microRNAs associated with disease pathogenesis

Preeti Chhabra, Ricardo Gehrau, Katherine Schiermeyer, Valeria Mas, Kenneth Brayman.
Surgery - Transplantation Division, University of Virginia, Charlottesville, United States.

Goal: Type 1 Diabetes (T1D) is hallmarked by beta-cell destruction. Our goal was to identify specific pancreatic microRNAs (miRNA) as potential biomarkers for T1D risk prediction and diagnosis, and to study the efficacy of Immunoglobulin M (IgM) therapy.
Background: At diagnosis, 80–90% of the beta-cells have already been destroyed. Current use of autoantibodies against islet antigens as biomarkers for disease prediction is unreliable. Therefore, identifying novel pancreatic miRNA biomarkers that predict the risk of T1D as well as its recurrence following pancreas or islet transplantation remains an important goal. IgM therapy prevents onset of T1D and studying its effect on the diabetic miRNA profile has immense therapeutic significance.
Methods: Female non-obese diabetic (NOD) mice (n=39) were used to identify differentially-expressed pancreatic miRNAs at various developmental stages of T1D. NOD mice were treated with IgM (n=15) or Saline (n=24) intraperitoneal and underwent pancreatectomy at 5-weeks (n=10), 14-weeks (n=15), and 21-weeks (n=14). T1D progression was categorized by blood glucose: normal (≤120mg/dL), prehyperglycemia (120-250mg/dL), and diabetes (≥250mg/dL). Labeled total pancreatic RNA was used for GeneChip miRNA v3.0 array hybridization and expression summaries obtained using RMA algorithm. Pairwise comparison was performed using two-sample t-test and P≤0.01 considered significant. IPA tool was used for biological analyses. Significant biologically relevant miRNAs were validated by RT-PCR.
Results: In non-treated mice, there was a significant increase in glycaemia at 14wk (p=0.005) and 21wk (p>0.001). In contrast, glycaemia in IgM-treated mice remained similar at 5wk (135mg/dL), 14wk (114mg/dL) and 21wk (113mg/dL). In non-treated mice, onset of diabetes occurred at 14 weeks. In total, 39 miRNAs were differentially expressed when comparing 5wk vs. 21wk. Differentially-expressed miRNA profiles were identified in non-treated (39 miRNAs) and IgM-treated (42 miRNAs) mice between 5wk and 21wk. A comparison between profiles identified 17 miRNAs specific for T1D and 30 miRNAs specific for IgM-treated mice. Downregulated T1D-specific miRNAs (miR-17-5p, -31-5p, -103-3p, and -379-5p) were related to pancreatic disorders and hyperglycemia. Upregulated IgM-treatment specific miRNAs (miR-155-5p, -21-5p, 16-5p, and 10a-5p) were associated with induction of monocytic and polymorphonuclear myeloid derived suppression cells and immunomodulation. Four miRNAs (miR-1224, -130b, -150, and -29a) overlapped between groups. The differential expression of miR-150 (which modulates B and T cells) was 7.6-fold higher in IgM-treated mice. Selected miRNAs (n=11) including miR-29a, -150, -31, and -103 were validated.
Conclusion: IgM therapy promoted normal glycaemia and upregulated immunomodulatory miRNAs in T1D compromised mice. This finding may be the basis for new therapeutic strategies for individuals at risk for T1D and patients undergoing transplantation.
Focus to Cure Diabetes Foundation

559.2

The role of calcineurin inhibition in the dendritic cell response to Aspergillus fumigatus infection in lung transplant recipients

Amit Adlakha[1,2], Darius P. H. Armstrong-James[2], Boris Lenhard[1].
[1]MRC Clinical Sciences Centre, Imperial College, London, United Kingdom; [2]National Heart and Lung Institute, Imperial College, London, United Kingdom.

Background: Invasive aspergillosis caused by *Aspergillus fumigatus* has high morbidity and mortality in the setting of lung transplant immunosuppression. The calcineurin inhibitor FK506 inhibits the calcineurin–NFAT axis in immune cells, and has been shown to impair fungal killing, phagocytosis and TNF-α production in recipient alveolar macrophages[1]. Pulmonary dendritic cells play a crucial immune signalling role by phagocytosing antigen, maturing, secreting cytokines and stimulating T-cell immunity[2]. We used transcriptomics, cytokine profiling and cell surface marker analysis to investigate the mechanisms by which the dendritic cell-mediated fungal response is impaired by FK506 treatment.

Methods: Healthy volunteer monocytes were differentiated into monocyte-derived dendritic cells (moDC's) in cell culture with GM-CSF and IL-4 for 7 days. MoDC's were matured with interferon-gamma before treatment with FK506 +/- inoculation with *A .fumigatus* swollen conidia. Anti-CD83 (a late maturation marker) and anti-CD209 (a DC-specific marker) stained cells were analysed with the ImageStream imaging flow cytometer (Amnis). IL-2 and IL-10 in cell culture supernatant were quantified by sandwich ELISA. RNA-Sequencing - total-RNA was extracted using phenol/chloroform, poly-A +ve mRNA was selected and cDNA libraries were sequenced on the Illumina HiSeq 2500. Post-sequencing read quantification, alignment and differential expression were performed with open source C++/R software (kallisto[3], edgeR[4] and RTN[5]).

Results and Discussion: *Imaging flow cytomtery:* 5000 cells per condition were analysed. MoDCs were identified by gating for CD209. We observed upregulation of CD83 with infection of both immature moDCs (29,888 mean fluorescence units [SEM 1,393] vs 12,534 [799]; p<0·0001), and IFNγ-matured moDCs (36,778 [1,356] vs 26,228 [1,462]; p<0·0001).

CD83 mean fluorescence intensity was reduced with FK506 treatment of both infected immature moDCs (29,888 [1,393] vs 24,289 [1,253], p=0·0028) and infected, IFNγ-matured moDCs (36,778 [1,356] vs 30,159 [1.279], p=0·0004), but unchanged for uninfected moDCs (12,534 [799] vs 11,942 [762], p=0·5921).

ELISA: the upregulation of IL-2 in response to infection (median 16.8 vs 13.5pg/ml; p<0.01) was impaired by FK506 (median 16.8 vs 12.8pg/ml; p<0.01). IL-10 increased with infection in FK506-treated cells (median 146.4 vs 246.9pg/ml; p<0.01), but not in untreated cells (median 106.6 vs 101.8pg/ml; p<0.01).

RNA-Seq: 19 genes were differentially expressed in untreated moDCs +/- infection and 17 in FK506-treated moDC's +/- infection. Treatment-effect analysis revealed 74 genes under FK506-control. Co-expression analysis of these genes revealed strong correlation with 3 transcription factors - Oct-4, pod-1 and FOXF2.

Conclusion: *Aspergillus fumigatus* infection stimulates phenotypic maturation of moDCs and FK506 treatment inhibits this maturation. FK506 also impairs IL-2 release by moDCs in response to infection and is required for IL-10 release during infection – this is consistent with IL-10's known inhibitory action on IL-2, though the significance of the FK506-dependence of this response is unclear. Both these findings suggest an inhibitory role of FK506 on dendritic cell signaling to the adaptive immune system. Our transcriptomic co-expression analysis has identified targets to investigate regulatory transcriptional factor control by ChIP-Sequencing and hence elucidate the gene regulatory mechanisms by which FK506 exerts its inhibitory effect on the DC response to aspergillosis in lung transplants recipients.

Genomics Laboratory, MRC Clinical Sciences Centre, Imperial College London, UK; Flow Cytometry Facility, MRC Clinical Sciences Centre, Imperial College London, UK; Lung Transplant Team, Royal Brompton and Harefield NHS Trust, London, UK

References:

[1] S. Herbst, A. Shah, M. Mazon Moya, V. Marzola, B. Jensen, A. Reed, M. A. Birrell, S. Saijo, S. Mostowy, S. Shaunak, and D. Armstrong-James, "Phagocytosis-dependent activation of a TLR9-BTK-calcineurin-NFAT pathway co-ordinates innate immunity to Aspergillus fumigatus," EMBO Mol Med, vol. 7, no. 3, pp. 240–258, Mar. 2015.
[2] T. Zelante, J. Fric, A. Y. W. Wong, and P. Ricciardi-Castagnoli, "Interleukin-2 production by dendritic cells and its immuno-regulatory functions," Front Immunol, vol. 3, p. 161, 2012
[3] http://pachterlab.github.io/kallisto
[4] http://bioconductor.org/packages/release/bioc/html/edgeR.html
[5] http://bioconductor.org/packages/devel/bioc/html/RTN.html

559.3

Human B cell cytokines predict renal allograft rejection and early transplant outcomes

Aravind Cherukuri, Rajil Mehta, Sunday Ogunde, Sundaram Hariharan, David Rothstein.
Thomas E. Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA, United States.

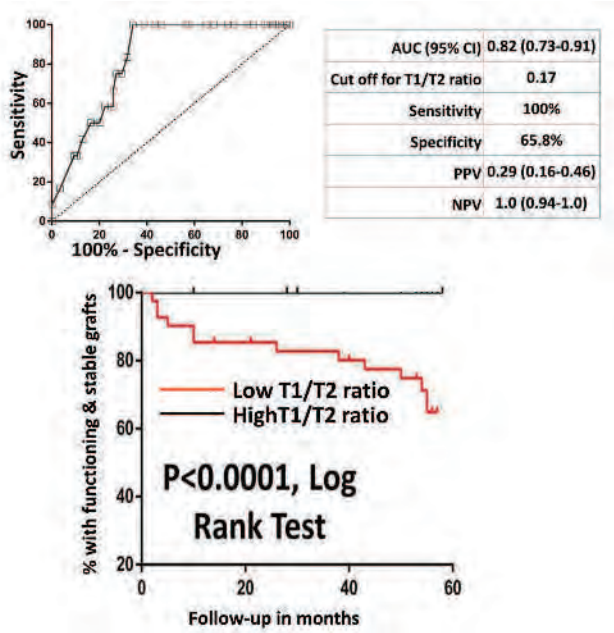
Introduction: In renal transplantation, non-invasive predictive biomarkers that allow for early intervention are limited. We have previously shown that Breg activity in human peripheral blood is best defined by the ratio of IL-10:TNFa expression, and that this ratio falls in renal allograft rejection. Here we report findings from a prospective longitudinal study using these B cell cytokines as a biomarker in renal transplant recipients to predict rejection and outcome.
Methods: 164/374 patients transplanted between dates 1/13-12/14 having serial biopsies (for-cause, and protocol at 3 and 12 months) and blood drawn at 0, 1, 3, 6 and 12 months, were examined (315 biopsies and 534 PBMC samples). We assessed the ratio of B cell IL-10:TNFa expression (after in vitro stimulation with CD40L & CpG X24h) as a biomarker for transplant outcomes.
Results: Patients received thymoglobulin induction with rapid steroid withdrawal and were maintained on tacrolimus and mycophenolate. 37% of the patients had acute rejection (AR) within the first year (all ACR and 3 ACR&ABMR; 21% subclinical, 16% clinical). Of patients with AR: 35% had early AR (≤3mos) that responded to therapy (no recurrence), 27% had early AR with persistence/recurrence at 12mos despite initial treatment, and 38% had late AR (6-12mos) despite a normal early biopsy. The utility of 3-month histology and currently available markers like creatinine or DSA was limited in that they were not predictive of response to treatment or late AR.
At every time-point, patients with AR (clinical or subclinical) had a significantly lower IL-10:TNFα ratio within all B cell subsets, especially within the T1 transitional subset (T1B). The T1B IL-10:TNFα ratio at 3 months was independently associated with rejection (OR 0.31 per increase in ratio by 1; p=0.0001) and predicted AR at any time (ROC AUC 0.88, Sensitivity 85%, Specificity 86%, p<0.0001). At 3months, even in patients with normal protocol biopsy, a low T1B IL-10:TNFα ratio predicted subsequent rejection with a sensitivity of 88% and specificity of 81% (AUC 0.86, p<0.0001). Moreover, in patients treated for early AR, the T1B cytokine ratio at 3mos was strongly predictive of response to therapy vs. persistent/recurrent AR (ROC AUC 0.79, Sensitivity 78%, Specificity 80%, p=0.004). Importantly, patients with a low T1B IL-10:TNFα (<1.26, determined by ROC analysis) ratio had significantly worse creatinine at 18 and 24 months post-transplant and this was independently associated with interstitial fibrosis with inflammation (IF+i) on 12 months biopsy (OR 0.31, p=0.03).
Importantly, this biomarker is not confounded by "inflammation" due to infection. Patients with BK or CMV viremia had significantly higher T1B IL-10:TNFa ratio compared to those with AR (4.9 vs. 0.97, P=0.02). Moreover, Tacrolimus trough levels and the total Mycophenolate dosage were comparable between the groups with high and low cytokine ratio.
Conclusion: T1B IL-10:TNFα ratio at 3 months appears to be a strong and a specific biomarker for the prediction and course of renal allograft rejection, and possibly long–term outcome. If confirmed, it may help inform individualized therapeutic intervention.
ROTRF, AST

559.4

Transitional B cell T1/T2 ratio is a prognostic marker for human renal allograft deterioration

Aravind Cherukuri[1], Richard Baker[2], David Rothstein[1].
[1]Thomas E. Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA, United States; [2]Leeds Transplant Centre, Leeds Teaching Hospitals NHS, Leeds, United Kingdom.

Introduction: Human transitional B cells (TrB) play a significant role in auto and alloimmune disease. We showed that TrB express a high IL-10:TNFα ratio and this falls in the presence of renal allograft rejection. TrB are comprised of less mature T1 and more mature T2 cells. We characterized the cytokine expression of T1 vs. T2 cells and investigated the utility of T1/T2 ratio as a biomarker for allograft deterioration.
Methods: Various B subsets were defined as described previously. Specifically T1 and T2 cells were differentiated by the level of expression of CD24 and CD38 and cytokine expression analysed after in vitro stimulation (CpG+CD40L X48hrs). In a training set, TrB were analysed in 84 patients (45 patients at time of for-cause biopsy for allograft dysfunction and 39 patients with stable allograft function) and followed for an additional 5 years. In a validation set, we also examined T1 and T2 cells 2 years post-transplant in 97 stable patients who were participating in a RCT comparing alemtuzumab (n=51) or basiliximab induction (n=46). The clinical outcome in both training and validation sets was a composite of graft loss or doubling of serum creatinine over a five year follow-up from the time of B cell analysis.
Results: While both T1 and T2 cells expressed similar IL-10, T1 cells expressed significantly less TNFα, resulting in a higher IL10:TNFα ratio. Thus, T1 cells are the B subset with the most anti-inflammatory profile. The number of T1 cells was found to fall in rejection (resulting in lower T1/T2 ratio), and this contributed to the low IL-10:TNFα ratio in this setting.



Training set: In 84 patients (with either prior graft dysfunction or stable baseline function), a low T1/T2 ratio was a strong predictor of allograft deterioration over 5 years (ROC AUC 0.84, P<0.001) and was independently associated with allograft deterioration (per increase in the ratio by 1: HR 0.9, P=0.007). The association remained statistically significant when the analysis was only included patients with prior graft dysfunction.

Validation set: T1/T2 ratio measured 2-years post-transplant in stable patients was a strong predictor of outcome over the subsequent 5 years (ROC AUC 0.82, P<0.001) and was independently associated with allograft deterioration (HR 0.88, P=0.02). Thus, the T1/T2 ratio can segregate clinically quiescent patients into two halves. Those with a high ratio had entirely stable function, whereas in the group with a low ratio, ~35% had allograft deterioration over the ensuing five years (Fig1).
In both the test and validation sets, the T1/T2 ratio was a much stronger predictor of graft deterioration than traditional markers like eGFR (test set, eGFR ROC AUC 0.76 vs. T1/T2 ratio 0.84; validation set, eGFR AUC 0.64 vs. T1/T2 0.82), or DSA (test set, DSA ROC AUC 0.68 vs. T1/T2 ratio 0.84; validation set, DSA AUC 0.66 vs. T1/T2 0.82).
Conclusion: We demonstrate that the T1 subset of TrB expresses a strongly anti-inflammatory cytokine profile. A decrease in T1:T2 ratio (due to selective loss of T1 cells) is a strong prognostic biomarker for subsequent renal allograft deterioration.

559.5

The unholy triad of inflammation, infection and immune rejection of the kidney allograft- partners in crime?

Franco B.Mueller, Hua Yang, Carol Li, Catherine Snopkowski, Liana Perry, Elly Varma, John Lee, Darshana Dadhania, Manikkam Suthanthiran, Thangamani Muthukumar.
Department of Medicine and Transplantation Medicine, Weill Cornell Medicine, New York, United States.

Introduction: The role of inflammation and infection in acute allograft rejection is emerging. Inflammsomes are multiprotein molecular complexes that are critical players regulating inflammation. DAMPs (Damage-Associated Molecular Pattern molecules) are host molecules that can initiate/perpetuate inflammation. PAMPs (Pathogen-Associated Molecular patterns) are molecules associated with pathogens that are recognized by the innate immune system. Thus, while cellular rejection of allograft is primarily driven by T-cells in response to alloantigens, infection and tissue damage in pre-clinical models trigger the activation of inflammasomes and shape the adaptive immune response (reviewed by Chong and Bromberg, Literature Watch, AJT 2013). Their role in rejection of human allograft has not been well characterized. We tested the hypothesis that inflmmasomes, DAMPs and PAMPs are hyperexpressed during acute rejection in human kidney allografts.
Methods: We studied 12 kidney transplant biopsies from 12 kidney recipients; 7 were for-cause biopsies categorized as Banff acute cell mediated rejection (ACR) and the remaining 5 were protocol/surveillance biopsies categorized as normal (Protocol/Normal). RNA from these biopsies was sequenced in Illumina HiSeq platform. The sequencing data analysis workflow using Galaxy consisted of FASTQC for read quality control, mapping of reads to reference genome assembly: Feb 2009 GRCh37.75/hg19, read alignment with TopHat2, generation of the raw counts with HTSeq, normalization with Cufflinks and differential gene expression analysis using the Bioconductor R package edgeR. We used KEGG and NCBI databases to identify genes implicated in inflammasomes, DAMPs and PAMPs.
Results: The mean (SD) age of recipients in the ACR group was 48 (14) years and was 45 (12) years in Protocol/Normal biopsy group. There were 3 women, 1 Black and 2 deceased donor transplants in ACR group and 3 women, 0 Blacks and 2 deceased donor transplants in Protocol/Normal group. Median time from transplant to biopsy: 12 months (ACR) and 6 months (Protocol/Normal). Median serum creatinine at biopsy: 2.63 (ACR) and 1.20 (Protocol/Normal). Median RIN (RNA integrity number) value: 7.2 (both groups). Single end reads per sample: >36 million. We identified 26 key inflammasome genes, 17 key DAMP genes and 33 key PAMP genes in KEGG and NCBI databases of which 16, 17 and 29, respectively, had >1 cpm in at least 1 biopsy sample. Among these, 9 inflammasome genes, 5 DAMP genes and 11 PAMP genes were differentially expressed (figure-1–genes overlapping among the categories are shown only once).

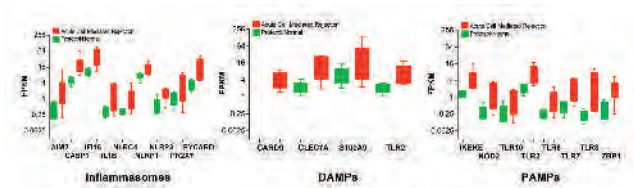
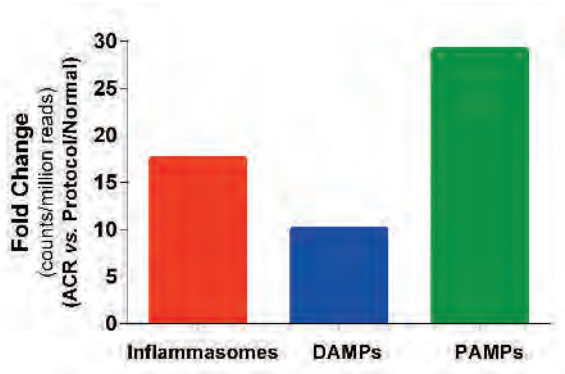


Figure-2 below shows the fold change of transcripts (ACR vs. Protocol/Normal).



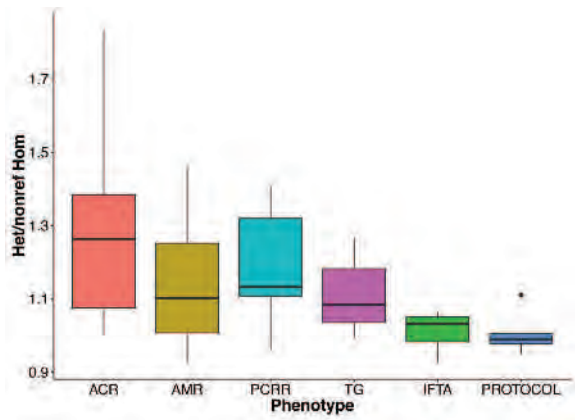
Conclusion: Our RNA-Seq and bioinformatics analyses have discovered that Inflammsomes, DAMPs and PAMPs are all hyperexpressed during ACR in the human kidney allograft suggest a possible causal link between inflammation, infection and immune rejection, and the need to target each of these components contributing to allograft damage and failure.

559.6

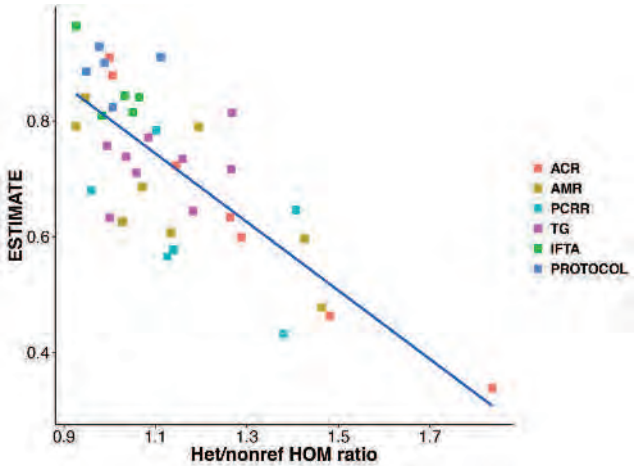
The Het/Hom ratio calculated from RNA-Seq data: a universal and unbiased quantitative measure of immune cell invasion into human allografts

Gaurav Thareja[1], Hua Yang[2], Shahina Hayat[1], Carol Li[2], Catherine Snopkowski[2], Liana Perry[2], Elly Varma[2], Mohamad Alkadi[2], John Lee[2], Darshana Dadhania[2], Karsten Suhre[1], Manikkam Suthanthiran[2], Thangamani Muthukumar[2].
[1]Physiology and Biophysics, Weill Cornell Medicine-Qatar, Doha, Qatar; [2]Department of Medicine and Transplantation Medicine, Weill Cornell Medicine-Qatar, New York, United States.

Introduction: Immune rejection continues to remain the Achilles heal of organ transplantation. The advent of RNA sequencing technology at affordable costs has brought this powerful tool to the bedside and has paved the way for personalized medicine. Newer bioinformatics approaches are being developed to analyze the sequencing data in order to better understand the mechanistic basis of rejection. However standard RNA sequencing analysis does not account for the invasion of the host immune cells into the allograft and the fact that the RNA sequences are generated from two different sets of DNA. The Het/Hom ratio, defined as the ratio of heterozygous to non-reference homozygous variant calls, has been traditionally used as a quality measure to identify contamination in variant calls using DNA sequencing data. RNA obtained from the allograft biopsies provides a classic example of "sample mix-up", as the allograft and immune cells on the allograft are from two different individuals. Herein, we propose the use of the Het/Hom ratio from the RNA sequencing data of the transplant kidney biopsies to resolve immune cell invasion into the allograft.
Methods: We studied 40 human kidney allograft biopsies, 35 showing abnormal findings based on the Banff classification, including acute cellular rejection (ACR, n=7), acute antibody mediated rejection (AMR, n=8), plasma cell rich acute rejection (PCRR, n=6), fibrosis (IFTA, n-=5), transplant glomerulopathy (TG, n=9) and 5 surveillance/protocol biopsies showing no abnormalities (Protocol). We isolated total RNA and sequenced using the Illumina HiSeq platform at high coverage (>36 million single ended reads per sample). Data was processed following GATK best practice protocols for RNA seq variant calling using the iGenome human Ensembl gene annotation. Individual Het/Hom ratios were calculated using an in-house python script. We used ESTIMATE software to infer the admixture of immune and donor kidney cells. These admixture fractions were converted into purity scores and a score of 1.0 describes no admixture of cells the biopsy.
Results: The median Het/Hom ratio of protocol biopsies, which have no/minimal invasion of immune cells, was 0.989. This observed Het/Hom ratio was lower compared to all rejection biopsy groups. The highest ratio of 1.263 was found in the ACR group.



The Het/Hom ratio was strongly correlated with the purity score from ESTIMATE (r=-0.77; p-value=5.3x10-9).



Conclusion: In the first of its kind study in kidney transplantation, we show the feasibility of using Het/Hom ratio as a measure of immune cell invasion into the kidney allograft. The Het/Hom ratio was significantly associated with fractional admixture of recipient immune and donor kidney cells in the allograft biopsies. Our results suggest that the Het/Hom ratio is an unbiased and universal biomarker for recipient immune cell invasion estimates in any type of allografts.

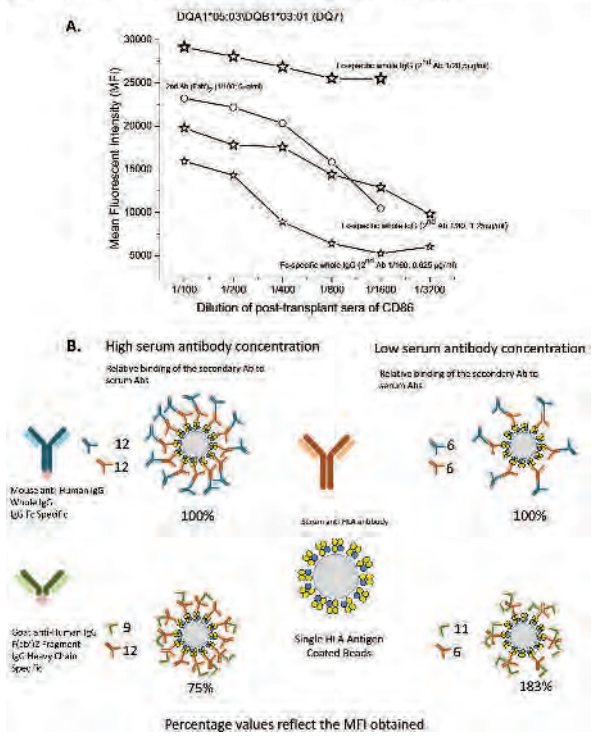
559.7

Importance of the nature and binding specificity of the secondary antibody used in Luminex single antigen bead assays for monitoring clinically relevant HLA IgG antibodies in pre- and post-transplant patients

Mepur H. Ravindranath, Vadim Jucaud, Nubia Banuelos, Anh Nguyen, Mathew Everly, Junchao Cai, Paul I.Terasaki.
HLA Immunology, Terasaki Foundation Laboratory, Los Angeles, CA, United States.

Antibodies (Abs) to HLA antigens are monitored to guide donor selection and kidney paired exchange programs, to assess de novo Ab-mediated rejection and to monitor desensitization therapies. Efforts have been made to provide insight into the sources of variability caused by commercially available HLA antibody testing kits. The PE-conjugated secondary antibody (2nd Ab) is least recognized as a factor that can influence the reproducibility and reliability of the assay.
In the 1960s, polyclonal (Fab')2 of IgG was developed as a 2nd-Ab for monitoring antigens on cell surfaces, tissue sections and Western blots, since its binding to multiple epitopes on the primary antibody promotes signal amplification when multiple (Fab')2 bind to antigen-bound antibody. For monitoring HLA-Abs, a dual-laser flow based detection flow cytometer (Luminex) is used with single antigen bead (SAB) assays, which defines the second laser as the one that "determines the magnitude of the PE-derived signal, which is in direct proportion to the amount of bound analyte" [1]. Fc-specific Abs are considered critical "for the quantitation of the heavy chain antibody only in samples containing other immune-globulins and proteins such as serum or tissue culture media"[2]. This implies that the signal amplification by multiple Fab or (Fab')2 may not be directly proportional to the HLA-bound antibodies on SAB, and hence not reliable or reproducible for monitoring the level of HLA-Abs.

Figure 1 **A.** Box titration (Primary Ab dilution X 2nd Ab dilution) profile of a *de novo* donor specific DQ antibody in a patient (CD86), examined with polyclonal $(Fab')_2$ 2nd Ab and monoclonal Fc-specific IgG 2nd Ab. Note the dilution of 2nd Ab required for titration. **B.** Theoretical model validates the hypothesis and illustrates the differences in the binding of $(Fab')_2$ *versus* Fc-specific IgG to high and low concentrations of the serum Ab. The superiority and reliability of the monoclonal Fc specific IgG 2nd Ab for monitoring high and low concentration primary Ab is indicated.



To test this hypothesis, the Mean Fluorescent Intensities (MFI) of HLA-Abs in the sera of healthy donors (n =13) and renal allograft recipients (n = 10) obtained with "constant heavy chain (CH)-binding" polyclonal PE-(Fab')2 and "Fc-specific" monoclonal PE-IgG (whole) as 2nd Ab in a Luminex SAB assay, are compared. IgG Isotype specific 2nd Abs were also employed. The 2-tailed paired-sample T-test was used to assess the significant differences.

Table 1 shows the number of HLA antigens reacting with (Fab')2 is higher than that with monoclonal Fc-specific IgG for both healthy and allograft recipients. For healthy donors, this difference is significant for all loci (1A). Among allograft recipients, significant difference is seen for HLA-B and HLA-DRB loci (1B). For both, the number of anti-HLA IgG isotypes are also lower than those recognized by (Fab')2 2nd Ab. Perhaps the reported prevalence of high numbers of "non-donor specific HLA antibodies" and even "pre-existing DSA" is a consequence of using (Fab')2 as the 2nd Ab. Strikingly, the MFIs (even titers) obtained for de novo DSA in allograft recipients are markedly higher with Fc-specific whole IgG than those obtained with (Fab')2 as 2nd Ab. An example and the mechanism underlying the differential binding of (Fab')2 and Fc-specific whole IgG as 2nd Ab are shown in Figure 1. These findings suggest the superiority of Fc-specific IgG over CH-binding (Fab')2 as the 2 Ab for SAB assays.

Table 1. **A.** The number of HLA antigens reacting for each HLA Loci is significantly higher with polyclonal CH-binding (Fab')$_2$ than with monoclonal Fc-specific IgG as 2nd Ab. **B.** The number of HLA antigens reacting for each HLA Loci is higher with (Fab')$_2$ than with Fc-specific IgG .

		Number of HLA reactive alleles											
		HLA-A*		HLA-I HLA-B*		HLA-Cw*		HLA-DR*		HLA-II HLA-DQ*		HLA-DP*	
	Number of HLA-antigens tested for each Loci	31		50		16		36		29		26	
A	Normal & Healthy Male (M) & Females (F)	(Fab')2	whole IgG	(Fab')2	whole IgG	(Fab')2	whole IgG	(Fab')2	whole IgG	(Fab')2	whole IgG	(Fab')2	whole IgG
	TFL-M01	0	0	1	0	1	0	4	3	0	0	4	4
	TFL-M02	0	0	2	0	10	5	5	4	2	2	9	4
	TFL-M03	0	0	0	0	7	4	6	4	7	2	10	2
	TFL-M04	1	0	6	0	7	0	9	2	1	0	5	2
	TFL-M05	2	0	1	0	10	2	7	2	0	1	5	4
	TFL-M06	4	0	9	0	15	9	4	4	5	1	7	7
	TFL-F01	18	7	15	0	15	12	17	16	10	6	9	5
	TFL-F02	26	0	26	0	16	12	12	5	12	6	12	8
	TFL-F03	0	0	1	1	9	7	4	5	0	2	4	6
	TFL-F04	3	0	1	0	15	3	3	3	1	1	5	5
	TFL-F05	24	0	37	0	16	14	22	6	13	4	13	9
	TFL-F06	0	0	2	1	15	11	5	3	3	1	6	5
	Paired Sample T p^2	0.05		0.036		0.0003		0.031		0.032		0.015	
B	Post-transplant Males												
	CD14	1	0	2	0	2	2	1	1	0	0	0	0
	CD40	0	0	0	0	0	0	11	10	0	0	0	0
	CD58	0	0	2	0	0	0	2	0	9	8	0	0
	CD61	0	0	4	4	0	0	0	0	0	1	0	0
	CD67	12	1	5	0	8	2	1	0	0	0	7	6
	CD86	10	10	28	19	0	0	10	9	11	9	0	0
	CD92	1	0	12	4	9	7	0	0	21	1	2	0
	CD85	1	1	11	2	0	0	2	0	5	1	0	0
	CD87	0	1	0	0	0	0	3	3	5	4	0	3
	CD815	0	0	1	1	0	0	0	0	3	2	0	0
	Paired Sample T p^2	NS		<0.02		NS		0.025		NS		NS	

(Fab')$_2$ = CH-binding polyclonal (Fab')$_2$ (1/100; 5μg/ml)
Whole IgG = Fc-specific Monoclonal whole IgG (1/20; 5mg/ml)

References:

[1] (1) www.rndsystems.com/resources/technical/luminex-bead-based-assay-principle
[2] (2) www.kpl.com/docs/techdocs/Antibodies%20in%20Immunoassays.pdf.

559.8

De-novo donor specific antibodies (DSA) monitoring in living related transplantation

Raj Kumar Sharma, Sonia Mehrotra, Mahabaleshwar Mayya, Narayan Prasad, Amit Gupta, Anupama Kaul, Dharmendra S.Bhadauria.
Nephrology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India.

Introduction: Development of donor specific antibody (DSA) could have an adverse effect on graft survival due to immunologically mediated acute or chronic allograft failure. Single antigen bead assay(SAB) on luminex platform is the gold standard for detection of post-transplant DSA. There are no reports of new onset DSA detected by solid phase cross match luminex assay using donor lysate as marker of rejection after transplantation.

Material and Methods: Present study is comparison of two methods (single antigen bead assay and solid phase cross match luminex assay using donor lysate), their sensitivity and specificity.121 living donor kidney transplant recipients were prospectively evaluated after transplantation for development of de-novo DSA against Class I and Class II HLA antigens. All recipients were complement dependant cytotoxicity (CDC) cross match negative at room, warm and cold temperatures at the time of kidney transplant. Eighty seven recipients (72%) were given induction with either Basiliximab (62 recipients) or ATG (25 recipients) at the time of kidney transplant.

Result: Twenty six recipients (21.4%) out of 121 developed dn-DSA within one to three months post-transplant by solid phase cross match luminex assay using donor lysate. Out of patients who were dn-DSA+ive, 11 were given Basiliximab and 9 got ATG induction. Sixteen out of 26 dn-DSA positive recipients (61%) developed only Class II DSA and seven patients (27%) developed only Class I DSA positivity; while in three patients (12%), both Class I and Class II dn-DSA developed. Eight recipients(31%) developed acute rejection in positive group; six (75%) of these patients with acute rejection had evidence of antibody mediated rejection (ABMR) with glomerulitis (5 C4d–ve, one C4d +ve), while two patients (25%) had T cell mediated AR. In the DSA negative group (78.5%): 18 recipients out of 95 (19%) developed acute rejection. Twenty two(18.2%) recipients out of 121 were positive for dn-DSA by single antigen bead assay and ninety nine (81.8%) were negative. All acute rejection episodes in dn-DSA negative group were responsive to treatment. There was more use of ATG induction as compare to Basaliximab induction in d-DSA positive group. On comparison of these two methods; the sensitivity of lysate assay was 100% (95% confidence interval 84.56 % to 100%) and specificity was 95.96% (95% confidence interval 89.98% to 98.89%) against SAB assay.There was increased dn-DSA development with ATG induction (36%) as compared to Basiliximab (17.7%) and no induction (17.6%).

Conclusion: Development of de-novo DSA was associated with more acute rejection episodes (31%) in positive group vs 19% in negative group. dn-DSA positive group had more ABMR (75% of AR) while 33.3% of AR in dn-DSA negative group were ABMR. DSA detected by luminex solid phase cross match developed in 18 patients (5 Class I, 11 Class II, both class I and II in 2 patients) without clinical rejection. These patients would need to be followed up to assess significance of dn-DSA on graft outcome.Lysate based solid phase cross match with 100% sensitivity and 95.96% specificity which is cheaper than SAB assay can be useful for post-transplant DSA monitoring.

559.9

Evidence for structural variants within an individual HLA-I molecule coated on Luminex single antigen bead assays: impact on monitoring HLA antibodies for organ allocation and de novo DSA in transplant patients

Vadim Jucaud, Mepur H.Ravindranath, Paul I.Terasaki.
Terasaki Foundation Laboratory, Los Angeles, CA, United States.

Recombinant HLA-I (rHLA-I) molecules coated on the single antigen beads (SAB) are used to screen HLA-I antibodies for pre-transplant organ allocation and post-transplant monitoring of de novo DSA. While a HLA-I trimeric complex, comprised of a glycosylated HLA heavy chain (HC), β2-microglobulin (β2m), and a peptide, is expressed on the cell surface, every single rHLA-I molecules on SAB are structurally diversified as β2m-associated HLA HC (β2aHC) and β2m-free HLA HC (β2fHC)[1]. The conventional LabScreen® HLA-I beads (LS1A04, Lot# 8, One lambda, USA) does not distinguish anti-β2aHC from anti-β2fHC. iBeads (Lot#1, One Lambda) specifically express β2aHC, and acid treated HLA-I beads (expressing β2fHC only) help to resolve the nature of HLA-I antibodies in patients[2],[3]. In addition, peptide dissociation from HLA HC results in peptide-free (pepF)-β2aHC and β2fHC molecules (Figure 1A).

There is a need to evaluate the relative distribution of the variants of an individual HLA-I molecule on SAB. We used anti-HLA-I monoclonal antibodies (mAbs), targeting the structural variants of rHLA-I: W6/32 (specific for β2aHC); TFL-006[4],[5] and HC-10 (both specific for β2fHC) (Figure 1B). mAbs were tested at 10µg/mL, MFI cutoff >1000 for positivity. The percentage (%) of β2fHC, coated on SAB, is based on the MFI of W6/32 and TFL-006, as HC-10 binds to alleles on iBeads whereas TFL-006 does not, but both bind to the same alleles on acid treated beads. Table 1A shows the median % of β2fHC on SAB.

HC-10 can bind HLA-I HC in the presence of β2m as HC-10 recognizes alleles on iBeads. The arginine (R) at position 62 on the α1 domain of a HLA HC is critical for HC-10 binding[6], and R62 is masked in the presence of peptide in the groove[7]; perhaps HC-10 recognizes a pepF-β2aHC. iBeads may carry peptide-associated and pepF-β2aHC, whereas HLA-I beads carry peptide-associated and pepF-β2aHC, as well as β2fHC. Table 1A shows the median % of pepF-β2aHC coated on iBeads. Acid treatment results in a homogeneous entity of pepF-β2fHC for all HLA-I alleles. Based on the reactivity of the mAbs, it is possible to identify the alleles with no or minimal β2fHC on HLA-I beads, as well as the alleles with β2fHC and pepF-β2aHC on iBeads (Table 1B).

Figure 1. A. rHLA-I conformation coated on SAB. **B.** Reactivity of conformational-specific mAbs against rHLA-I molecules

A

	Trimer (HC+pep+β2m)	Dimer (HC+β2m)	Dimer (HC+pep)	Monomer (HC)
	β2aHC		β2fHC	

B

W6/32	+++	+++	-	-
TFL-006	-	-	+?	+++
HC-10	-	+	-?	+++

The structural variants of any single HLA-I allele (presence and absence of β2m and a peptide in the groove), on HLA-I beads or iBeads, creates an awareness of similar variants in the binding specificities of de novo DSA or non-DSA, and also provides an explanation for reports on DSA associated with negative Flow-XM[8],[9]. Anti-β2fHC are found in non-alloimmunized healthy males[10], and are highly prevalent in HLA-sensitized patients awaiting donor organs[2]. The clinical evaluation of pre- or post-transplant DSA (using allele-specific MFI cutoff based on TFL-006 reactivity against HLA-I beads and HC-10 reactivity against iBeads) may improve the organ allocation for patients awaiting a transplant and the management of post-transplant patients, by discriminating β2fHC HLA-I DSA.

Table 1. A. Median Percentage of β2fHC coated on SAB. Median Percentage of peptide-free β2aHC coated on iBeads. **B.** Percentage of β2fHC on HLA-ClassI beads, iBeads, and percentage of peptide-free β2aHC on iBeads.

A

	HLA-ClassI Beads	iBeads	Acid treated HLA-ClassI beads
HLA-A Alleles (n=31)	7 ± 5.7%	1.5 ± 1.9%	98.3 ± 3.2%
HLA-B Alleles (n=50)	11.7 ± 7.5%	0.1 ± 0.3%	98.6 ± 2.7%
HLA-Cw Alleles (n=16)	28.2 ± 10.6%	0.2 ± 0.5%	98.8 ± 1.2%
	iBeads		
HLA-A Alleles* (n=20)	12.8% ± 20.9%		
HLA-B Alleles (n=50)	6.7 ± 9.5%		
HLA-Cw Alleles (n=16)	13.2 ± 8.1%		

B

HLA-ClassI beads specificities with NON-detectable β2fHC (% β2fHC) W6/32 positive and TFL-006 negative (Cutoff MFI>1000)		
HLA-A Alleles	HLA-B Alleles	HLA-Cw Alleles
A*02:01 (3%) A*02:06 (4%) A*03:01 (3%) A*23:01 (1%) A*25:01 (5%) A*26:02 (4%) A*32:01 (4%) A*34:02 (4%) A*66:02 (3%) A*74:01 (5%)	B*15:01 (3%) B*27:05 (4%) B*27:08 (4%) B*42:01 (4%) B*48:01 (3%) B*67:01 (1%)	None
iBeads specificities with detectable β2fHC (% β2fHC) W6/32 and TFL-006 positive (Cutoff MFI>1000)		
A*11:01 (8%) A*24:02 (5%) A*24:03 (5%)	None	None
iBeads specificities* with detectable peptide-free β2aHC (% pepfree-β2aHC) W6/32 and HC-10 positive (Cutoff MFI>1000)		
A*25:01 (18%) A*26:01 (21%) A*33:01 (36%) A*33:03 (18%) A*34:01 (32%) A*34:02 (21%) A*66:01 (24%) A*66:02 (22%) A*68:01 (12%) A*68:02 (25%) A*68:01 (28%)	B*13:01 (48%); B*14:02 (7%) B*15:02 (15%); B*15:03 (5%) B*15:10 (8%); B*15:11 (25%) B*[illegible] (33%); B*15:16 (16%) B*18:01 (15%); B*35:01 (13%) B*37:01 (26%); B*38:01 (21%) B*39:01 (9%); B*40:01 (13%) B*40:02 (6%); B*40:06 (11%) B*41:01 (7%); B*44:02 (8%) B*44:03 (9%); B*45:01 (22%) B*47:01 (30%); B*48:01 (15%) B*49:01 (15%); B*52:01 (12%) B*51:02 (10%); B*52:01 (11%) B*58:01 (26%); B*59:01 (9%)	Cw*01:02 (18%) Cw*03:02 (9%) Cw*03:03 (14%) Cw*03:04 (14%) Cw*06:02 (20%) Cw*12:03 (12%) Cw*15:02 (12%) Cw*16:01 (14%) Cw*17:01 (41%) Cw*18:02 (13%)

*HC-10 NON-reactive alleles. A*01:01, A*03:01, A*11:02, A*23:01, A*24:02, A*24:06, A*30:01, A*30:02, A*31:01, A*36:01, and A*80:01. The percentage of peptide-free β2aHC cannot be asseed and were not included.

References:

[1] El-Awar N, Terasaki PI, Nguyen A, et al. Epitopes of human leukocyte antigen class I antibodies found in sera of normal healthy males and cord blood. *Hum Immunol*. 2009;70(10):844–53. doi: 10.1016/j.humimm.2009.06.020.

[2] Visentin J, Guidicelli G, Bachelet T, et al. Denatured class I human leukocyte antigen antibodies in sensitized kidney recipients: prevalence, relevance, and impact on organ allocation. *Transplantation*. 2014;98(7):738–44. doi: 10.1097/TP.0000000000000229.

[3] Otten H, Verhaar M, Borst H, et al. The significance of pretransplant donor-specific antibodies reactive with intact or denatured human leucocyte antigen in kidney transplantation. *Clin Exp Immunol*. 2013;173(3). doi: 10.1111/cei.12127.

[4] Ravindranath MH, Terasaki PI, Pham T, et al. Therapeutic preparations of IVIg contain naturally occurring anti–HLA-E antibodies that react with HLA-Ia (HLA-A/-B/-Cw) alleles. *Blood*. 2013;121(11):2013–28. doi: 10.1182/blood-2012-08-447771.

[5] Ravindranath MH, Terasaki PI, Pham T, et al.Suppression of blastogenesis and proliferation of activated CD4+ T cells: intravenous immunoglobulin (IVIg) versus novel anti-human leucocyte antigen (HLA)-E monoclonal antibodies mimicking HLA-I

reactivity of IVIg. *Clin Exp Immunol*. 2014;178(1):154–177. doi: 10.1111/cei.12391.
[6] Perosa F, Luccarelli G, Prete M, et al. Beta 2-microglobulin-free HLA class I heavy chain epitope mimicry by monoclonal antibody HC-10-specific peptide. *J Immunol*. 2003;171(4):1918–26.
[7] Nurzia E, Narzi D, Cauli A, et al. Interaction pattern of Arg 62 in the A-pocket of differentially disease-associated HLA-B27 subtypes suggests distinct TCR binding modes. *PLoS ONE*. 2012;7(3):e32865.doi: 10.1371/journal.pone.0032865.
[8] Jacob EK, Goey SR De, Gandhi MJ. Positive virtual crossmatch with negative flow crossmatch results in two cases. *Transpl Immunol*. 2011;25(1):77–81. doi: 10.1016/j.trim.2011.05.007.
[9] Visentin J, Guidicelli G, Nong T, et al. Evaluation of the iBeads assay as a tool for identifying class I HLA antibodies. *Hum Immunol*. 2015;76(9). doi:10.1016/j.humimm.2015.09.012.
[10] Morales-Buenrostro LE, Terasaki PI, Marino-Vázquez LA, et al. "Natural" human leukocyte antigen antibodies found in nonalloimmunized healthy males. *Transplantation*. 2008;86(8):1111–5. doi: 10.1097/TP.0b013e318186d87b.

570.1

Evaluation of PK profile and clinical outcomes with Hexal® versus Prograf® in de novo renal transplant recipients: Results from SparTacus - a multicentre, prospective randomized study

Wolfgang Arns[1], Andrea Huppertz[1], Thomas Rath[1], Stephan Ziefle[1], Lars C.Rump[1], Anita Hansen[1], Daniel Baeumer[2], Klemens Budde[1], Lukas J.Lehner[1], Thomas Klein[1], Peter Schenker[1].
[1]Spartacus Study Group, Koln, Germany; [2]Novartis Pharma GmbH, Nuernberg, Germany.

Background: Little data exists for pharmacokinetic (PK) profile and safety and efficacy of generic Tacrolimus (TAC) vs the reference drug in renal transplant recipients (RTxR). SparTacus (NCT01649427) study was designed to compare PK profile of Tacrolimus Hexal® with Prograf® in RTxR and to evaluate whether the comparable exposure results in similar renal function.
Methods: Overall 76 *de novo* RTxR were randomized to this prospective two-phase open-label study, to receive TAC Hexal® (n = 35) or Prograf® (n = 41), with enteric-coated mycophenolate sodium + corticosteroids + basiliximab induction therapy. TAC was initiated at a dose of 0.15 mg/kg/day and further adjusted to target trough levels (C0) of 8–12 ng/mL from Tx to month (M) 1; 5–10 ng/mL up to M3; and 5–8 ng/mL up to M6. Primary objective of this phase I study was to demonstrate comparable PK at M1 post-Tx and non-inferior renal function (GFR, Nankivell) at M6 in TAC Hexal® vs Prograf®. Renal function was analyzed using ANCOVA model with treatment, donor status and center as factors and GFR at baseline as covariate. Incidences of efficacy and safety outcomes at M6 were also assessed.
Results: Overall 44 patients were included in PK analysis. At M1, PK parameters [dose-normalized TAC 12-h-AUC (h/10^{3}xL), Cmax (1/10^{3}xL) and mean 12 h TAC C0 (µg/L)] were comparable between TAC Hexal® vs Prograf® (Figure 1a). A total of 24 patients in the TAC Hexal® and 29 patients in Prograf® group were analyzed at M6. Renal function was better with TAC Hexal® vs Prograf® (unadjusted mean: 72.1 vs 63.0 mL/min). Mean GFR after adjusting (for the treatment, donor status and center, ANCOVA model) was also better with TAC Hexal® (47.9 mL/min) vs Prograf® (37.9 mL/min) and the between treatment difference of 10.0 mL/min (95% CI 1.10 – 18.90) was non-inferior (P = 0.004) and superior (P = 0.0285) in TAC Hexal® vs Prograf®. TAC Hexal® vs Prograf® had a comparable incidence of composite efficacy events and its individual components (Figure 1b). Incidence of adverse events (AEs) was comparable between TAC Hexal® vs Prograf® (AEs: 97.1 % vs 100%; serious AEs: 37.1% and 42.1%).
Conclusion: TAC Hexal® vs Prograf® had similar PK profile along with better renal function and comparable efficacy and safety in *de novo* RTxRs.

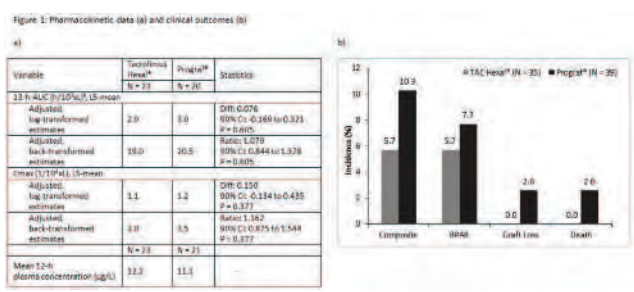


Figure 1: Pharmacokinetic data (a) and clinical outcomes (b)

a)

Variable	Tacrolimus Hexal®	Prograf®	Statistics
	N = 23	N = 20	
12-h AUC (h/10³xL), LS-mean			
Adjusted, log-transformed estimates	2.9	3.0	Diff: 0.076 90% CI: -0.169 to 0.321 P = 0.605
Adjusted, back-transformed estimates	19.0	20.5	Ratio: 1.079 90% CI: 0.844 to 1.378 P = 0.605
Cmax (1/10³xL), LS-mean			
Adjusted, log-transformed estimates	1.1	1.2	Diff: 0.150 90% CI: -0.134 to 0.435 P = 0.377
Adjusted, back-transformed estimates	3.0	3.5	Ratio: 1.162 90% CI: 0.875 to 1.544 P = 0.377
	N = 23	N = 21	
Mean 12-h plasma concentration (µg/L)	12.2	11.1	

b)

570.2

Reduced incidence of cytomegalovirus infection in kidney transplant recipients receiving everolimus: A cost-effectiveness analyses

Hélio Tedesco-Silva, Claudia Rosso Felipe, Alexandra Nicolau Ferreira, Marina Pontello Cristelli, Juliana Mansur Siliano, Mayara Ivani de Paula, Adrieli Barros Bessa, Priscila Ruiz Ruppel, José Medina - Pestana.
Nephrology Division, Hospital do Rim - UNIFESP, São Paulo, Brazil.

A cost-effectiveness analysis was performed using data from a prospective single center trial where de novo kidney transplant recipients received a single 3 mg/kg dose of rabbit antithymocyte globulin, tacrolimus, everolimus and prednisone (r-ATG/EVR, n=85); basiliximab, tacrolimus, everolimus and prednisone (BAS/EVR, n=102) or basiliximab, tacrolimus, mycophenolate and prednisone (BAS/MPS, n=101). This analysis was developed from the perspective of the Public Healthcare System. The clinical outcomes considered for this analysis were: acute rejection, CMV viremia/disease, graft dysfunction, surgery complications, graft loss and death. The economic outcomes contemplated in the analyses were direct medical costs, including: immunosuppression, treatment of adverse events and follow-up of patients. To estimate costs and outcomes for each treatment a Markov model was used to follow patients during 12 months after transplantation.
Compared to the standard of care regimen, r-ATG/EVR and BAS/EVR were associated with 36% and 15% reduction in costs. This was primarily due to lower costs associated with the management of adverse events as there were no significant differences in the costs of immunosuppressive drugs. Compared to the standard of care regimen, r-ATG/EVR showed higher effectiveness in all clinical outcomes while BAS/EVR showed higher effectiveness for the prevention of CMV infection and graft loss and comparable effectiveness for the other clinical outcomes. A probabilistic sensitive analysis with 1000 simulations, using ±20% variation in costs and beta distributions for transition probabilities, efficacy and safety data, confirmed these observations.
Conclusion: In this cohort of de novo kidney transplant recipients receiving tacrolimus and prednisone and no CMV pharmacological prophylaxis, everolimus containing regimens were dominant (cost-effective) compared to the standard of care regimen.

570.3

Abbreviated thymoglobulin and basiliximab induction therapy in patients undergoing kidney transplantation results in superior graft and patient survival with less infectious complications

Vaughn Whittaker, Angelika Gruessner, Rainer Gruessner.
Surgery, SUNY Upstate Medical University, Syracuse, United States.

Purpose: We report the two year outcomes from our single center experience with the novel induction regimen using Thymoglobulin (3 mg/kg) in divided doses given on the operative day (post operative day zero and post-operative day one and Basiliximab (20 mg) given on post operative day two, followed by steroid-free maintenance therapy for patients undergoing kidney transplantation.
Methods: We retrospectively reviewed 168 consecutive patients receiving thymoglobulin plus basiliximab induction therapy (cohort A) transplanted between August 2013 to December 2015 and compared outcomes against a prior five year series August of 2008 to August 2013 with 144 consecutive patients who received a basiliximab-based regimen followed by steroid maintenance (cohort B) for low immunologic risk patients PRA <20% and Thymoglobulin 6mg/kg for those with PRA >20%. The two groups were compared against the length of hospital stay, rejection events, infectious complications, graft failure, and death, for a period of up to 2 year.
Results: Patients receiving the thymoglobulin plus basiliximab induction therapy experienced shorter length of hospital stay compared to our historical cohort (mean 4.63 d vs. 6.25 d, median 4 d vs. 5 d). The biopsy proven rejection rate was similar between the two groups (11/168 (6.5%) vs. 14/144 (9.7%)), p <0.05. Infectious complications were similarly comparable between the two groups (42/168 (25%) vs. 59/144 (41.0%)), p<0.05. Graft failure rate (4/164 (2.38%) vs. 7/144 (4.86%)), p<0.05, and death rate for primary transplants (1/168) (0.59%) vs. 4/144 (2.7%)), p <0.05, in the thymoglobulin plus basiliximab group compared to our historical cohort. There were only rejection episode in the group with high immunologic risk PRA >20.
Conclusions: Two year results from utilizing a Thymoglobulin plus basiliximab for induction in kidney transplantation demonstrate that the combination is associated with decreased length of hospital stay, comparable infectious complication rates, and superior graft and patient survival. It may be associated with cost savings as well.

570.4

Comparison of mycophenolic acid (MPA) exposure after conversion from enteric-coated mycophenolate sodium (EC-MPS) twice-daily to once-daily in adult kidney transplant recipients

Eric Dailly[1], Claire Garandeau[2], Matthieu Grégoire[1], Guillaume Deslandes[1], Pascale Jolliet[1], Diego Cantarovich[2].
[1]Clinical and Pharmacological Department, Nantes University Hospital, Nantes, France; [2]Institute of Transplantation-Urology-Nephrology (ITUN), Nantes University Hospital, Nantes, France.

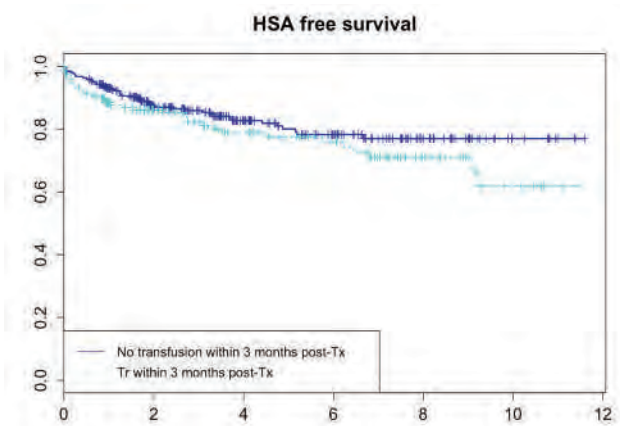
The variation of mycophenolic acid (MPA) exposure was investigated after the administration of the enteric coated formulation of mycophenolate sodium (EC-MPS), 720 mg twice-daily versus 1440 mg once-daily, in association with tacrolimus (Advagraf®) and prednisone, following adult kidney transplantation.
MPA full pharmacokinetic profiles were assessed 3 months after transplantation in 8 patients, before and after switching EC-MPS from 720 mg twice-daily to 1440 mg once-daily (morning intake). The pharmacokinetic parameters of MPA were calculated by a non-compartmental analysis. The log transformed values of MPA pharmacokinetic parameters were compared between periods using a paired t-test ($p < 0.05$ for significance).
No significant variations of MPA area under the plasma concentration–time curve from 0 to 24 h, plasma concentration at the end of the dosing interval, maximum observed drug concentration (Cmax) and apparent clearance were observed. Time to reach Cmax (Tmax) was significantly increased (3.29 h versus 2.16 h; p= 0.03) after administration of EC-MPS once-daily versus twice-daily.
The significant increase in Tmax but not Cmax when EC-MPS was given once-daily, confirmed the non-linear pharmacokinetic profile of MPA. This result could be explained by a saturable absorption of MPA and/or by a high inter-individual variability. EC-MPS once-daily (1440 mg) was well tolerated and seemed to be sufficient and efficacious as 720 mg twice-daily.

570.5

Impact of early red blood cell transfusion after kidney transplantation on de novo HLA sensitization in the setting of antithymocyte globulin induction

Thomas JOUVE[1,2], Guillaume Rigault[1,2], Benedicte Janbon[2], Rachel Tetaz[2], Nicolas Terrier[2], Dominique Masson[3], Lionel Rostaing[1,2], Paolo Malvezzi[2].
[1]Health department, Grenoble Alpes University, Grenoble, France; [2]Nephrology and kidney transplantation unit, Grenoble University Hospital, Grenoble, France; [3]HLA laboratory, Etablissement Francais du Sang, Grenoble, France.

Introduction: HLA specific antibodies detection (HSA) and that of Donor Specific Antibodies (DSA) were revolutionized by the introduction of solid phase assays (Luminex®), therefore making these antibodies a powerful biomarker for humoral injuries to the allograft. Determinants of HSA development entail non-adherence to immunosuppressive drugs but also allosensitizing events such as red blood cell (RBC) transfusions. Recent works showed a higher incidence of DSA in transplanted patients following RBC transfusion events, but no specific data exist for AntiThymocyte Globulin (ATG) induction.
This work aims at assessing whether peri-transplant RBC transfusion resulted in post-transplant HLA sensitization, in the setting of ATG induction therapy.
Patients and Methods: All consecutive patients benefiting from a first ATG-induced kidney allograft between 2004 and 2014 at our center with no history of HLA immunization were included retrospectively, provided transfusion history and HSA history were available. Our primary endpoint was immunization-free survival. Early transplantation was defined as a RBC transfusion within the first three months after transplantation. We defined de-novo HLA sensitization as at least one positive Luminex test post-transplantation (whether class I or II), without sensitization history before transplantation.
Results: Among the 1018 kidney transplants performed at our center, 432 patients corresponded to our inclusion criteria (82.7% of our patients received ATG induction). Of these, 152 (35%) received at least one RBC unit within the first three months after transplantation. With a median follow-up of 3.9 years, de-novo HLA sensitization occurred in 46 patients (16%) in the no early RBC transfusion group VS 34 patients (22%) in the early RBC transfusion group (chi-squared test: p=0.176, log-rank test: p=0.172, fig. 1). A multivariate Cox survival analysis (adjusting for recipient age, donor-status (living or brain-dead), pre-transplantation RBC transfusion and CNI use) shows that HSA development is not associated with early RBC transfusion (p=0.18).
Conclusion: We found that early RBC transfusion in the setting of ATG induction therapy does not result in HLA sensitization.



Sabine Noviant

570.6

Evaluation of genetic variants in diabetes susceptibility genes and disposition-related genes as risk factors for the development of new-onset diabetes after transplantation in Singapore asian adult kidney transplant recipients

Wai-Ping Yau[1], Diana Chee-Siang Teh[1], Anantharaman Vathsala[2,3].
[1]Department of Pharmacy, Faculty of Science, National University of Singapore, Singapore, Singapore; [2]National University Centre for Organ Transplantation, National University Hospital, Singapore, Singapore; [3]Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore.

Introduction: New-onset diabetes after transplantation (NODAT) is a clinically significant metabolic complication of transplantation contributing to increased risks of graft failure and mortality. Previous studies suggest that specific genetic variants increase the risk of NODAT. This study aimed to evaluate 14 single nucleotide polymorphisms (SNPs) in 8 diabetes susceptibility genes and disposition-related genes as potential risk factors for NODAT development in Singapore Asian adult kidney transplant recipients on tacrolimus- or cyclosporine-based immunosuppression.
Materials and Methods: A nested case-control study was conducted at National University Centre for Organ Transplantation, Singapore. Cases included kidney transplant recipients without pre-existing diabetes who developed NODAT while controls included those who did not develop NODAT. Blood samples were collected from study participants and DNA was extracted for genotyping at the following SNPs: *KCNQ1* rs2237892 and rs2237895; *HHEX* rs1111875, rs5015480 and rs7923837; *CDKAL1* rs10946398 and rs7756992; *TCF7L2* rs4506565; *CCL5* rs2107538, rs2280789 and rs3817655; *ADIPOQ* rs2241766; *CYP3A5* rs776746 *(CYP3A5*3)*; and *ABCB1 (MDR-1)* rs1045642. Odds ratio (OR) and 95% confidence interval (CI) were calculated to estimate the risk of NODAT development with each evaluated SNP.
Results and Discussion: Of the 101 patients who received kidney transplants between 1994 and 2014 (79.2% Chinese, 11.9% Malays and 8.9% Indians), 53 (52.5%) received tacrolimus-based immunosuppression while 48 (47.5%) received cyclosporine-based immunosuppression. A total of 36 patients (35.6%) developed NODAT over a mean (± standard deviation) follow-up duration of 3.2 ± 3.7 years. NODAT generally occurred earlier in patients receiving tacrolimus (1.7 ± 2.3 years, n=17) than in those receiving cyclosporine (4.6 ± 4.2 years, n=19). Overall, NODAT patients (n=36) had a larger pre-transplantation body weight than non-NODAT patients (n=65) (66.5 ± 13.2 kg vs. 60.1 ± 12.3, p=0.044). Among the 14 SNPs evaluated, increased NODAT susceptibility was observed for the *KCNQ1* rs2237895 AC genotype as compared with the AA genotype in the overall population (OR: 2.56; 95% CI: 1.02 – 6.44). Though not statistically significant, the *CYP3A5**1/*3 genotype seemed to confer an increased risk of NODAT development as compared with the *CYP3A5**3/*3 genotype among patients receiving tacrolimus (OR: 3.81; 95% CI: 0.99 – 14.67).
Conclusion: Our data suggest that pre-transplantation body weight and the *KCNQ1* rs2237895 AC genotype are associated with an increased risk of NODAT development in Singapore Asian adult kidney transplant recipients on tacrolimus- or cyclosporine-based immunosuppression. While the *CYP3A5**1/*3 genotype was not found to be significantly associated with NODAT development, the observed trend suggests possible genetic predisposition to NODAT among patients on tacrolimus-based immunosuppression that warrants further evaluation in larger studies.

571.1

Management of humoral rejection in renal transplantation: Results of a single center

Ebru H.Ayvazoglu Soy, Mahir Kirnap, Murat Yilmaz, Gokhan Moray, Mehmet Haberal.
Transplantation Center, Baskent University, Ankara, Turkey.

Introduction: Humoral rejection (HR) following renal transplantation (RT) is still major concern for graft survival despite improvements in surgical technique and immunotherapy. We had various protocols for HR with various success rates. In this study we evaluated three different protocols according to effects on graft survival.
Materials and Methods: Between October 1985 and December 2015 we performed 2248 RT at our centers. We diagnosed humoral rejection by renal biopsy and confirmed with PRA levels, USG findings and C4d deposition ratio. The choice of HR treatment protocols are done according to the changes in Turkish government health insurance payment system. HR are grouped according to treatment protocols; pulse steroid followed by anti-thymocyte globulin (PS+ATG), plasmapheresis followed by intravenous immunoglobulin (PF+IVIG), plasmapheresis followed by rituximab (PF+RX). We evaluated all RT patients with preoperative and postoperative creatinine levels (at the 6th and 12th month of RT). RT patients with HR were evaluated with C4d deposition ratio, creatinine levels before and after HR treatment and also preoperative and postoperative creatinine levels (at the 6th and 12th month of RT).
Results: We detected HR in 574 RT patients (29.8%). We applied PS+ATG in 124 (21.7%), PF+IVIG in 206 (35.8%), and PF+RX in 244 (42.5%) RT patients. In PS+ATG group we had no PRA positivity; we detected PRA positivity in 33 (16%) patients in PF+IVIG group, 39 (16%) patients in PF+RX group (p=0,394). No statistically significance is detected between groups' responses to rejection therapy with respect to creatinine levels (before and after rejection therapy at the first week, at the 6th and 12th month of RT). In all groups creatinine levels are significantly decreased after HR therapy (p <0,001).
Conclusions: Current treatment protocols for humoral rejection following RT are developed with new agents. There was no difference in response to treatment between these different protocols.

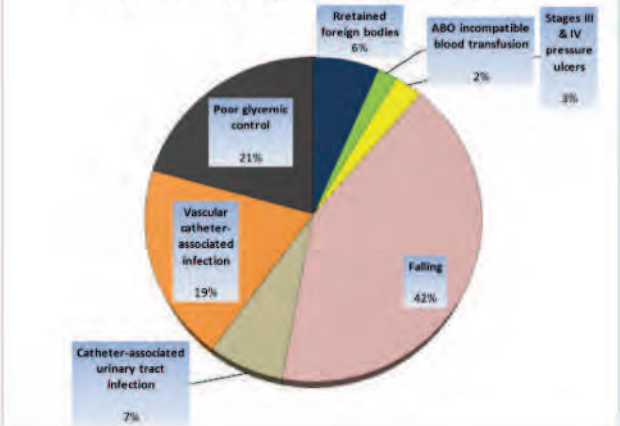
571.2

Never events and hospital acquired conditions after kidney transplant

Zhobin Moghadamyeghaneh, Mahmoud Alameddine, George Burke, Gaetano Ciancio, Linda Chen.
Miami Transplant Institute, University of Miami/ Jackson Memorial Hospital, Miami, United States.

Introduction: Never Events (NE) and Hospital Acquired Conditions (HAC) after surgery have been designated as quality metrics in health care by the centers for Medicare and Medicaid Services (CMS).
Methods: The NIS database 2002-2012 was used to identify patients who underwent kidney transplant. Multivariate analysis using logistic regression was used to identify outcomes and risk factors of HAC and NE after transplant.
Results: Among 172,586 patients who underwent kidney transplant alone there were 48 Never Events, all of which were due to retained foreign bodies. Among HAC after surgery, falling was the most common (42%) followed by poor glycemic control (21%), vascular catheter-associated infection (19%), catheter-associated urinary tract infection (7%), stages III & IV pressure ulcers (3%), and ABO incompatible blood transfusion (2%)(Figure 1). HAC and NE after surgery lead to a significantly increased length of stay (13 days vs. 7 days, P<0.01), hospital charges ($233128 vs. $147312, P<0.01), mortality (AOR: 2.78, P<0.01), and a trend towards increased need for reoperation (AOR: 2.05, P<0.01). A significantly higher risk of HAC or NE events was seen for patients who were expected to have higher mortality (AOR: 1.51, P=0.01) and morbidity (AOR: 2.02, P<0.01) before operation.
Conclusions: HAC and NE after kidney transplant are uncommon, but they are associated with a significant increase in mortality, hospitalization length of stay, hospital charges, and reoperation of patients. Quality improvement initiatives should target HAC and NE in order to successfully reduce or prevent these events.

Figure 1: Never Events and Hospital Acquired Conditions(HAC) after Kidney Transplant



571.3

Clinical outcomes of percutaneous coronary intervention on patients with cardiac allograft vasculopathy

Raymond Fung, Katherine Y.Y.Fan, Man Hong Jim.
Cardiac Medical Unit, Grantham Hospital, Hong Kong, Hong Kong.

Introduction: Cardiac allograft vasculopathy (CAV) is the Achilles heel of heart transplant. CAV accounts for nearly 25% of all deaths between year 1 and 10[1]. Although retransplantation is the only definite treatment for CAV, due to limited donor pool, it is rarely performed in our locality[2]. Bypass surgeries were also not commonly performed because of the suboptimal results. Another treatment option is percutaneous coronary intervention (PCI). The effect of PCI on native coronary artery disease has been extensively studied. There were relatively few studies on the clinical effect of PCI on CAV patients[3]-[6]. Most of the studies only involved early generation devices. In this retrospective study, patients with either angiographically or intravascular ultransound diagnosed CAV treated with PCI (both old and newer generation devices) were included. The primary objective is to determine the incidence of major cardiac events after the procedure.

Table 1. Baseline , clinical and procedural characteristics

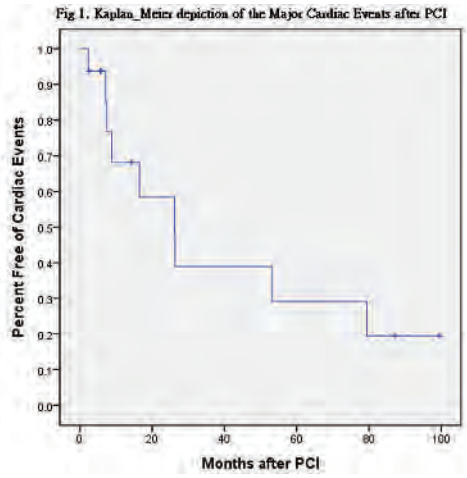
Baseline characteristics		
Age		51.4 ± 12.1
Male, gender n (%)		17 (87.5%)
Body weight, kg		61.5 ± 14.0
Body height, m		1.61 ± 0.06
Body mass index, kg / m^2		22.7 ± 3.23
Clinical characteristics		
Reasons for transplant	Ischaemic cardiomyopathy	6 (37.5%)
	Chronic rheumatic heart disease	4 (25%)
	Dilated Cardiomyopathy	3 (18.8%)
	Myocarditis	1 (6.3%)
	Tetralogy of fallot	1 (6.3%)
	Sarcoidosis	1 (6.3%)
Clinical presentations	Annual examination	11 (68.8%)
	ST elevation myocardial infarction	3 (18.8%)
	Stable angina	1 (6.3%)
	Congestive heart failure	1 (6.3%)
Cytomyegalovirus status	Positive	2 (12.5%)
Peripheral vascular disease		0 (0%)
Diabetes mellitus		6 (37.5%)
Hypertension		9 (56.3%)
Hyperlipidaemia		15 (93.8%)
Renal failure		2 (12.5%)
Old stroke		0 (0%)
Ejection fraction	> 55%	14 (87.5%)
	35-55%	2 (12.5%)
Smoking	Current smoker	2 (12.5%)
	Exsmoker	7 (43.8%)
	Nonsmoker	6 (37.5%)
	Unknown	1 (6.3%)
Aspirin		16 (100%)
Clopedogrel / Ticagrelor		16 (100%)
Beta-blocker		8 (50%)
Calcium channel blocker		5 (31.3%)
Angiotensin converting enzyme inhibitor		5 (31.3%)
Angiotensin II receptor blocker		3 (18.8%)
Statin		14 (87.5%)
Insulin		1 (6.3%)
Oral hypoglycaemic agents		5 (31.3%)
Calcineurin inhibitors	Tacrolimus	4 (25%)
	Cyclosporin	8 (50%)
Antimetabolites	Azathioprine	4 (25%)
	Mycophenolate mofetil	10 (62.5%)
Prednisolone		8 (50%)
Proliferative signal inhibitors	Everolimus	6 (37.5%)
Procedural characteristics		
Vessel(s)	Left main artery	1 (6.3%)
	Left anterior descending artery	13 (81.3%)
	Left circumflex artery	3 (18.8%)
	Right coronary artery	5 (31.3%)
Number of vessels	1	12 (75%)
	2	2 (12.5%)
	3	2 (12.5%)
Type of devices	Bare metal stents	2 (12.5%)
	First generation drug eluting stents	3 (18.8%)
	Second generation drug eluting stents	8 (50.1%)
	Second generation drug eluting stent + drug eluting balloon	1 (6.3%)
	Drug eluting balloon	1 (6.3%)
	Bioresorbable vascular scaffold	1 (6.3%)

Materials and Methods: Consecutive CAV patients treated with PCI in Grantham Hospital between 1 Jan 2005 and 31 Dec 2015 were included. Patients treated with medical therapy / CABG or retransplantation excluded. Methods of intervention and type of devices used were subjected to the discretion of operators. Clinical and procedural data were retrieved from the computer based electronic clinical records, PCI reports. The primary endpoint was the composite of cardiac death, target lesion revascularization (TLR), non-target lesion revascularization (nonTLR), congestive heart failure (CHF), acute

coronary syndrome (ACS) and stent thrombosis. Data were analyzed using SPSS software. Kaplan-Meier analysis was used to examine the major cardiac events.

Results and Discussions: A total of 16 patients were identified. Table 1 shows the baseline, clinical and procedural characteristics of the population. Most patients have CAV diagnosed during their annual examinations. Mean follow up duration was 5.14 ± 4.22 years. The mean time of PCI from transplant was 7.74 ± 3.88 years. The majority of patients were implanted with newer generation devices; eight (50.1%) patients had second generation drug eluting stents (DES), one (6.3%) patient had mixed second generation DES and drug eluting ballon (DEB), one (6.3%) patient had DEB and one (6.3%) patient had biosresorbable vascular scaffold . The incidence of major cardiac events was 9 (56.3%) (Fig 1). There were 1 (7.2%) cardiac deaths, 4 (25%) TLR, 3 (18.8%) nonTLR and 1 (6.3%) CHF. Mean time of cardiac event from PCI was 12.9 ± 3.8 years.

Conclusion: This study included patients implanted with newer generation devices. Despite improvement in interventional devices, CAV patients still have a high cardiac event rate (56.3%). The majority of the events were due to repeat revascularization which reflected progressive nature of the condition. There was only 1 death in our series. However this study is limited by its small sample size. The overall effect of PCI in CAV may be better shown in study with larger number of patients.



References:

[1] Costanzo MR, Naftel DC, Pritzker MR, Heilman JK,3rd, Boehmer JP, Brozena SC, et al. Heart transplant coronary artery disease detected by coronary angiography: a multiinstitutional study of preoperative donor and recipient risk factors. Cardiac Transplant Research Database. J Heart Lung Transplant 1998 Aug;17(8):744-753.

[2] Halle AA, DiSciascio G, Massin EK, Wilson RF, Johnson MR, Sullivan HJ, et al. Coronary angioplasty, atherectomy and bypass surgery in cardiac transplant recipients. J Am Coll Cardiol 1995;26(1):120-128.

[3] Heublein B, Pethig K, Maaß C, Wahlers T, Haverich A, Vasculopathy CA. Coronary artery stenting in cardiac allograft vascular disease. Am Heart J 1997;134(5):930-938.

[4] Jain SP, Ramee SR, White CJ, Mehra MR, Ventura HO, Zhang S, et al. Coronary stenting in cardiac allograft vasculopathy. J Am Coll Cardiol 1998;32(6):1636-1640.

[5] Lee MS, Kobashigawa J, Tobis J. Comparison of percutaneous coronary intervention with bare-metal and drug-eluting stents for cardiac allograft vasculopathy. JACC: Cardiovascular Interventions 2008;1(6):710-715.

[6] Lee MS, Yang T, Kandzari D, Mahmud E, Liao H, Kirtane A. Long−term clinical outcomes in patients treated with drug−eluting compared to bare−metal stents for the treatment of transplant coronary artery disease. Catheterization and Cardiovascular Interventions 2012;80(4):533-538.

571.4

NKG2A+ natural killer cells dominate the response to latent Epstein-Barr virus infection

Olivia Hatton[1], Dara Strauss-Albee[2], Sheri M.Krams[1], Catherine Blish[2], Olivia Martinez[1].

[1]Surgery/Abdominal Transplantation, Stanford University School of Medicine, Stanford, CA, United States; [2]Medicine/Infectious Disease, Stanford University School of Medicine, Stanford, CA, United States.

Introduction: EBV infection elicits a strong host immune response that is essential for preventing EBV-associated lymphomas in transplant recipients, including post-transplant lymphoproliferative disorder (PTLD). Natural killer (NK) cells are phenotypically heterogeneous lymphocytes capable of responding to viral infection and are known to participate in control of primary EBV infection, when the virus is in a lytic cycle phase. In contrast, the role of NK cells in the immune response to latently-infected B cells, as is seen in EBV+ PTLD, has not been established.

Materials and Methods: EBV+ lymphoblastoid cell lines (LCL), a model of EBV+ B cell lymphomas, were generated in vitro from a cohort of healthy donors (n=11). IL-2-primed primary human NK cells were co-cultured with primary, normal B cells or autologous EBV+ LCLs. Co-cultures were assayed by multi-parameter flow cytometry for target cell cytotoxicity or the functional markers CD107a and IFN-γ, the NK cell marker CD56, the NK maturation marker CD57, and the NK cell receptors NKG2A, NKG2C, NKG2D, 2B4, and CD16.

Results and Discussion: NK cells were able to induce greater target cell killing of autologous, EBV+ LCLs than uninfected B cells (8±2.1% vs 2.8±0.6%, p=0.016). In response to the autologous EBV+ LCL, 4.1±2% of NK cells produced IFN-γ, 13.5±6% degranulated, and 2.6±1.2% of NK cells both degranulated and produced IFN-γ above background levels. Individual and combinatorial analysis revealed that a higher percent of EBV-specific responding NK cells are enriched for NKG2A+ and display a NKG2A+2B4+CD16-CD57-NKG2C-NKG2D+phenotype.

Conclusion: EBV-specific NK cells constitute an NK cell subset that is NKG2A+2B4+CD16-CD57-NKG2C-NKG2D+. More broadly, these data provide direct evidence for a functional role for NK cells in the response to latent EBV infection. Finally, these studies lay the groundwork for harnessing this subset for mechanism-based NK cell immunotherapies for EBV+ B cell lymphomas that arise after solid organ and bone-marrow transplantation.

571.5

Erythropoietin level and post-transplant anemia: A comparison of living related kidney transplant, standard deceased donor kidney transplant, and expanded criteria deceased donor kidney transplant.

Kanin Thammavaranucupt, Atiporn Ingsathit, Surasak Kantachuvesiri, Bunyong Phakdeekitcharoen, Chagriya Kitiyakara, Arkom Nongnuch, Montira Assanatham, Vasant Sumethkul.
Division of Nephrology, Department of Medicine, Faculty of medicine Ramathibodi hospital, Mahidol university, Bangkok, Thailand.

Aim: Kidney transplantation is followed by restoration of excretory function and endogenous erythropoietin production. The purpose of this study was to investigate factors that impact the time resolution of anemia among kidney transplant recipients who received from living related donors (LRKT), standard deceased donors (SCD) and expanded criteria donors (ECD).
Methods: All consecutive kidney transplant patients were enrolled during a six months period and followed up to 3 months after kidney transplantation. They were classified as LRKT (Gr1), SCD-KT (Gr2), and ECD-KT (Gr3). Erythropoietin levels (ELISA) were measured at pre-operative day, immediate post-operative day (day0), day1, 3, 7, 14, 21, 28, 60, and 90. Data of transplant associated factors that can impact hemoglobin level were recorded and examined. These included renal function (serum creatinine, eGFR), cold ischemic time, blood loss, presence or absence of delayed graft function, presence or absence of bacteremia, type of immunosuppressive agents, and reticulocyte counts.
Results: A total of 53 patients were enrolled. There were 32 patients (60%) in Gr1, 11 patients (21%) in Gr2 (SCD-KT) and 10 patients (19%) in Gr3 (ECD-KT). Mean eGFR at day90 of Gr1, 2 and3 were 61.7±13.5, 60.36±19.5 and 51.21±26.3 ml/min/1.73m2, respectively (p=0.27). Mean EPO levels at day3, 90 of Gr1 were 14.81±9.5 and 16.66±8.6 IU/mL (p=0.43). Mean hemoglobin levels in Gr1 increased from 9.71±1.8 to 13.09 ±1.7 g/dL from day3 to day90. Mean EPO levels at day3, 90 of Gr2 were 11.76±7.6 and 14.94±6 IU/ml (p=0.48). Mean hemoglobin levels in Gr2 increased from 9.35±2 to 13.07±2.2 g/dL from day3 to day90. Mean EPO levels at day3, 90 of Gr3 were 7.68±10 and 17.49±12.2 IU/mL (p=0.12). Mean hemoglobin levels in Gr3 increased from 8.99±0.9 to10.53±2.5 g/dL from day3 to day90. Peak of serum EPO level in group1, 2, 3 occurred on day14, 28 and day90, respectively. The mean times to achieve Hb level more than 12 g/dl were at 8 weeks in recipients with immediate graft function but were calculated as more than 90 days for recipients with delayed graft function (p=0.006). The mean times to achieve Hb level more than 12 g/dl in patients who have normal range of EPO levels (3.7-29.5IU/mL) were 12 weeks. This is significantly longer than patients who have an EPO level less than 3.7 (mean times=2 weeks) (p=0.0003).
Conclusion: Serum EPO levels gradually increase with time after renal transplantation and were indifferent at day 90 among the three groups. However, only LRKT and SCD-KT can achieve Hb level more than 12 g/dl at day 90. ECD-KT have significant lower Hb level at day 90. These results suggest that the quality of graft function have higher impact on Hb level than endogenous serum EPO level.

571.6

MDR1 C3435T, C1236T and G2677T/A polymorphisms in renal transplant patients with tremor

Maria Macias[1], Pilar Salvador[1], Isaura Pedreira[1], Constantino Fernández[2], Angel Alonso[2].
[1]Pharmacy Department, A Coruña University Hospital, A Coruña, Spain; [2]Nephrology Department, A Coruña University Hospital, A Coruña, Spain.

Background: P-glycoprotein (P-gp) is expressed in brain and blood-brain barrier. Tacrolimus (TAC) is known to be a substrate of P-gp and may cause adverse neurotoxic effects, including tremor. Aim: To analyze the relationship between MDR1-C3435T, C1236T and G2677T/A polymorphisms with the manifestation of tremor in adult renal transplant patients.
Patients and Methods: A retrospective study of 53 patients who underwent a cadaveric renal transplantation treated with a TAC-based immunosuppressive regimen and with a follow-up period of 6 months. These patients had no history of tremors before transplantation. In the pharmacy service, while extracting blood samples for TAC measurement, a hospital pharmacist interviewed the patients using the Fahn-Tolosa-Marin Tremor Rating Scale in order to determine the tremor grade. Patients with grade 3 (marked) or 4 (severe) were considered to have tremor.
Blood levels were determined using CMIA on an Architect™ C8000 analyzer. All patients were genotyped for MDR1-C3435T, C1236T and G2677T/A with DNA chip technology using genomic DNA extracted from a peripheral blood sample. Patients were stratified according to genotype.
A statistical analysis was performed using SPSS version 19.0. The association of the MDR1 C3435T, C1236T and G2677T/A genetic polymorphisms with tremor was assessed using the Mann-Whitney test. $P<0.05$ was considered statistically significant.
All patients provided their consent.
Results: The study included 53 patients (30 men/23 women), aged 51 years and weighing 72 kg.
The genetic variants identified for C3435T were as follows: CC in 12 patients (23%), CT in 23 (43%), TT in 18 (34%); C1236T: CC in 14 patients (26%) and CT in 27 (51%) and TT in 12 (23%) and G2677T/A were: GG in 16 patients (30%), GT in 24 (45%) and TT in 13 (25%). This distribution was in accordance with the Hardy-Weinberg equilibrium.
14 patients (26%) showed grade 3 and 4 tremors. None of the MDR1 polymorphisms studied (C3435T, C1236T and G2677T/A) were individually associated with tremors; however, a non-statistical trend was observed towards the presence of tremors in mutant (T-allele) carriers.
When the analysis was limited to patients with tremor, 13 patients were at least one-T allele carriers in two of the MDR1 genetic polymorphisms assessed (6 of them were mutant for the three genes –TTT-) and the other patient was wild-type for them (CCG) ($p<0.05$).
When analyzing the possible influence of TAC trough blood concentrations on tremor at 15 days, 1 and 6 months after transplantation, no significant associations were found, and similar trough concentrations were recorded in patients with tremor (12.29±4.13, 12.81±3.52 and 7.47±2.14 ng/mL, respectively) and those without (11.53±4.82, 11.46±4.70 and 8.00±2.63 ng/mL.
Conclusions: This study showed an association between TAC-related tremor and the mutant TTT haplotypes (3435TT, 1236TT and 2677TT) of MDR1 in renal transplant patients. This could indicate an accumulation of TAC in brain due to a reduced efflux.

572.1

The value of interferon-gamma releasing assays (IGRA) determining latent tuberculosis infection in renal transplant candidates

Füsun Eyüboğlu[1], Balam Er Dedekargınoglu[1], Özlem Salman Sever[1], Aydıncan Akdur[2], Mehmet Haberal[2].
[1]Pulmonary Diseases, Baskent University Medical School, Ankara, Turkey; [2]General Surgery, Baskent University School of Medicine, Ankara, Turkey.

Objectives: Tuberculosis (TB) has 20-75 times higher incidence among patients with organ transplantation with high mortality. Latent tuberculosis infection (LTBI) is an asymptomatic condition that persists for many years in individuals,and may progress to active TB disease . Tuberculin skin test (TST) and Interferon-gamma release assays (IGRA) both depend on the specific immune response of the patient to Mycobacterium tuberculosis. Immunosupression increases in end stage renal disease (ESRD) due to high urea levels, causing lymphocyte dysfunction and this condition deepens during post transplant period. For that reason, detection of LTBI in transplant candidates is important because active TB secondary to reactivation of latent TB infection is a preventable disease. IGRA is known to have higher sensitivity among immunocompromised patients than TST. The aim of our study is to investigate the factors that influence IGRA test results in renal transplant candidates who are immunocompromised due to uremia.
Methods: 202 patient (76 women, 126 men) records of renal transplant candidates tested with IGRA were evaluated retrospectively (between 2011 to 2015 records evaluated. Patients with no IGRA tests or history of lung surgery (to avoid misleading lung radiology) were excluded. Chest X-rays and thorax computed tomographies were evaluated to detect thoracic sequelae of tuberculosis. Also, TB exposure and TB diagnosis history of the patients were noted. Patients with both negative IGRA results and positive thoracic sequelae of TB or history of TB / exposure of TB were noted as 'false negative' IGRA. Positive and indefinite IGRA results were also noted.
Results: IGRA tests were 68,3% negative. When IGRA results evaluated with radiology and positive history or exposure of TB 31 of the negative IGRA results were 'false negative' (15,3%). Of these false negative patients, 67, 7% had smoking history. And the relation was significantly positive between false negative IGRA and smoking history (rho=0, 26 and p=0). Alsofalse negative IGRA were positively correlating with lymphocyte count (rho=-0, 35 and p=0).
Conclusion: IGRA sensitivity is lower in renal failure patients and smoking and lymphocyte cell count has a role in IGRA false negative results. There is not a gold standard test for LTBI but IGRA is known to be a better test than TST in immunocompromised patients. Even IGRA have gaps in detecting LTBI, new tests are needed for acurrate detection of LTBI to prevent reactivation TB in transplant candidates.

572.2

Predictors and outcome of influenza A/H1N1 infection in kidney transplant recipients in Kuwait

Tarek Mahmoud, Khaled A. T.Atea, Torki Al-Otaibi, Osama Gheith, Jude Yagan, Magdalena Cerna, Medhat Abdel-Halim, Nair Prasad, M.R.N. Nampoory.
Nephrology, Hamed Al-Essa Organ Transplant Center, Kuwait, Kuwait.

Introduction: Following the pandemic of Influenza A/H1N1 in 2009, reports describing the epidemiological and clinical data in solid organ transplantation from different centers were variable. The correlation between patient's transplant characteristics, medical co-morbidities and presentation is crucial to predict the overall outcome.
Patients and Methods: We collected the data of 241 kidney transplant recipients (KTR) who presented with symptoms of probable H1N1 infection including demographic and transplant data, co-morbid conditions and presenting symptoms. The outcome was assessed and compared between positive and negative cases.
Results: There was no significant difference between the demographic characteristics for the positive (n=69) and negative (n=172) groups. Although the type of induction immunosuppression and the use of mycophenolate and tacrolimus did not increase the incidence of the infection, steroids (p=0.001) and cyclosporine (p=0.003) did increase and sirolimus reduced the incidence (p=0.053). Calcineurin inhibitor free regimens were associated with less incidence of infection (p=0.001). Among medical co-morbidities, diabetes was found to be less likely associated with infection (p=0.013). Presenting symptoms of significance were cough (p=0.0001), headache (p=0.0001) and malaise (p=0.0001). Out of the positive group, 24 patients required hospitalization. Fever was a presenting symptom (p=0.001). Four patients developed complications in the form of pneumonia in three and urinary tract infection in fourth. One of the pneumonia patients required endotracheal intubation and ventilatory support, and two patients developed renal graft rejections. All complications were treated adequately without any graft or patient loss.
Conclusion: Influenza A/H1N1 is highly predictable in KTR receiving cyclosporine or steroid and less likely in patients on sirolimus. Cough, headache and malaise are more likely presenting symptoms in H1N1 positive patients. Morbidity and mortality did not increase with the infection.

572.3

Catheter-associated urinary tract infection (CAUTI) in kidney transplant recipients is high cost burden and prolong hospital stay, report from a single center in the northeastern Thailand

Ubonrat Toimamueang[1], Chitranon Chan-on[2], Ekkarin Chotikawanich[3], Vayune Vajanaopath[4].
[1]Nursing Division , Srinagarind Hospital, Khon Kaen University, Khon Kaen , Thailand; [2]The Nephrology Division, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand; [3]Department of Surgery, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand; [4]Faculty of Medicine, Siriraj Hospital, Mahidol University, Khon Kaen, Thailand.

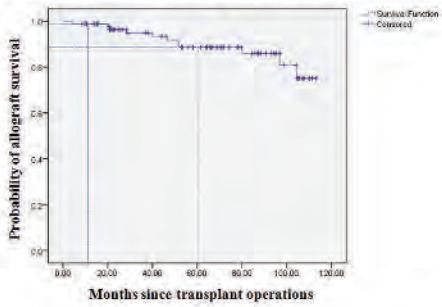
Introduction: CAUTI is a common complication in post-operative kidney transplant recipients because of immunocompromised status and urinary catheter has to be retained for a while after surgery. There are limited studies has done in this field to present outcome and burden of this complication. The purpose of this study is to determine the allograft outcomes and burden of CAUTIs in kidney transplant (KT) recipients.
Materials and Methods: This single center retrospective cohort was conducted in Srinagarind hospital, the Faculty of Medicine, Khon Kaen University, Thailand. Data were collected from medical and related records. Inclusion criteria included aged 18-60 years old transplant recipients which primary kidney transplant operations have done from January, 2003 to December, 2011. All has asymptomatic or symptomatic bacteriuria that evident by positive urine culture more than 105 CFU/ml during post-operative urinary catheter insertion until 48 hours after catheter removal. Record forms were reviewed by 3 experts. The data were analyzed using descriptive statistic.
Results and Discussion: Ninety-two recipients with 131 CAUTI events met the criteria. 63% were male, mean age was 41.11 ±10.3 (mean ± S.D.), 11.8% were diabetes, 93.5 % used hemodialysis as pre-transplant dialysis and 89.1 % were deceased donor type. Mean duration of urinary catheter retained was 13.3 ± 13.3 days (mean ± S.D.) There were 20 patients had recurrent bacteriuria. All patients had got three days antibiotic prophylaxis and vesico-ureteric stenting as protocol. 33.7 % have urinary catheter re-insertion after first episode removal. 63.4 % of urine cultures were positive for Gram negative organisms, 19.1 % were Gram positive organisms, 1.5 % were fungus and 16 % were mixed organisms. 17.4 % suffered from septicemia and mean length of hospital stay (LOS) was 32.63 ±19.56 (mean ± S.D.) day. 21.7 % were re-admission within 4 weeks from any cause. Mean follow-up time was 62.4 month ± 33.6 (mean ± S.D.). There was no graft loss after 3 months. Mean eGFR at first month and third month were 60.85 ± 19.72 ml/min/1.73m2 and 61.85 ± 20.06 ml/min/1.73m2. 1-years graft survival was 97 % and 5-years graft survival was 86%. Mean hospital cost was 229,200 ±1.05 (mean ± S.D.)Thai baht (6,409 US dollars).This result shown CAUTI in our center can results in septicemia and major organism was Gram negative.

Table 1. Demographic clinical data from kidney transplant recipients with CAUTI

Clinical data	n	%
Patients	92	100
Mean age (years old ± S.D.)	41.11 ± 10.3	
Male gender	58	63
DM	8	8.7
Deceased donor type	82	89.1
CSA-based IS regimen	61	66.3
TAC-based IS regimen	31	33.7
Mycophenolate Mofetil usage	60	64.3
Mycophenolic acid usage	22	23.9
IL-2 receptor blocker induction	18	19.6
DGF (dialysis in 1st wk)	36	39.1
Mean duration of urinary catheter (day ± S.D.)	13.79 ± 13.49	
Min-Max (days)	4 - 89	
Urinary catheter re-insertion	31	33.7
Bladder irrigation	7	7.6
Kidney allograft fluid culture	7	7.6
Urological complication	20	21.7
Antibiotic prophylaxis: Ceftriazone	92: 86	100: 95.7
Vesicoureteric stent retention	92	100
Mean follow-up time	62.41 ± 33.61	

Conclusion: Even though short-term graft outcome does not seem affected, there is high burden from prolong hospital stay and cost. Prospective intervention studies are needed to reduce CAUTI in our transplant recipients.

Figure 1. Kaplan-Meier curve of allograft survival in post-operative CAUTI subjects whose transplanted during January, 2003 to December, 2011



References:

[1] Saad SD, Hussein SM, Elbarassi SM, Zunni M. Urinary tract infection in renal transplant patients followed up at Benghazi Nephrology Centre. *The libyan journal of Infectious Diseases* 2010; 4 (1): 31-34.
[2] Sorto R, Irizar SS, Delgadillo G, Alberú J, Correa-Rotter R, Morales- Buenrostro LE. Risk factors for urinary tract infections during the first year after kidney transplantation. *Transplantation Proceedings* 2010; 42: 280–281.
[3] Rivera-Sanchez R, Delgado-Ochoa D, Flores-Paz RR, García-Jiménez EE, Espinosa-Hernández R, Bazan-Borges AA, Arriaga-Alba M. Prospective study of urinary tract infection surveillance after kidney transplantation. *BMC Infectious Diseases* 2010 ; 10: 245.
[4] Dharnidharka VR, Agodoa LY , Abbott KC. Effects of urinary tract infection on outcomes after renal transplantation in children. *Clin J Am Soc Nephrol* 2007 ; 2: 100–6.
[5] Lqbal T, Naqvi R, Fazal Akhter SF. Frequency of urinary tract infection in renal transplant recipients and effect on graft function. *J Pak Med Assoc* . 2010, 60(10) : 826-9.
[6] Menegueti MG, Pereira MF, Bellissimo-Rodrigues F, Tania Marisa Pisi Garcia TM , Santamaria Saber LT, Maria Estela Papini Nardim ME, Muglia VA, Neto MM, Romão EA. Study of the risk factors related to acquisition of urinary tract infections in patients submitted to renal transplant. *Rev Soc Bras Med Trop* . 2015, 48(3):285-290.
[7] Toimamueang U., Chotikawanich E., Thepsuthammarat K, Anunnatsiri S, Vajanaopath V. Incidence and Risk Factors of Catheter Associated Urinary Tract Infection among Kidney Transplant Recipients. *Journal of Nursing and Health Care* . 33 (1) :115-26.

572.4

Mycobacterium tuberculosis in solid organ transplantation, impact of expanded isoniazid prophylaxis

Hassan Aleid[1], Suad Al Mukhaini[2], Fatma Alduraibi[2], Hanan Hakami[2], Haifa Al Talhi[2], Mohamed Shoukri[3], Abdulrahman A. Alrajhi[2].
[1]Department of Kidney and Pancreas Transplantation, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia; [2]Medicine, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia; [3]Biostatistic, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia.

Background: Tuberculosis is a major challenge in solid organ transplantation, with increased risk of activation, complications and mortality. We report frequency and incidence of Mycobacterium and tuberculosis infection in transplant recipients and the results of expanded isoniazid chemoprophylaxis.
Methods: Recipients of solid organ transplantation during a ten-year period (January 2003-December 2012) are included. Tuberclosis-free transplantation follow-up is used for incidence rates calcultation. The impact of expanded isoniazid chemoprophylaxis in renal transplant recipients on tuberculosis rates is reported.
Results: Of 1966 solid organ transplantation recipients as kidney (1391), liver(426), heart (114), and lung (35), 20 recipients (1.02%) developed tuberculosis. Incidence is 2.48 cases per 1000 transplant-years. Twelve cases (60%) developed tuberculosis within one year of transplantation. The frequency and incidence of tuberculosis per 1000 transplant-years for specific organs are: kidney:0.58% and 1.27, liver, 1.88% and 5.94, heart:1.75% and 5.7, and lung: 5.71% and 47.5. On survival analysis, only lung transplant recipients had significant difference compared to recipients of kidney from living donor (p value 0.0001), rate ratio 45.3 times (95% CI: 7-313). Case fatality was 5%. Fourteen patients (70%) were cured without any documented relapse. Two transplanted kidneys were lost. After the expanded isoniazid chemoprophylaxis among deceased-donor kidney recipients, no tuberculosis occured in 177 recipients, compared to 3 out of 155 (2%) recipients before the program.
Conclusions: Tuberculosis among our solid transplant recipient population is reduced. The expanded isoniazid chemophrophylaxis among renal transplant recipients resulted in reduction of tuberculosis.%%

References:

[1] 1. Munoz P, Rodriguez C and Bouza E. Mycobacterium tuberculosis infection in recipients of solid organ transplants. Clin Infect Dis 2005;40:581-7.
[2] 2. Lattes R, Radisic M, Rial M, Argento J and Casadei D. Tuberculosis in renal transplant recipients. Transpl Infect Dis 1999;1:98-104.
[3] 3. Malhotra KK, Dash SC, Dhawan IK, Bhuyan UN and Gupta A. Tuberculosis and renal transplantation—observations from an endemic area of tuberculosis. Postgrad Med J 1986;62:359-62.
[4] 4. Qunibi WY, al-Sibai MB, Taher S, et al. Mycobacterial infection after renal transplantation—report of 14 cases and review of the literature. Q J Med 1990;77:1039-60
[5] 5. Naqvi SA, Hussain M, Askari H, et al. Is there a place for prophylaxis against tuberculosis following renal transplantation? Transplant Proc 1992;24:1912.

572.5

Bloodstream infections after haematopoietic stem cell transplantation: Risk factors, etiologic spectrum and predictors of fatal outcome

Igor Stoma[2], Anatoly Uss[1], Natalia Milanovich[1], Igor Karpov[2], Igor Iskrov[1].
[1]Department of Bone Marrow Transplantation, City Clinical Hospital No. 9, Minsk, Belarus; [2]Department of Infectious Diseases, Belarusian State Medical University, Minsk, Belarus.

Background: Bacterial bloodstream infections (BSI) remain a frequent complication in the preengrafment period after haematopoietic stem cell transplantation (HSCT), demonstrating high levels of mortality. Knowledge of risk factors and causes of BSI after HSCT is one of the key steps in choosing the appropriate antibacterial treatment and prophylaxis regimen.
Objectives: to determine the risk factors and etiologic agents of BSI in haematopoietic stem cell transplant recipients; to define possible clinical predictors of fatal outcome in case of BSI after HSCT.
Methods: A prospective case control study was performed at the center of haematology and bone marrow transplantation in Minsk, Republic of Belarus. Data relating to age, gender, date and type of transplantation, conditioning chemotherapy regimen, antibacterial therapy/prophylaxis, microorganisms isolated from blood and mucous membranes were prospectively collected in all adult haematopoietic stem cell transplant recipients during a 3-year period. Logistic regression was performed to establish the risk factors for BSI and predictors of 30-days fatal outcome after the onset of febrile neutropenia.
Results: Totally 135 patients had a microbiologically proven BSI after HSCT, with 65.19% caused by gram-negative microorganisms, including 21.48% by non-fermenting bacteria. Among independent risk factors for developing a BSI after HSCT were: massive colonization of mucous membranes of oral cavity by A. baumannii, P. aeruginosa, K. pneumoniae and E. coli (OR 2,44; 95% CI 1,04–5,74; p=0,0401) and absence of antibacterial prophylaxis (OR 10,94; 95% CI 2,49–48,14; p=0,0016). Inadequate empirical antibacterial therapy, isolation of carbapenem-resistant non-fermenting gram-negative bacteria (A. baumannii and P. aeruginosa) and acute myeloid leukemia as a primary diagnosis were independently associated with higher level of all-cause 30-days mortality in adult patients with BSI after HSCT.
Conclusion: Over 65% of BSI in adult patients after HSCT were caused by gram-negative pathogens. Massive colonization of oral cavity by A. baumannii, P. aeruginosa, K. pneumoniae, E. coli and absence of antibacterial prophylaxis were shown to be the risk factors for BSI after HSCT, while inadequacy of empirical antibacterial therapy, isolation of carbapenem-resistant A. baumannii or P. aeruginosa and acute myeloid leukemia as a primary diagnosis were established as clinical predictors for fatal outcome.

572.6

The frequency of pretransplant HHV-8 and BKV in renal transplant recipients and donors and the effect of renal transplantation on HHV-8 and BKV replications

Aydın Turkmen, Özlem Güven, Sevim Meşe, Ali Ağaçfidan, Berna Yelken, Serpil Görçin, Yaşar Çalışkan, Gülden Çelik, Salih Türkoğlu, Burak Koçak.
Nephrology, Istanbul Medical Faculty, Istanbul, Turkey.

Human herpes virus type 8 (HHV-8) and polyomavirus (BKV) are important viral infections that may cause significant morbidity. Posttransplant monitorization is very useful for these viral infections. In this study, we aimed to determine HHV-8 and BKV seroprevalance in renal transplant donors and recipients. We also aimed to discover the effect of renal transplantation in these virus replications.
Patients and Methods: 49 renal allograft recipents and 43 donors were included to study. The blood samples were collected before renal transplantation in recipients and donors for HHV-8. HHV-8 antibodies were tested by IFA and ELISA methods in serum samples and HHV-8 DNA was tested by real time PCR methods. HHV-8 DNA was screened posttransplant 2. weeks and 1-3-6-12-18-24 months. BKV-DNA was also screened by real time PCR in donor and recipient in pretransplant period and regularly after transplantation in recipients.

	Donors (n: 43)					Recipients (n:49)				
	HHV-8			BKV-DNA		HHV-8			BKV-DNA	
	ELISA	IFA	PCR	Urine	Plasma	ELISA	IFA	PCR	Urine	Plasma
Pretx	(3-6.9%)	(2-4.6%)	0	(1-0.43%)	0	(4 - 8.2%)	(5-10.2%)	(1-0.49%)	0	0
Posttx – 1. month						0	0	0	**5(10.2%)**	0
Posttx 12 months						0	0	0	0	0

Results: In pretransplant period, HHV-8 seropozitivity was 8.2% in recipients and 6.9 % in donors by ELISA, 10.2% and 4.6% by IFA. In donors, we did not detect HHV-8 DNA in pretransplants tests. HHV-DNA was detected only one patient with 44 copy/ml. This titer was screened 828 and 134 copy/ml, second week and 1 month respectively. In recipients group there were no patients with HHV-8 DNA positive except that patients.
BKV-DNA was detected in urine only one (2.3%) donor before transplantation by 12 700 copy/ml. The plasma sample of same donors was negative. In recipient group, BKV- DNA was not detected in the same time period.
BKV- DNA viruria was found in 5 male patients in posttransplant out patients controls. In one patient, BKV- DNA was detected over 107 copy/ml (46 800 000 copy/ml) in posttransplant first month and this titer gradually decreased by the manipulation of immunosuppressive medications. Plasma samples of these patients were found negative. BKV reactivation was observed 10.2% in recipient group.
Conclusion: Immunosuppressive therapy did not cause HHV-8 reactivation in our renal transplant cohort. Posttransplant BKV reactivation and viruria may be related to immunosuppressive treatment
This work supported by Scientific Researchs Projects of Istanbul University (Project number 2424)

573.1

Serial transverse enteroplasty for patients with short bowel syndrome secondary to radiation therapy

Yousheng Li, Qi Mao, Danhua Yao, Jian Wang, Jian Wang, Yong Chen.
Center of Intestinal Rehabilitation and Transplantation, Jinling Hospital, Nanjing University School of Medicine, Nanjing, People's Republic of China.

Backgrounds: Numerous studies have demonstrated the feasibility of serial transverse enteroplasty (STEP) in short bowel syndrome (SBS). The objective of this study was to evaluate the outcome of patients with postresection SBS secondary to radiation therapy (XRT) who underwent STEP.
Methods: The observational cohort study population consisted of 5 adults developing SBS caused by XRT and underwent STEP simultaneously between September 2014 and February 2015. Patient demographics, and data from the time of STEP and at follow-up were collected and analysed.
Results: There was no death at a median follow-up of 15 (range 12-17) months and no patient had small bowel transplantation. All patients were weaned from parenteral nutrition (PN) at a median of 2.3 (range 1.1-3.8) months. There was one patient developed gastrointestinal bleeding and resolved after one month observation and total parenteral nutrition (TPN) without reoperation.
Conclusions: The preliminary results show STEP procedure for patients with SBS after XRT facilitate weaning from PN with few surgical complications. The study is limited by the small sample size and lack of a control group.

573.2

The prophylactic effect of secretin for intestinal failure-associated liver disease

Yousheng Li, Wencheng Kong, Jian Wang, Jieshou Li.
Center of Intestinal Rehabilitation and Transplantation, Jinling Hospital, Nanjing University School of Medicine, Nanjing, People's Republic of China.

Objective: To explore the effect of secretin to intestinal failure-associated liver disease(IFALD) by supplying physiological doses of secretin to SBS rat model.
Methods: Using 24 male SD rats, we divided them into four groups randomly: SBS+secretin (n=6), SBS+ placebo (n=6), Sham+secretin group (n=6), Sham+placebo (n=6). SBS group underwent 80% intestinal resection and anastomosis. All rats underwent jugular vein cannulation and accepted TPN support for 14 days. Sham group underwent sham resection and anastomosis. We detected blood biochemistry of all rats at preoperative day, 7th day and 14th day. Finally, before rats were sacrificed, we detected bile flow and kept liver tissue specimens.
Results: the ALT (95.28±18.25 U/L), AST (211.96±19.27 U/L), TB (211.96±0.30μmol/L), TBA (23.78±3.59 μmol/L) of SBS+placebo group were significantly higher than those of SBS+secretin group (ALT, 76.35±8.12 U/L;AST, 175.35±7.53 U/L;TB, 175.35±7.53 μmol/L;TBA, 19.59±1.54μmol/L). In addition, the bile flow of SBS+placebo group (6.14±3.04, μml/min)? was also lower than that of SBS+secretin group (14.11±4.97μml/min). The pathological score and TUNEL apoptosis semiquantitative of SBS+secretin group were significantly lower than those of SBS+placebo group.
Conclusions: The secretin can significantly decrease IFALD by supplying physiological doses of secretin. This protective effect was associated with the inhibition of hepatocyte apoptosis. The specific mechanism needs further study.

573.3

Surgical challenges in the longterm follow-up after intestinal and multivisceral transplantation

Undine Gerlach[1], Georgios Vrakas[2], Henk Giele[2], Srikanth Reddy[2], Peter Friend[2], Johann Pratschke [1], Andreas Pascher[1], Anil Vaidya[3].
[1]Department of General, Visceral and Transplant Surgery, Charité-Universitaesmedizin Berlin Campus Virchow Klinikum, Berlin, Germany; [2]Oxford Transplant Centre, Oxford University Hospitals and University of Oxford, Oxford, United Kingdom; [3]Transplant Centre, Apollo Hospital, Chennai, India.

Background: Intestinal and multivisceral transplant recipients have often undergone mulitple operations prior to and early after transplantation. In addition, they receive high amounts of immunosupression. Thus abdominal surgery at a later stage posttransplant is very challenging and may increase the morbidity and mortality of these fragile patients.
Methods: We retrospectively studied 69 patients (27 female, 42 male, 37±9 years) with isolated intestinal (ITX, n=48), modified (mMVTX, n=7) or typical multivisceral transplantation (MVTX, n=15) in 2 large european transplant centres. 4 grafts included a kidney and 17 included the abdominal wall. Maintenance immunosuppression consisted of either Tacrolimus Monotherapy in 31 patients or a double combination of Tacrolimus/MMF, Tacrolimus/Sirolimus, Tacrolimus/Everolimus.
We recorded all intraabdominal interventions, which were performed after the first year posttransplant, and subdivided them into 2 groups depending on whether or not they were related to the intestinal graft.
Results: The median follow-up time posttransplant was 8 years [1;15].
20 intraabdominal operations were reported in 15 patients after a median of 5 years [1;13] posttransplant.
81% were emergency operations: Graft-related surgery was required due to graft ischeamia (n=2), adhesive ileus (n=3) and encapsulating ileus (n=2). Surgery for these patients included partial graft resection (n=5), graft explantation (n=4) and intestinal retransplantation (n=1).
Non graft-related operations did not affect the graft or graft function and were: native colon resection due to Volvulus (n=1), native nephrectomy due to outflow obstruction (n=1), kidney transplantation due to CNI-toxicity (n=1), cholecystectomy due to necrotic cholecystitis (n=1), caesarian (n=1), post-mortem kidney/liver donation (n=1).
There were no deaths related to surgery.
19% was elective surgery: Incisional herniotomy at the previous stoma-site (n=3) and a bilateral inguinal herniotomy without incarceration(n=1).
A conservative treatment was attempted in 4 patients with: rectovaginal fistula in a stapled rectum stump (n=1), neutropenic colitis (n=1) and upper GI-haemorrhage (n=2). The latter 2 patients died.
The median hospital stay following surgery was 27 days [7;210]. Six patients had to undergo repeated surgery (median number of operations 3 [2;13]).
Conclusion: Abdominal surgery in ITX and (m)MVTX-recipients is challenging but feasible. Especially graft-related surgery was accompanied with a high risk of graft-loss but not with mortality. Non graft-related surgery did not affect graft function or longterm survival.

573.4

Clinical presentation and outcome of norovirus enteritis in small bowel transplant recipients compared to non-small bowel transplant recipients

Diana Florescu[1,2], Jessica L.Sonderup[4], Pearlie P.Chong[3], David van Duin[3], Wendy Grant[2], Andre C.Kalil[1].
[1]Transplant Infectious Diseases Program, University of Nebraska Medical Center, Omaha, NE, United States; [2]Transplant Surgery Program, University of Nebraska Medical Center, Omaha, NE, United States; [3]Division of Infectious Diseases, University of North Carolina, Chapel Hill, NC, United States; [4]Department of Internal Medicine, University of Nebraska Medical Center, Omaha, NE, United States.

Background: Norovirus enteritis (NVE) is increasingly recognized among solid organ transplants. The clinical manifestations and outcomes of NVE in small bowel transplant recipients compared to other allografts is currently unknown.
Methods: This is a 4-year retrospective review of adult and pediatric solid organ transplant recipients with NVE at 2 institutions. Statistical analysis was performed with t-tests for continuous variables and chi-square statistics for categorical variables. Linear and logistic regressions were done to compare NVE in small bowel transplant vs non-small bowel transplant recipients.
Results: 45 small bowel transplant recipients were compared to 107 other allograft recipients (kidney [56%], liver [14%], kidney-pancreas [12%] heart [11%], lung [4%], kidney-liver [1%], kidney-stem cell transplant [1%], pancreas [1%] recipients).
Patients' characteristics are presented in figure 1.

Variables	Small bowel transplant (45)	Non-small bowel transplant (107)
Pediatric recipients	40	12
Adult recipients	5	95
Induction therapy	43	74
Rejection	9	5
Co-infections	25	19
Hospitalized	40	81

Factors associated with small bowel transplantation at presentation included: younger age (OR=0.90;p<0.0001); shorter time from transplant to NVE (OR=0.99; p=0.008); lower use of anti-lymphocyte induction therapy (OR=0.15;p<0.0001); less frequent nausea (OR=0.11;p<0.0001) and vomiting (OR=0.36;p=0.01); higher stool volume (OR=1.06;p=0.003); higher WBC count (OR=1.09;p=0.001); and higher GFR (OR=1.02;p<0.0001). Small bowel transplant recipients were more likely to have diarrhea resolution (OR=1.94;p=0.15); to be longer on IV fluids (OR=1.18;p<0.0001); to receive anti-motility agents (OR=9.6;p<0.0001) and TPN (OR=13.5;p<0.0001); to relapse NVE (OR=4.25;p<0.0001); to have acute rejection (OR=5.1;p=0.006); longer hospital stay (OR=1.07;p<0.0001) and lower survival (OR=0.28;p=0.006) than other allograft recipients.

Results of the multivariate analysis are presented in figure 2.

Variables	OR	95% CI	p-value
Age	0.93	0.88-0.97	0.0001
Anti-motility agent administration	1274.6	2.97-547009.4	0.021
Number of days on IV fluids	1.77	1.10-2.83	0.018
Length of hospital stay (days)	0.81	0.64-1.00	0.06
Patient survival	0.17	0.02-1.27	0.08

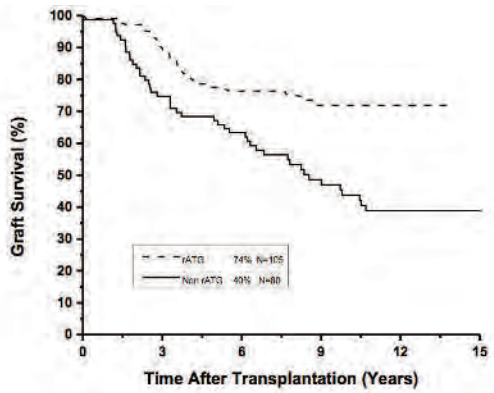
Conclusions: Compared to other allograft recipients that did not include small bowel, small bowel transplant recipients with NVE were significantly younger, received more anti-motility agents, required more IV fluids, and had longer hospital stay. There was a trend for lower survival with NVE in small bowel transplant recipients.

573.5

Conditional long term intestine patient and graft survival is improving under rATG immunosuppression in pediatric intestine transplantation

Neslihan Celik[1], Frederick Vyas[1], Geoffrey J.Bond[1], Kyle A.Soltys[1], Jeffrey Rudolph[2], Rakesh Sindhi[1], George V.Mazariegos[1].
[1]Hillman Center for Pediatric Transplantation, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, United States; [2]Intestinal Care and Rehabilitation Center, Department of Pediatrics, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, United States.

Aim: We reviewed conditional graft survival and short and long term improvements in children receiving rATG (rabbit anti-thymocyte globulin) immunosuppression at a single center over 15 years.
Methods: All children undergoing primary intestine transplantation under rATG and non rATG immunosuppression were analyzed. rATG immunosuppression was with 5 mg/kg rATG/ Tacrolimus/ Prednisolone (n=126) and other immunosuppression consisting of Tacrolimus/ Prednisolone (n=50), Tacrolimus/ Prednisolone/ Daclizumab (n=23), Tacrolimus/ Prednisolone/ Cyclophosphamide (n=16), Alemtuzumab (n=21) and Tacrolimus/ Prednisolone/ Basiliximab (n=1). Patients with intact grafts at one year were followed for subsequent long term outcomes of patient and graft survival, retransplant outcomes and significant morbidities.
Results: 242 children underwent primary intestine transplantation between 1990 and 2015 consisting of isolated small bowel (n=94, 39%), liver and small bowel (n=109, 45%), modified multivisceral (n=7, 3%) and multivisceral type transplantations (n=32, 13%). The major causes of intestinal failure were gastroschisis (n=62, 26%), volvulus (n=51, 21%), necrotizing enterocolitis (n=28, 12%), pseudo obstruction (n=30, 12%), intestinal atresia (n=23, 10%), microvillus inclusion disease (n=17, 7%) and Hirschsprung's Disease (n=16, 7%). Conditional graft survival at 15 years for rATG and non rATG groups is illustrated in Figure 1.
Figure 1. Conditional graft survival at 15 years for rATG and non rATG groups.



Patients with intact grafts at one year had the subsequent outcomes, stratified by rATG and non rATG immunosuppression in the Table below.

Initial induction Immunosuppression	rATG n	non- rATG n
Patients	126	111
Intact grafts at one year	105/126 (83%)	80/111 (72%)
Alive with intact graft	80 (76%)	34 (43%)
Alive with retransplant	7 (7%)	5 (6%)
Alive with graft removed	4 (4%)	6 (8%)
Deceased	17 (16%)	37 (46%)
PTLD	13/126 (10.3%)	36/111 (32.4%)
Chronic rejection	20/126 (16%)	21/111 (19%)

Conclusion: Conditional long term survival in intestine transplantation has improved under rATG in our experience. Chronic rejection remains the most significant challenge to more dramatic improvements in long term outcomes.

573.6

The effect of probiyotics on the healing of colon anastomosis after ischemia and reperfusion injury in the rat

Necdet Ozcay[1], Mehmet Haberal[1], Eda Yılmaz Akcay[2], Elif Buse Altun[3], Yurdagul Deveci[3], Ece Ermin[3], Seray Gulay[3], Cem Haberal[3].
[1]General Surgery, Baskent University, Ankara, Turkey; [2]Pathology, Baskent University, Ankara, Turkey; [3]Medical Student, Baskent University, Ankara, Turkey.

Introduction: Intestinal transplantation (IT) is one of the important cause of intestinal ischemia and reperfusion (I/R) injury. It is well known that anastomotic problems seen after IT is mainly related to reperfusion injury. Probiotics are live microorganism that are using for various bowel diseases due to their beneficial effects to bowel functions. Wound healing is an important bowel function and impaired healing is a serious trouble after IT and ischemic bowel. In this study we aimed to investigate the effect of probiotics on intestinal healing proces. For this purpose we used a colonic anastomosis model since probiotics mostly colonized to the colon.
Material and method: Forty male SD rats were divided into four groups: Group I(n:10)colonic anastomisis only, Group II (n:10) colonic anastomosis after I/R injury, Group III (n:10) probiotic and colonic anastomosis, Group IV (n:10) probiotic and colonic anastomosis after I/R injury. Probiotics (Lactobasillus rhamnus and acidophilus, Enterococcus faecium, Bifidobacterium bifidum and longum) are given 250 milion/day,po, started 7 days before surgery, and continued 7 days after surgery. Ischemia was achieved by the occlusion of superior mesenteric artery for 60 minutes.Left colon is cut and anastomosed with continuous 6/0 nylon suture after 60 minutes reperfusion. Animals were followed up for 7 days, then sacrificed. Macroscopic findins (Adhesion, abscess, or anastomotic leak) are scored (0 to 3), anastomotic bursting pressures measured as a mmHg, and histopathologic findins were scored (0 to 2).The data are reported as the mean ±SE. The differences between the groups were analyzed by the Kruskal-Wallis test followed by the Dunn test. Probability values $p<0.05$ were considered significant.
Results: Probiotics are well tolerated in the treatment groups (Group III and IV). There were no side effect during the study period. Results are summarized in the table. Macroscopically there were no anastomotic leak or abscess formation in both probiotic groups (Groups III and IV). Bowels of these animals looked healthy even in the I/R group (Group III). Anastomotic bursting pressures of probiotics groups were tend to be high compared with ischemic control group (Group II), although the difference did not reach the statistacally difference. Histologic scores of probiotic groups were very similar to control groups.
Conclusion: Probiotic groups; had better results acording to macroscopic findings, anastomotic bursting pressures were tend to be high and histologycally they had almost equivalent healing scores

	Macroscopic scores (0 to 3)	Bursting pressure(mmHg)	Histologic scores (0 to 2)
Group I	0.9±0.1	168±11	8.2±0.4
Group II	1.4±0.2 *	159±11	8.0±0.5
Group III	0.6±0.1	190±13	7.2±0.4
Group IV	0.5±0.1	167±14	7.8±0.4
	* $p<0.05$	$P=0.4$	$P=0.3$

574.1

Public promotion of unspecified live kidney donation: an ELPAT view

Lisa Burnapp[1,2], Kristof Van Assche[3], Annette Lennerling[6], Nizam Mamode[2], Dorthe Slaats[5], David Van Dellen[7], Franco Citterio[4], Willij Zuidema[5], Willem Weimar[5], Frank Dor[5].
[1]Organ Donation and Transplantation, NHS Blood and Transplant, Bristol, United Kingdom; [2]Nephrology and Transplantation, Guy's & St.Thomas' NHS Foundation Trust, London, United Kingdom; [3]Health law and Kinship, University of Antwerp, Antwerp, Belgium; [4]Department of Surgery, Universita Cattolica del Sacro Cuore, Rome, Italy; [5]Internal Medicine, Erasmus University, Rotterdam, Netherlands; [6]Transplant Institute, University of Gothenburg, Gothenburg, Sweden; [7]Transplantation Surgery, Manchester University Hospitals, Manchester, United Kingdom.

Living donor kidney transplantation (LDKT) is the preferred treatment for patients with end stage kidney disease (ESKD) and unspecified live kidney donation (UKD) is morally justified. However, despite the excellent results of LDKT, UKD is limited to a minority of countries in Europe due to both legal and moral reasons. Consequently, there are significant variations in practice and approach between countries and the potential contribution of UKD is under-valued. Where UKD is accepted as a routine part of living organ donation (LOD), an increasing number of patients in the domino paired exchange programme are successfully transplanted when a 'chain' of transplants is triggered by a single UKD. Our Working Group has examined the limitations on UKD in Europe and recommend strategies to increase transplant opportunities by raising awareness and engaging with key audiences across nations, recognising that the maturity and characteristics of individual (LDKT) programmes will impact on levels of engagement. Key audiences include the public, healthcare professionals, policy makers and society or religious and cultrual leaders. Their roles and responsibilities are defined and discussed in the context of the following recommendations:

1. Countries wishing to undertake UKD must have a legal framework to support LOD and be committed to LDKT and UKD.
2. UKD offers the best opportunity to maximise transplant opportunities for patients with ESKD through the domino paired exchange (donor chains) programme.
3. Raising awareness by providing stratified information that is country specific, culturally sensitive and relevant across all sectors of society offers a sustainable option for increasing UKD activity.
4. The content and context of raising awareness initiatives must be appropriate for both mature and emerging programmes and relevant to all target audiences.
5. Collaboration between dedicated groups: previous UKDs, healthcare professionals, and procurement organisations, is the most effective model for engaging with target audiences.
6. Competent authorities with support of dedicated groups are best placed to achieve legislative change in individual countries.

Increasing UKD by raising awareness contributes more kidneys to the shared living donor pool, extending the benefit of LDKT to more patients, even if they do not have a suitable living donor of their own.

574.2

Does privacy exist in directed organ donation

Alvin Wee[1], Amy De Carlo-Frepan[2], Mary Ann Palumbi[2], Abdul Moiz[1], Tami Rader[2], Islam Ghoneim[1].
[1]Transplant , Cleveland Clinic, Cleveland, OH, United States; [2]Transplant, St Vincent Indianapolis, Indianapolis, IN, United States.

Introduction: In today's age, there has been a push to increase awareness about organ transplantation through social media. Particularly, in direct donation, is there privacy breach when both families of the donor and recipient communicate through social media and in close proximity?
Case: A directed donation became available for 64 year old renal transplant candidate. The donor and donor's spouse were admitted secondary to an MVA to the same institution where the recipient was listed. The donor's daughter contacted the recipient's daughter via social media regarding donation. Offer was accepted and transplant was completed uneventfully.
Discussion: Two issues were encountered in this situation. First, following social media notification of donation, recipient was contacted directly by the Organ Program Organization (OPO) coordinator for verification. In effect, bypassing the institution that can maintain boundaries. Second, the donor's spouse remained inpatient throughout the recipient's recovery. Due to proximity, the recipient reported feelings of guilt, stress and obligation towards the donor's family. Donor's family visited recipient several times, inviting the recipient to attend funeral services and other events. It was communicated that the recipient was now a part of their family with an implied obligation. After multiple intensive intervention with our team, the recipient was able to handle the different issues and felt confident setting boundaries to protect their personal privacy.
Summary: The utilization of social media to spread organ donation awareness has had a major positive impact. However, in the realm of directed donation, it takes a well-coordinated effort between the OPO and transplant team to ensure recipient privacy. The multidisciplinary team will play an important role in aiding recipients in setting boundaries thus protecting their privacy.
References:

[1] Lancet 2015:385
[2] Transplantation. 2015 99(():1894-9
[3] Transplant Proc 2014 46(10):3647-52
[4] Am J Transplant 2013 13:(8): 2059-65
[5] Prog Transplant 2012. 22(2):169-74

574.3

Transplant volume in China

David Matas.
Law, University of Manitoba, Winnipeg, Canada.

In looking at the sources for organ transplants in China, researchers have taken Chinese government official statements of overall transplant volumes at face value and focused on attempting to identify the sources for those asserted volumes. However, Chinese government statistics for transplant volumes are not necessarily reliable. An effort which needed to be made and which this presentation would make is to determine what Chinese transplant volumes in fact have been.
Many claims of human rights abuses suffer from too little evidence. With the claim of transplant abuse in China, the volume problem is the opposite, too much evidence.
It may seem counterintuitive in this context to add to this problem by presenting for consideration yet more evidence. What the presentation would address is not more of the same, more data in fields already explored. It explores here a whole new data field -individual hospitals. Researchers had looked at individual hospitals in the past to point out that their websites were promoting transplant tourism. This presentation looks to them for transplant volumes.
Reliable transplant volume aggregates in China do not exist. The Chinese health system runs four transplant registries, one each for liver, kidney, heart and lung. The aggregate data for these registries is accessible only to those who have registry issued login names and passwords.
Access to the aggregate data for these four registries would be useful, if for no other reason than to cross check the proliferation of contradictory statements Chinese officials make about the specifics of transplant volumes. Yet, any registry is as reliable as the reports it receives. These registries accumulate their data from reporting hospitals. We can not assume that every transplant hospital reports all its transplants to these four registries.
Considering transplant volumes, hospital by hospital, is, in China, a mammoth task, since there are over one thousand hospitals which have been engaged in transplants. Moreover, cover up is not just in China an aggregate, national phenomenon. It exists as well at the hospital level. Nonetheless, to get a grip on transplant volume figures, it is a task which must be undertaken and this presentation undertakes.
The official Chinese figures of 10,000 transplants a year, with some years higher, bring the official total transplant volume to date to 150,000 to 200,000 transplants since transplant volumes increased dramatically in China in the year 2000. The conclusion of this presentation is that the actual number of transplants is far larger than the official figure, in the order of hundreds of thousand larger. Given the systematic efforts at cover up, it is impossible to affix an exact number to transplant volumes.
The duty to explain the sourcing of organs for transplantation falls on China. The figures from this presentation means that Chinese authorities have a massive amount of explaining to do.

References:

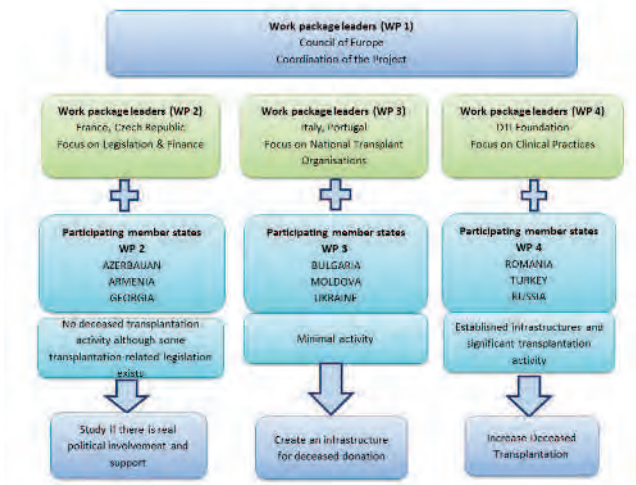
[1] David Matas and David Kilgour, "Bloody Harvest: The Killing of Falun Gong for their Organs" Seraphim Editions 2009
[2] David Matas and Torsten Trey, editors, "State Organs: Transplant Abuse in China", Seraphim Editions 2012
[3] Ethan Gutmann "The Slaughter:Mass Killings, Organ Harvesting, and China's Secret Solution to Its Dissident Problem" Prometheus Books, 2014

574.4

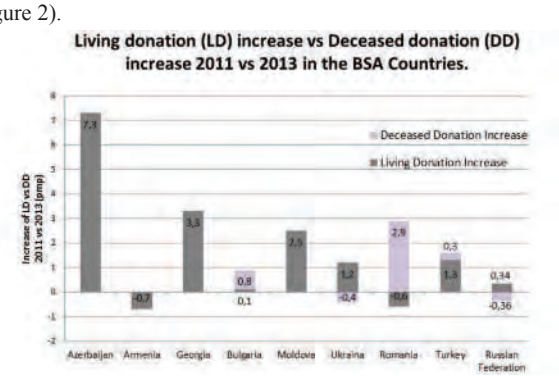
International initiative to enhance the organ donation and transplantation systems in the Black Sea area: The BSA project

Estephan Arredondo[2], Marti Manyalich[2], Chloë Ballesté-Delpierre[2], Marta López-Fraga[3], Alessandro Nanni Costa[1], Efstratos Chatzixiros[1]. [1]Centro Nazionale Trapianti, Rome, Italy; [2]DTI Foundation, Barcelona, Spain; [3]European Directorate for the Quality of Medicines & Healthcare (EDQM), Council of Europe, Strasburg, France.

Background: The Council of Europe(CoE) launched in 2011 a three year collaboration project that aimed to battle organ shortage and improve the access to transplant health services in the Council of Europe BSA member states (Armenia, Azerbaijan, Bulgaria, Georgia, Moldova, Rumania, Russian Federation, Turkey and Ukraine) through the development of safe and ethical Donation and Transplantation (D&T) programmes.
Objective: To support the development of D&T programmes through close inter-state cooperation between national health organisations and relevant stakeholders.
Methodology: Focused in the level of development of D&T in each Black Sea Area (BSA) member state, the following working packages (WP) were established: a)WP1: Coordination of the Project (CoE), b)WP2: Development and implementation of an effective legislative and financial framework (Czech Republic and France), c)WP3: Establishment of National Transplant Authorities (Italy and Portugal), d)W4 Clinical Practices (Spain and DTI Foundation) (Figure 1)



Data collection, surveys and experts visits were performed to get first-hand information in each participant country at national, regional and hospital level by detecting problems and proposing solutions.
Results: Data analysis showed a positive impact of the project represented by a tendency to raise the D&T rates in all the countries (Figure 2).



Conclusions: The increase of the donation rates is a result of an implementation of legislative, organizational and institutional recommendations performed by the CoE, the efforts of the Ministry of Health (MOH) of each country and synergies with other European projects placed in the BSA area.
BSA project made possible to analyse, assess and compare the different legal and organizational systems of the BSA countries. As a consequence of the outcomes, BSA countries should invest themselves in the implementation of the recommendations resulting from this project in order to achieve their organ donation and transplantation system self-sufficiency.

574.5

The effect of the syrian crisis on organ transplantation in Syria

Bassam Saeed.
Pediatric Nephrology, Kidney Hospital, Damascus, Syrian Arab Republic.

The war in Syria that started in March 2011 has destroyed much of the country's infrastructure including many hospitals. The total number of kidney transplants performed in Syria in 2010 was 385 transplants before the number gradually declined to 154 transplants in 2013, a decrease of 60%. In addition, the number of operational kidney transplant centers has decreased from 8 to 4 centers. Unrelated-donor kidney transplant decreased from 70% during the years that preceded the crisis to 47% in 2013. More than 50% of physicians and surgeons involved in kidney transplant are not practicing transplant currently in their centers. Difficulties in the provision of immunosuppressive drugs for all patients in all provinces constitute a major challenge for the health authorities and transplant patients, especially patients who cannot arrange an alternate source. The project to initiate liver transplant came to a halt because foreign trainers could not visit Syria. The autologous bone marrow transplant program continued to function, but in a smaller and irregular manner. The commitment of transplant teams despite the large challenges was, and still is, extraordinary.
Conclusion: all aspects of organ transplant have been affected, paralyzing new projects and negatively affecting existing programs.

574.6

Establishing credible evidence of human organ trafficking. The need to effectively manage purported organ trafficking claims originating from special interest groups

Campbell Fraser.
Asian Studies, Griffith University, Brisbane, Australia.

The global transplant community is often confronted with claims of organ trafficking from special interest groups (SIGs) lobbying on behalf of political and spiritual based organisations. This paper details a set of guidelines that may assist in assessing such claims, specifically in relation to establishing the credibility of the evidence presented.
Firstly, it is essential that evidence is confirmed to relate to recent rather than historical alleged organ trafficking activity. Evidence provided by SIGs, purported to be current, on further investigation, often relate to events occurring several years previously.
Secondly, evidence presented is often based principally on the testimony of individuals, whose membership of a political, spiritual or social organisation may place them under the duress of peer-pressure to provide such evidence. This is particularly relevant in the case of individuals who give testimony that they had been evaluated for organ donation but did not ultimately have any organ removed.
Thirdly, SIGs predominately provide evidence from the supply side of the trafficking equation, either from a donor or an individual surgeon claiming to have been involved in organ trafficking. There is often no evidence provided from the demand side, from individuals who can claim they have purchased an organ under such circumstances. Organ trafficking environments are ideally identified and verified through the discovery and testimony of both buyers and sellers, where the location and approximate date of transplant procedures match.
Finally, care has to be taken to differentiate between circumstances which may be indicative of having potential for organ trafficking and those which, beyond reasonable doubt, provide compelling evidence that organ trafficking has indeed occurred. Individuals or groups may well have been persecuted, unjustly detained or trafficked, but not necessarily for the purpose of removal of their organs.
Investigators must have confidence in quality of their evidence before making specific accusations based on the lobbying of SIGs. Failure to do so risks their professional reputation and, more importantly, jeopardizes ongoing productive relationships with transplantation authorities in jurisdictions where anti-trafficking initiatives are in progress.
SIGs continue to make claims of human organ trafficking in order to leverage support both from the media and the public to further their wider political objectives. While investigators should consider every potential organ trafficking case presented to on its own merits, regardless of its sponsor, they must remain focused on establishing and verifying credible evidence rather than simply responding to appeals targeting emotions. While individual organ trafficking investigators may choose to champion the cause of particular SIGs, it is imperative that they separate personal convictions from their professional responsibility to report objectively back to the transplant community.

620.1

Pregnancy outcomes in female solid organ transplant recipients with exposure to mycophenolic acid products

Michael J. Moritz[1,2], Lisa Coscia[2], Dawn P. Armenti[2], Serban Constantinescu[2,3].
[1]Surgery, Lehigh Valley Health Network, Allentown, PA, United States; [2]National Transplantation Pregnancy Registry (NTPR), Gift of Life Institute, Philadelphia, PA, United States; [3]Medicine, Temple University, Philadelphia, PA, United States.

The purpose of this study was to describe pregnancy outcomes of female transplant recipients who took a mycophenolic acid (MPA) product during a portion of the first trimester (exposed group). MPA was continued throughout 53% of these pregnancies, although 75% resulted in miscarriages. These outcomes were also compared with those where no MPA was taken since 6 weeks prior to the estimated date of conception (unexposed group). Data were collected by the National Transplantation Pregnancy Registry (NTPR) via telephone interviews and review of medical records. Of the 1428 female recipients reporting to the NTPR as of 2/1/16, there are 154 recipients in the exposed group (101 kidney, 19 liver, 19 heart, 9 kidney-pancreas, 5 lung, 1 pancreas alone) who had 221 pregnancies (224 outcomes, including multiple births). This is the largest such cohort reported to date. The exposed conceptions (63% unplanned) occurred between October 1996 and February 2015. The unexposed group was limited to conceptions that occurred during the same time period. Among the 99 live births (LB) in the exposed group, the mean gestational age was 35.5±3.4 wks (52% preterm; <37 wks) and mean birthweight was 2394±736 g (49% low birthweight; <2500 g), both similar to the unexposed group. Fetal loss and birth defect rates were significantly higher in the exposed group compared to the unexposed group (Table). The MPA phenotype described in the literature consists of a set of malformations often occurring in tandem: microtia/external auditory canal defects, clefts, cardiac and skeletal anomalies, eye issues, trachea/esophageal and brain anomalies. In the exposed group, 8 infants (53%) with birth defects clearly exhibit this phenotype, while 1 infant (2%) in the unexposed group born with a small soft cleft palate and mild micronagthia might be considered to exhibit aspects of this phenotype. While the birth defect rate in the unexposed group of 4.7% was within the range found in the general population (3-5%), the birth defect rate in the exposed group was significantly higher (15.3%). This birth defect rate does not include MPA-phenotypic anomalies found in 1 stillbirth and 1 termination in the exposed group.

NTPR: MPA Exposed vs. Unexposed Pregnancy Outcomes

	Exposed	Unexposed	p-value (Fisher's exact test)
Pregnancy Outcomes	n=224	n=1249	
Live births	44.2%	75.5%	<0.0001
Miscarriages	52.7%	19.8%	<0.0001
Fetal loss*	57%	22%	<0.0001
Birth defects	15.3%	4.7%	0.0002
MPA Phenotype	53%	2%	0.0001
*miscarriages, terminations due to fetal anomalies, stillbirths, neonatal deaths, and child deaths due to congenital issues.			

Conclusions: To date, this is the largest reported cohort of pregnancies exposed to MPA. The birth defect and fetal loss rates in pregnancies exposed to MPA are significantly higher than those not exposed to MPA. This confirms the importance of counselling women of child bearing age about the risks of conceiving while taking MPA.
The NTPR is supported by grants from Astellas Pharma, US Inc., Bristol-Myers Squibb Co. and Pfizer Inc

620.2

Evaluation of immunosuppressive regimens on the incidence of new-onset diabetes mellitus after kidney transplantation: 24-Month analysis of the ELEVATE study

Steve Chadban[1], Johan W.de Fijter[2], Hallvard Holdaas[3], Lionel Rostaing[4], Eduardo Mancilla Urrea[5], Peter Bernhardt[6], Patricia Lopez[6], Gaohong Dong[7], Josep Cruzado[8], Markus van der Giet[9]. [1]Royal Prince Alfred Hospital, Camperdown, Australia; [2]Leiden University Medical Center, Leiden, Netherlands; [3]Oslo University Hospital, Oslo, Norway; [4]Hôpital de Rangueil, Toulouse, France; [5]Instituto Nacional de Cardiología Ignacio Chávez, Mexico City, Mexico; [6]Novartis Pharma AG, Basel, Switzerland; [7]Novartis Corporation, East Hanover, NJ, United States; [8]Bellvitge University Hospital, Barcelona, Spain; [9]Charité - Universitätsmedizin Berlin, Berlin, Germany.

Introduction: New-onset diabetes mellitus after transplantation (NODAT) is a common comorbid condition affecting patient survival and kidney function. Development of NODAT is multifactorial and the choice of immunosuppressive regimen in general calcineurin inhibitors (CNI), particularly tacrolimus (TAC), has been identified as one of the major risk factor. Here, we present the 24-month (M) results from the ELEVATE study (NCT01114529) which prospectively evaluated the impact of early CNI to EVR conversion vs standard CNI in the development of NODAT in kidney transplant recipients (KTxRs).

Methods: ELEVATE was a 24-M, multicenter, open-label trial, in which *de novo* KTxR were randomized at 10–14 weeks post-Tx to convert from CNI to EVR (N = 360; C0 6–10 ng/mL) or continue standard CNI (N = 357; TAC: n = 231, C0 5–10 ng/mL and cyclosporine [CsA]: n = 126, C0 100–250 ng/mL); all received enteric-coated mycophenolate sodium + steroids. For patients without diabetes prior to randomization (RND), NODAT was considered if any of the following conditions were met: DM reported as adverse event (AE); random glucose ≥11 mmol/L; DM reported as reason for medication; two consecutive fasting glucose measurement ≥7 mmol/L; two HbA1c ≥6.5%. HbA1c, fasting plasma glucose and random glucose were measured centrally at baseline, Week 4, RND, and at M6, M12, M18 and M24.

Results: Of 526 patients without DM at RND, a total of 55 patients developed NODAT. Overall incidence of NODAT was similar between the treatment arms (10.9% in EVR vs 10.1% in CNI arm; $P = 0.78$) (Table). Incidence of NODAT was numerically lower in CsA (4.1%; $P = 0.06$ vs EVR) arm compared with TAC (13.3%; $P = 0.46$ vs EVR) or EVR (10.9%). Similarly, lower proportion of patients in CsA (0.0%) and EVR (0.8%) arm required treatment for NODAT compared with TAC (6.1%). Regardless of treatment, among the patients who developed a NODAT, there were more males (77.8% in EVR, 66.7% in TAC, and 75.0% in CsA). Overall, EVR vs CNI had comparable incidence of hyperglycemia/DM reported as AE (11.0% in EVR vs 11.7% in CNI; 6.6% in CsA and 14.3% in TAC) or serious AE (1.7% in EVR vs 2.2% in CNI; 1.7% in CsA and 2.5% in TAC).

Conclusion: Given the high cumulative incidence of NODAT reported for patients receiving mTORi in registry studies (particularly sirolimus)[1], the findings from ELEVATE study shows that early conversion to EVR therapy at 3M post-Tx is not associated with a higher incidence of NODAT compared with TAC.

Table: Incidence rate of NODAT by treatment at month 24

	EVR N=346 n (%)	Standard CNI			P value* (vs EVR)
		Total N=359 n (%)	TAC N=238 n (%)	CsA N=121 n (%)	
Without DM at randomization	248 (100.0)	278 (100.0)	181 (100.0)	97 (100.0)	
NODAT-total	27 (10.9)	28 (10.1)[a]	24 (13.3)[b]	4 (4.1)[c]	[a]P = 0.78 [b]P = 0.46 [c]P = 0.06
DM reported as AE	2 (0.8)	10 (3.6)	10 (5.5)	0 (0.0)	
DM as a reason for medication	2 (0.8)	11 (4.0)	11 (6.1)	0 (0.0)	
Two consecutive FPG ≥7 mmol	2 (0.8)	4 (1.4)	3 (1.7)	1 (1.0)	
HbA1c ≥6.5%	26 (10.5)	20 (7.2)	16 (8.8)	4 (4.1)	

AE, adverse event; CNI, calcineurin inhibitors; CsA, cyclosporine; DM, diabetes mellitus; EVR, everolimus; FPG, fasting plasma glucose; NODAT, new-onset diabetes mellitus after transplantation; TAC, tacrolimus

*P value of Fisher's exact test

References:

[1] Johnston O *et al*. Sirolimus is associated with new-onset diabetes in kidney transplant recipients. *J Am Soc Nephrol*. 2008; 19(7): 1411-1418.

620.3

Rapid discontinuation of prednisone (RDP) in kidney transplant (KTx) recipients: 15-year outcomes

Oscar Serrano[1], Raja Kandaswamy[1], Kristen J.Gillingham[1], William D.Payne[1], Ty B.Dunn[1], Srinath Chinnakotla[1], Erik B.Finger[1], Hassan N.Ibrahim[2], Aleksandra Kukla[2], Richard Spong[2], Naim Issa[2], Timothy L.Pruett[1], ArthurJ. Matas[1].
[1]Surgery, University of Minnesota, Minneapolis, MN, United States; [2]Medicine, University of Minnesota, Minneapolis, MN, United States.

Purpose: RDP after KTx is associated with increased early rejection; no decrease in medium-term patient (PS) or graft survival (GS); and significantly decreased prednisone-related side effects. Concern remains that RDP will be associated with increased late graft loss.
Methods: Between 10/1/1999 and 6/1/2015, 1553 adult 1st KTx (1021 LD; 532 DD) were done utilizing RDP (discontinuation of prednisone on POD #6). Outcomes were compared between RDP and standard long-term prednisone therapy (control). We studied: a) actuarial 15-year PS, GS, and death-censored graft survival (DC-GS); and b) for patients with 5 year GS, outcomes between 5-15 years.
Results: a) PS for LD recipients was similar among the RDP and control groups; for DD, PS was significantly better for the RDP group ($p<0.01$; Table 1a). GS for patients on RDP (vs controls) was significantly better for both LD ($p<0.05$) and DD ($p<0.01$) recipients. Similarly, DC-GS for patients on RDP was significantly better for both LD ($p<0.05$) and DD ($p<0.01$) recipients. Ten-year RDP outcomes were similar to contemporaneous SRTR data. b) For LD and DD recipients with 5-year graft survival, subsequent outcomes (5-15 years) were similar between RDP and controls [Table 1b]. eGFR in RDP-treated groups (DD or LD) was initially greater than eGFR in control groups [Fig. 1]. However, this difference dissipates over time. Causes of graft loss did not differ between groups.
Conclusion: In 1st KTx, RDP (vs long-term prednisone) is not associated with increased rates of late graft functional deterioration or loss.

Table 1a. All patients

	Patient Survival		Graft Survival		DC GS	
	RDP	Control	RDP	Control	RDP	Control
1 year						
LD	98.2%	95.2%	96.7%	91.1%	98.3%	93.4%
DD	97.9%	91.4%	96.9%	86.3%	98.6%	93.3%
5 years						
LD	90.7%	88.0%	83.9%	76.8%	91.2%	84.5%
DD	88.7%	75.7%	80.4%	66.2%	87.9%	80.8%
10 years						
LD	76.7%	71.4%	66.4%	58.2%	82.0%	73.3%
DD	63.8%	55.3%	53.4%	43.1%	79.6%	66.7%
15 years						
LD	59.6%	54.7%	51.3%	36.5%	72.0%	59.8%
DD	54.9%	40.1%	38.7%	25.8%	65.8%	53.0%
	LD, $p=$ 0.22		LD, $p<$ 0.05		LD, $p<$ 0.05	
	DD, $p<$ 0.01		DD, $p<$ 0.01		DD, $p<$ 0.01	

Table 1b. Patients with >5 year graft survival

	Patient Survival		Graft Survival		DC GS	
	RDP	Control	RDP	Control	RDP	Control
5 years						
LD	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
DD	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
10 years						
LD	85.7%	83.5%	79.2%	75.8%	90.0%	86.7%
DD	72.8%	76.2%	66.5%	65.1%	90.6%	82.5%
15 years						
LD	67.0%	63.8%	61.1%	52.5%	79.0%	70.7%
DD	62.2%	56.1%	48.2%	38.9%	74.8%	65.5%
	LD, $p=$ 0.59		LD, $p=$ 0.24		LD, $p=$ 0.31	
	DD, $p=$ 0.70		DD, $p=$ 0.79		DD, $p=$ 0.12	

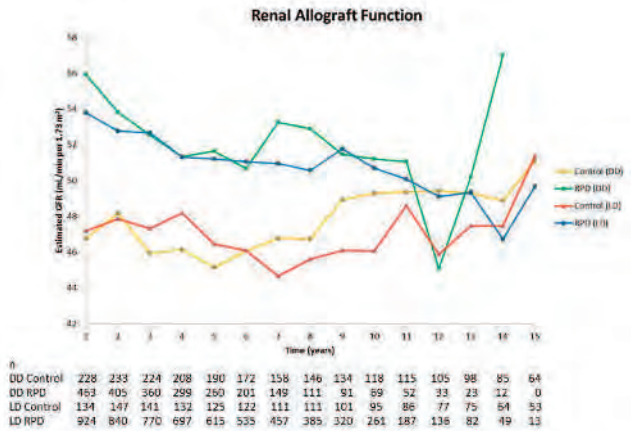


Figure 1. Estimated glomerular filtration rate (eGFR) for historical control and RPD kidney transplant recipients from deceased donors (DD) and live donors (LD). Each data point represents mean eGFR.

620.4

High intrapatient variability of tacrolimus concentrations predicts accelerated progression of chronic histologic lesions in renal recipients.

Thomas Vanhove[1], Tim Vermeulen[1], Pieter Annaert[2], Evelyne Lerut[3], Dirk R.J.Kuypers[1].
[1]Nephrology and Renal Transplantation, University Hospitals Leuven, Leuven, Belgium; [2]Pharmaceutical and Pharmacological Sciences, KU Leuven – University of Leuven, Leuven, Belgium; [3]Pathology, University Hospitals Leuven, Leuven, Belgium.

Introduction: High intrapatient variability (IPV) of tacrolimus plasma concentrations is increasingly recognized as a predictor of poor outcome in solid organ recipients. How it relates to evolution of histology has not been explored.
Methods: We analyzed tacrolimus IPV using coefficient of variability (CV) from months 6-12 posttransplant in a cohort of 220 adult renal recipients for whom paired protocol biopsies at 3 months and 2 years were available. CV was calculated from outpatient tacrolimus trough levels. All biopsies were semiquantitatively rescored according to revised Banff 1997 criteria by a single blinded pathologist.
Results: Fibrosis, tubular atrophy, chronic glomerulopathy and chronicity score (sum of ci, ct, cv, cg and ah scores) increased significantly by year 2 ($p < 0.01$ for all). Recipients in the highest CV tertile had an increased risk of moderate to severe fibrosis and tubular atrophy by 2 years compared with the low IPV tertile (OR 2.47 [1.09-5.60; $p = 0.031$] and OR 2.40 [1.03-5.60; $p = 0.043$], respectively). Other predictors were donor age, severity of chronic lesions at 3 months and presence of borderline or subclinical rejection at 3 months. Chronicity score increased significantly more in the high CV tertile group than in the middle and low tertiles (mean increase 1.97 ± 2.03 vs. 1.18 ± 2.44 and 1.12 ± 1.80, respectively; $p < 0.05$). CV did not correlate with or predict interstitial inflammation or tubulitis, nor did it predict evolution of renal function, which did not deteriorate within the 2-year follow-up period (average estimated glomerular filtration rate 46 ± 16 ml/min/1.73m² at month 3 vs. 53 ± 17 ml/min/1.73m² at year 2).
Conclusion: High IPV is independently related to accelerated progression of chronic histologic lesions, before any evidence of renal dysfunction.

620.5

Everolimus (EVR) versus mycophenolate sodium (MPS) for recipients of kidney transplants from expanded criteria donors (ECD) receiving anti-thymocyte globulin (r-ATG) and tacrolimus (TAC)

Hélio Tedesco-Silva[1], Claudia Rosso Felipe[1], Marina Pontello Cristelli[1], Laila Almeida Viana[1], Alexandra Nicolau Ferreira[1], Adrieli Barros Bessa[1], Mayara Ivani de Paula[1], Juliana Mansur Siliano[1], Priscila Ruiz Ruppel[1], Geovana Basso[1], Marcello Franco[3], Wilson Ferreira Aguiar[2], José Medina - Pestana[1].
[1]Nephrology Division, Hospital do Rim - UNIFESP, São Paulo, Brazil; [2]Urology Division, Hospital do Rim - UNIFESP, São Paulo, Brazil; [3]Pathology Division , Hospital do Rim - UNIFESP, São Paulo, Brazil.

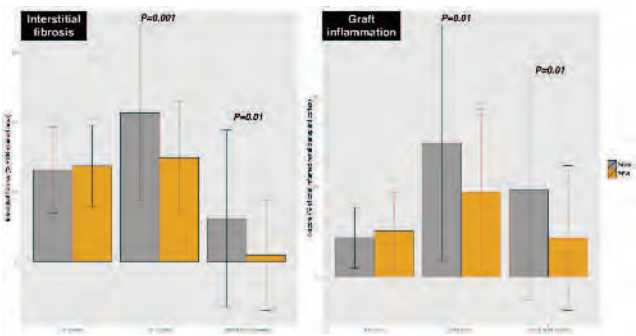
Introduction: There is no standard of care immunosuppressive regimen for recipients of kidneys recovered from expanded criteria donors. Several centers use r-ATG induction due to the higher risk of delayed graft function and acute rejection, increasing the risk of CMV infection.
Objectives: This study compares the efficacy and safety of EVR or MPS in ECD kidney transplant recipients.
Methods: This is a prospective, randomized, single center study designed to enroll 200 patients (1:1) to receive r-ATG induction (4 doses of 1.5 mg/kg every other day), prednisone, EVR or MPS and delayed introduction of TAC (day 7) to maintaim trough blood concentrations around 5 ng/ml. This preliminary analysis was performed with 127 randomized patients (EVR n=68 and MPS n=59) who completed 6 months of follow up. Preemptive strategy using pp65 antigenemia test was use to manage CMV infection.
Results: There were no differences in mean kidney donor profile index (KDPI, 89±9 vs. 89±8%) and mean kidney donor risk index (KDRI, 1.7±0.4 vs. 1.7±0.3) comparing EVR and MPS groups, respectively. There were no differences in main demographic characteristics except for the incidence of diabetes mellitus (EVR, 38% vs. MPS, 15%). There was no difference in incidence of delayed graft function (54 vs. 64%, p=0.253) but duration was lower in MPS group (11.1 ±11.9 vs. 5.0±4.6 days, p= 0.004). The incidence of first CMV infection was lower in EVR group (12 vs. 76%, p=0,000). Furthermore, 36% of patients in MPS (n=21) developed at least one recurrent event of CMV infection. Higher incidence of treated acute rejection (36 vs. 23%, p=0.165) or treated biopsy confirmed acute rejection (18 vs. 10%, p=0.197) was observed in the EVR group. At 6 months mean estimated glomerular filtration rate was higher in the MPS group (MRDR, 38.1±16.2 vs. 48.1±14.8 mL/min, p=0.003). Treatment discontinuation was higher in the MPS group (18% vs. 32%), primarily due to recurrent CMV infections.
Conclusions: This preliminary analysis indicates that patients receiving EVR show lower incidence of CMV infection but higher incidence of acute rejection and lower renal function compared to patients receiving MPS.

620.6

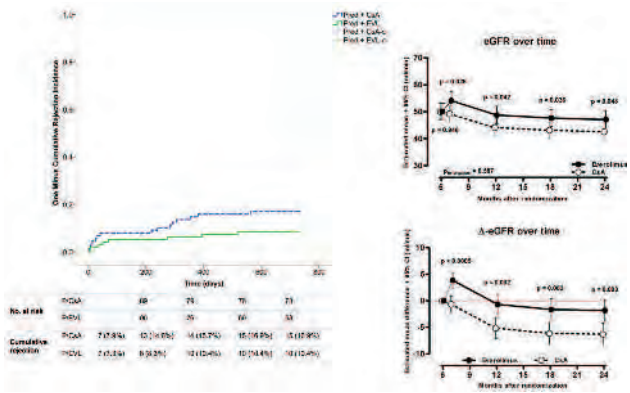
Early conversion to prednisolone and everolimus from a calcineurin-inhibitor-containing triple drug therapy as an alternative weaning regimen associated with beneficial renal transplant histology and function: the randomized-controlled MECANO trial

Frederike J.Bemelman[1], Johan W.de Fijter[2], Jesper Kers[1], Charlotte Meyer[1], Eltjo F.de Maar[3], Karlijn A.M.I.van der Pant[1], Aiko P.J.de Vries[2], Jan-Stephan Sanders[3], Aiko Zwinderman[1], Mirza M.Idu[1], Stefan Berger[3], Marlies E.J.Reinders[2], Christina Krikke[3], Ingeborg M.Bajema[2], Marcory C.van Dijk[3], Ineke J.M.ten Berge[1], Jan Ringers[2], Junior Lardy[4], Dave Roelen[2], Sandrine Florquin[1], Jaap J.Homan van der Heide[1].
[1]Academic Medical Center, Amsterdam, Netherlands; [2]Leiden University Medical Center, Leiden, Netherlands; [3]University Medical Center Groningen, Groningen, Netherlands; [4]Sanquin Diagnostic Services, Amsterdam, Netherlands.

Introduction: In renal transplant recipients, prolonged use of calcineurin inhibitors (CNIs) is associated with nephrotoxicity. The cumulative load of immunosuppressive drugs is associated with malignancies and infections. This trial aimed to minimize both CNI exposure and the burden of immunosuppression while maintaining efficacy.
Materials and Methods: Randomized controlled, open-label multicenter trial with early cyclosporine A (CsA) elimination. Patients started with quadruple therapy consisting of basiliximab, prednisolone (P), mycophenolate sodium (MPS) and CsA. Drug exposure was monitored by area-under-the-concentration-over-time curve (AUC12). At 6 months, immunosuppression was tapered to double therapy with P/CsA, P/MPS or P/EVL in patients free of subclinical rejection. Primary outcomes were renal fibrosis and inflammation and secondary outcomes were estimated glomerular filtration rate (eGFR) and cumulative incidence of rejection at 24 months.
Results and Discussion: The P/MPS arm was prematurely halted due to an increase in acute rejection rate at interim analysis[1] and the trial continued with P/CsA (N = 89) and P/EVL (N = 96). Interstitial fibrosis and inflammation were decreased in the P/EVL arm with a mean difference of 6% (95% CI 3-10%, P = 0.001) and 14% (95% CI 3-24%, P = 0.01), respectively.



The eGFR was higher in the P/EVL arm (51±20 versus 45±16 ml/min, P = 0.001). Cumulative rejection rates were 13% (P/EVL) and 19% (P/CsA), (P = 0.08).



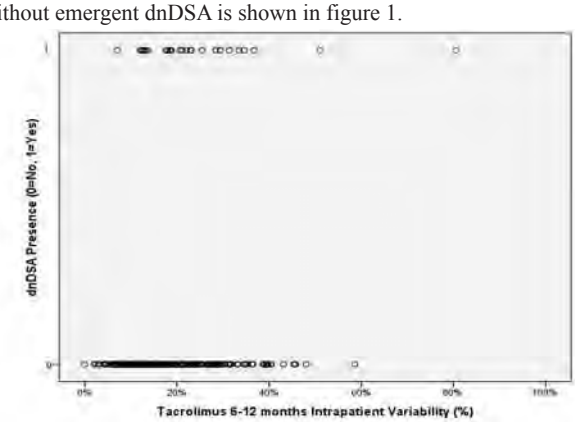
More side effects were reported in the P/EVL group. A post hoc analysis of anti-HLA class I and II donor specific antibodies at one year after transplantation revealed no differences between the groups.
Conclusions: An individualized immunosuppressive strategy of early CNI-elimination to dual therapy with everolimus associated with decreased allograft fibrosis, preserved allograft function and good efficacy and can be a valuable alternative regimen in patients suffering from CNI toxicity. However, dual therapy with everolimus had more side effects (NTR1615).
References:
[1] Bemelman FJ, et al. Minimization of maintenance immunosuppression early after renal transplantation: an interim analysis. *Transplantation*. 2009; 5;88(3):421-8.

620.7

High tacrolimus variability is associated with the emergence of de-novo donor specific antibody

Vanessa Teng, Julie Glen, Kathryn Stevens, Ann-Margaret Little, Marc Clancy.
West of scotland Renal Transplant unit, NHS Greater Glasgow & Clyde/University of Glasgow, Glasgow, United Kingdom.

Background: Tacrolimus is the cornerstone of modern immunosuppression for renal transplantation. As well as varying between individuals his drug exhibits significant intrapatient variability (IPV) in observed trough levels and high IPV has been associated with adverse renal outcomes including graft failure. However, any causal aspect to this relationship remains incompletely understood. This association may involve denovo DSA development. Our study assessed whether tacrolimus variability correlated with the emergence of dnDSA.
Methods: Prospective clinical, demographic and post transplant antibody data for 305 consecutive adult renal transplant recipients in the West of Scotland between 2007-2012 was collected from the Manzen tissue-typing and renal unit database. Serum samples were collected and screened for Class I and II HLA IgG antibodies, using LABScreen PRA and Single Antigen Bead test. dnDSA is defined as specificity that corresponded to current HLA mismatch and was absent pre-transplant, with a cutoff of MFI≥500. Logistic regression was used to analyze variables associated with dnDSA development.
Results: Of 305 patients, 26 patients (8.6%) developed dnDSA with a mean follow-up of 4.0 ± 1.3 years post transplant. Based on the population median of 16.0% for tacrolimus IPV, 157 patients (51.5%) were assigned to the high variability (HV) group whereas 148 patients (48.5%) were assigned to the low variability (LV) group. As compared to LV group, HV group were more likely to have previous late acute rejections (13.5% vs 5.4%, p=0.016), dnDSA development (12.2% vs 7%, p=0.02) and had poorer allograft survival rate (HR=6.43, p=0.005). After adjusting for late acute rejections, a high tacrolimus IPV was found to be independently associated with dnDSA development (OR=1.04, p=0.032). Tacrolimus variability for patients with and without emergent dnDSA is shown in figure 1.



Conclusion: The association between dnDSA and graft dysfunction/failureis well established. Since high tacrolimus IPV is associated with both worse clinical outcomes and the emergence of dnDSA, High variability patients may manifest lapses in immunosuprression which allow breakthrough of the alloimmune response detected as dnDSA. Specific strategies to redcue tacrolimus variability have the potential to reduce dnDSA and merit study in the context of clinical trials.

620.8

Comparison of the effects of brand name versus generic immunosuppressants on long-term graft failure risk among U.S. kidney transplant recipients: analysis of SRTR and Medicare claims data

Jarcy Zee[1], Ghalib A.Bello[1], Murewa Oguntimein[2], Charlotte A.Beil[1], Qian Liu[1], Jeong Park[3], Adam R.Saulles[4], Sangeeta Goel[4], Rajesh Balkrishnan[5], Melissa Fava[1], Pratima Sharma[6], Robert M.Merion[1], Alan B.Leichtman[1].
[1]Arbor Research Collaborative for Health, Ann Arbor, MI, United States; [2]Food and Drug Administration, Silver Spring, MD, United States; [3]Clinical Pharmacy, University of Michigan, Ann Arbor, MI, United States; [4]Specialty Pharmacy Services, University of Michigan Health System, Ann Arbor, MI, United States; [5]Public Health Sciences, University of Virginia, Charlottesville, VA, United States; [6]Medicine, University of Michigan, Ann Arbor, MI, United States.

Introduction: The use of generic immunosuppressants (IS) for kidney transplantation (KT) has increased in some countries following expiration of brand name patents. However, the reported risks and benefits of generic substitution have been inconsistent, particularly because bioequivalence requirements differ across countries and only small, short-term studies have examined the effects of substitution on transplant outcomes. Our aim was to compare the effects of brand name and generic tacrolimus (TAC) and mycophenolate (MYC) on the long-term risk of graft failure in a large, national cohort of U.S. KT recipients.
Methods: We used the Scientific Registry of Transplant Recipients (SRTR) to identify KT recipients transplanted from 2008-2013 and Medicare Part D claims data to identify patterns of generic and brand name use of TAC and MYC. Recipients were followed from the time of transplant until the earliest of graft failure, death, loss to follow-up, or end of the study period. Generic or brand name TAC and MYC use was recorded at each month of follow-up. Analysis was limited to recipients who had Medicare Part D coverage at any time during the study period, TAC or MYC claims at any time after transplant, and graft function at 30 days after transplant. For each drug, a joint model was used to test the association between brand/generic use and the outcome, defined by graft failure, repeat transplant or death. The model was constructed by linking a longitudinal model of long-term brand name or generic use with a Cox proportional hazards model of graft failure, adjusted for recipient, donor, and transplant program characteristics.
Results: Table 1 shows descriptive summary statistics on the study cohort (N=9,331). The sample was predominantly male, white, and aged 50-64 years. The most common primary cause of end-stage renal disease (ESRD) was diabetes.

Table 1: Descriptive Statistics on Study Cohort

Age, years, Mean (SD)	51.1 (16.46)
Male, % (N)	58.5 (5460)
Race/Ethnicity, % (N)	
White	47.0 (4384)
Black	27.6 (2571)
Asian/Other	8.1 (756)
Hispanic/Latino	17.4 (1620)
Primary Cause of ESRD, % (N)	
Diabetes	28.3 (2637)
Hypertension	22.9 (2132)
Polycystic Kidney Disease	7.0 (652)
Lupus/Nephritis	22.9 (2132)
Other	18.8 (1756)
Missing	0.2 (22)
Follow-up Time, years, Median (IQR)	3.6 (2.2-4.8)

SD: Standard Deviation, IQR: Interquartile Range (25th-75th percentiles)

Table 2 shows the model results, which indicate that for KT recipients, there was no significant difference in long-term graft failure risk arising from generic use of TAC or MYC, compared to the corresponding brand name drugs.

Table 2: Association between Generic/Brand Use and Graft Failure

	HR (95% CI)	p-value
Tacrolimus, Generic vs. Brand	1.001 (.997-1.004)	0.633
Mycophenolate, Generic vs. Brand	1.000 (.999-1.002)	0.958

HR: Hazard Ratio, CI: Credible Interval; Each estimate is from a separate joint longitudinal & survival model, adjusted for recipient, donor and transplant program characteristics

Conclusion: Our study of a large, national cohort of U.S. KT recipients showed that generic substitution of TAC or MYC did not result in a significant difference in the risk of long-term graft failure. Generalizability of the study results beyond the U.S. may be limited by differences between generic TAC and MYC formulations used in the U.S. compared with those used in other countries.

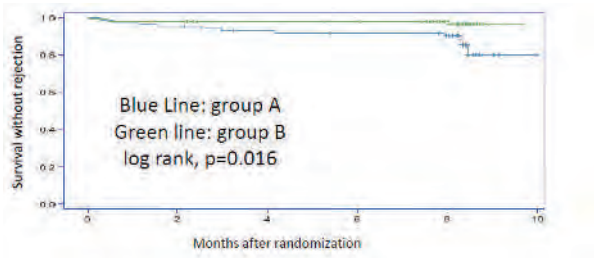
Funding for this research was made possible by the Food and Drug Administration through grant 1U01FD005274-01. Views expressed in written materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Food and Drug Administration or the Department of Health and Human Services; nor does any mention of trade names, commercial practices, or organization imply endorsement by the United States Government

620.9

Efficacy and safety of a 4-month post-renal transplant dose reduction of tacrolimus prolonged -release

Philippe Gatault[1], Nassim Kamar[2], Matthias Büchler[1], Charlotte Colosio[3], Dominique Bertrand[4], Antoine Durrbach[5], Laetitia Albano[6], Joseph Rivalan[7], Marie Essig[8], Yann Le Meur[9], Nicolas Bouvier[10], Bernard Charpentier[5], Lionel Rostaing[2], Eric Thervet[11], Yvon Lebranchu[1].
[1]CHU, Tours, France; [2]CHU, Toulouse, France; [3]CHU, Reims, France; [4]CHU, Rouen, France; [5]AP-HP, Kremlin Bicêtre, France; [6]CHU, Nice, France; [7]CHU, Rennes, France; [8]CHU, Limoges, France; [9]CHU, Brest, France; [10]CHU, Caen, France; [11]AP-HP - HEGP, France.

Introduction: The aim of the study was to determine the efficacy and safety balance of 2 different doses of tacrolimus prolonged-release (TacPR) in kidney transplant recipients between 4 and 12 months post-transplantation.
Methods: This clinical trial (NCT01744470) compared non-immunized stable patients randomized 4 months after in two groups (1:1): Group A : 50 % reduction of the daily dose of TacPR and with targeted Tacrolimus trough level (TacC0) > 3 µg/L ; Group B : continuation of the pre randomization dose and targeted TacC0 between 7 and 12 µg/L. Steroids were withdrawn before randomization. Primary Outcome was renal function (GFR estimated by MDRD) at 1 year. Secondary end points included the assessment of 1-year efficacy and safety data.
Results: Among 300 patients included between 10/2012 and 06/2014, 188 were randomized (group A: 89, group B: 99). TacC0 were lower in group A than in group B at 6 (4.1±2.7 vs 6.7±3.9µg/L, p<0.0001) and 12 months (5.6±2.0 vs 7.4±2.1µg/L, p<0.0001). All patients continued to receive MMF/ EC-MPS during the first posttransplant year. Renal function was similar in both groups at 4, 6 and 12 months. Patients with reduced TacPR daily doses experienced more acute cellular rejection episodes between month 4 and 12 (Figure, p=0.016). In addition, interstitial infiltrate was more frequently observed at 12 months in group A than in group B (21.4 vs 8.8%, p=0.047). Of note, IF/TA and chronic vascular lesions were similar in both groups. De novo DSA were found only in reduced dose group (6 patients vs. 0). BK virus viremia and CMV infection rates were similar in both groups.
Conclusion: Reduced daily dose and TacC0 during the first year post-transplantation impaired the efficacy of immunosuppressive regimen based on TacPR, even in low immunologic risk kidney transplant patients.



This study was supported by Astellas

621.1

CNI vs CNI-free immunosuppression: Impact on 1-year kidney protocol biopsy following simultaneous pancreas-kidney transplantation

Diego Cantarovich, Karine Renaudin, Anne Moreau, Cécile Guillot-Gueguen, Julien Branchereau, Georges Karam.
Institut de Transplantation, Urologie, Néphrologie (ITUN), Nantes University Hospital, Nantes, France.

Background and Aim: Current standard immunosuppression after SPK Tx consists of CNI/MMF/Pred, preceded by a short ATG induction. CNI-free as well as Pred-free regimens are under investigation. Histological features of different immunosuppressive combinations are not well documented. The objective of this study was to compare kidney histology (protocol biopsies) 1-year after SPK Tx in patients included in a prospective and randomized study comparing Tacrolimus vs Rapamycine.
Patients and Methods: A total of 100 type-1 diabetic patients (mean age 40 years; range 21-56) received a cadaver SPK-Tx (whole pancreas with portal and enteric drainages) and were randomly assigned at the time of Tx to continue Tac (n=50; mean age 40.5 years) or to replace Tac by Rapa (n=50; mean age 39.4 years) 60-90 days after surgery. Immunosuppression consisted of Thymoglobulin induction for 5 days, MMF and low-dose Pred (i.e. withdrawn on day 90). The study design included a 1-year protocol kidney biopsy and 5-yer follow-up.
Results: The following table summarizes histological kidney data. At the time of biopsy, serum creatinine (µmol/L) and proteinuria (gr/day) was 122 (range 61-289) and 0.32 (0-1.03) in Tac patients, and 115 (57-412) and 0.38 (0-1.5) in Rapa patients.
Conclusion: One-year protocol kidney histology among SPK Tx recipients under Tac or Rapa showed similar degree of IF/TA (1 and 2) and CNI toxicity in both groups. However, more active immune lesions were observed in Rapa patients requiring additional therapy and/or switch to Tac. Long-term (5-year) follow-up of patients is currently under evaluation.

	Tac	Rapa
Patient/biopsy	34/34	44/44
Normal	14 (41%)	15 (34%)
Borderline	2 (6%)	1 (2.3%)
Cellular rej.	0	2 (4.5%)
Vascular rej.	0	3 (6.8%)
CNI toxicity	2 (6%)	2 (4.5%)
C4d positive	1 (3%)	3 (6.8%)
IF/TA-1	7 (20%)	14 (32%)
IF/TA-2	1 (3%)	1 (2.3%)
BKV	1 (3%)	0
Pyelonephritis	1 (3%)	0
Undetermined	3 (9%)	4 (9%)

621.2

Rabbit anti-thymocyte in simultaneous kidney-pancreas transplantation: Does duration matter?

Karen Hardinger[1], Pranav Dalal[2], Daniel Murillo[2].
[1]Pharmacy Practice, University of Missouri Kansas City, Kansas City, MO, United States; [2]Transplant Institute, Research Medical Center, Kansas City, MO, United States.

The optimal dose and duration of rabbit anti-thymocyte globulin (rATG) induction in simultaneous kidney pancreas transplant recipient has not been defined.
Methods: Patients transplanted between January 2012 through December 2013 received rATG 1.5 mg/kg for four doses. Patients transplanted between January 2014 through September 2015 received rATG 2 mg/kg for three doses. We compared the safety and efficacy of rATG 2 mg/kg for 3 days (n=9) versus 1.5 mg/kg for 4 days (n=8) in a retrospective study. Patients also received immunosuppression with tacrolimus, mycophenolate sodium and prednisone.
Results: One year rejection free survival was 88% in the 1.5 mg/kg group and 87.5% in the 2 mg/kg group. In the 1.5 mg/kg group, one patient suffered pancreas allograft loss and one patient had a 2A kidney rejection. One patient in the 2 mg/kg group had a pancreas allograft rejection. One-year serum creatinine (1.1±0.2 mg/dl vs 1.2±0.3 mg/dl, P=NS) and the incidence of serious infection (11% vs 0%, P=NS) were similar in the 1.5 mg/kg and 2 mg/kg group, respectively. Cytomegalovirus viremia was low (11% in the 1.5/kg mg group vs 0% in the 2 mg/kg group). White blood cell count was lower in the 1.5 mg/kg group at 30 days after transplant (7.2 ±2.3 vs. 10.4 ±2.7, P=0.03), but similar at 7 days, 3 months, 6 months and 12 months. Platelets were similar throughout the one year follow-up. Polyoma viremia developed in 3 patients in the 1.5 mg/kg group and none in the 2 mg/kg group. There were no cases of BK-nephropathy or malignancy. The average length of initial transplant admission was higher in the 1.5 mg/kg group; 12.2+5.0 versus 8.1+0.9 days, P=0.054.
Conclusions: Shorter, more intense dosing of rATG is safe and effective. The 3 day dose-strategy resulted in a clinically, but not statistically, different length of the initial transplant hospitalization and may result in cost savings.

621.3

Association of autoreactive memory CD4 T cells expressing the chemokine receptor CXCR3 in the peripheral blood of pancreas-kidney transplant recipients with type 1 diabetes recurrence

Helena Reijonen[1], Francesco Vendrame[2], Alberto Pugliese[2,3], Linda Chen[4], George Burke[4].
[1]Benaroya Research Institute, Benaroya Research Institute, Seattle, United States; [2]Department of Medicine/Division of Diabetes and Metabolism, University of Miami Miller School of Medicine, Miami, United States; [3]Diabetes Research Instituite, University of Miami, Miami, United States; [4]Miami Transplant Institute/Department of Surgery, University of Miami Miller School of Medicine, Miami, United States.

Type 1 diabetes (T1D) is a T cell-mediated autoimmune disease leading to the destruction of pancreatic beta cells. Autoantibodies to beta cell molecules are diagnostic and predictive biomarkers of T1D. Patients with T1D may become recipients of simultaneous pancreas-kidney (SPK) transplants to restore insulin secretion and kidney function. We reported that 5-6% of SPK recipients develop T1D recurrence (T1DR) on follow-up (Diabetes, 2010), despite immunosuppression that prevents rejection. In our cohort of 223 SPK patients, T1DR has similar frequency to chronic rejection; we have shown that T1DR is preceded by seroconversion for multiple autoantibodies and these are risk factors for T1DR (AJT, 2016). We are investigating the role of memory T cells in T1DR. We report initial data from 5 SPK recipients with T1DR and 11 who had normal glucose tolerance (NGT). We used tetramer assays to detect autoreactive T cells in peripheral blood samples. We used a pool of MHC class II tetramers loaded with T1D-associated peptides from multiple autoantigens, and a viral antigen (flu), as a control. T cells were stained with lineage and phenotypic markers. Patients with T1DR had higher frequency of autoreactive T CD4 cells than NGT patients (p=0.0018). Regardless of diabetes status, patients with autoantibody conversions had higher frequency of autoreactive CD4 T cells than patients who were autoantibody negative or had persisting autoantibodies from prior to transplant (stable) (p=0.0326). These increased frequencies were observed in the memory compartment but not in the naïve compartment.
Our results support an association of memory autoreactive CD4 T cells with T1DR and autoantibody conversion. Most converters in the NGT and T1DR groups (all T1DR patients were converters) had higher proportions of CXCR3+, autoreactive CD4 T memory T cells than stable SPK patients (p=0.0099). Thus, while the frequency of autoreactive T cells may not be always increased in NGT converters, those cells express CXCR3. It is also possible that prior to the development of hyperglycemia CXCR3+ cells may migrate to the transplanted pancreas and this may be a factor in the lower frequency in this group. Significant increases in memory autoreactive T cells are more likely near diagnosis, perhaps representing a later event that precipitates beta cell destruction. Consistently, only partial beta cell loss is noted at biopsy in most SPK patients with T1DR, and that clinical symptoms manifest several years after autoantibody conversion (Diabetes, 2010; AJT, 2015). If validated by more extensive studies, CXCR3 may be a potentially suitable therapeutic target to antagonize islet autoimmunity, and we have initial evidence that memory T cells infiltrating the pancreatic islets in these T1DR patients do indeed express CXCR3.

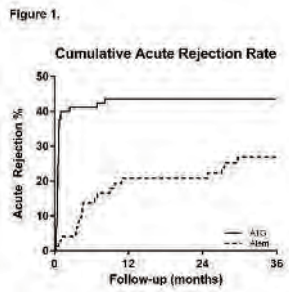
621.4

Empiric dose reduction is a risk for late acute rejection despite alemtuzumab induction in steroid-free simultaneous pancreas and kidney transplant recipients

J.R. Bank[1], S. Heidt[2], D. L.Roelen[2], M. J.K.Mallat[1], P. J.M.van der Boog[1], M. Vergunst[2], C. M.Jol-van der Zijde[3], R. G.Bredius[3], A. E.Braat[4], J. Ringers[4], F. H.J.Claas[2], M. E.J.Reinders[1], J. W.de Fijter[1].
[1]Nephrology, Leiden University Medical Center, Leiden, Netherlands; [2]Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, Netherlands; [3]Pediatrics, Leiden University Medical Center, Leiden, Netherlands; [4]Surgery, Leiden University Medical Center, Leiden, Netherlands.

Introduction: Simultaneous pancreas-kidney transplantation (SPKT) has been associated with relative high acute rejection rate compared to kidney transplantation alone. Recent large prospective studies in renal transplantation have shown a lower incidence of acute rejections with alemtuzumab induction compared to conventional induction therapy. In this retrospective cohort study we investigate incidence and time to acute rejection in SPKT recipients after alemtuzumab induction. Patients treated with ATG induction served as control. In addition, we provide mechanistic insights in acute rejections after alemtuzumab induction, including repopulation of specific alloreactive immune cells, maintenance immunosuppression and plasma alemtuzumab levels.
Methods: 158 consecutive SKPT recipients were included, and clinical data up to three years after transplantation of patients treated with alemtuzumab (30 mg SC; n=73) were compared to historical controls treated with ATG (single high-dose 9 mg/kg; n=85). All patients received Tacrolimus (Tac) and mycophenolate mofetil (MMF) maintenance therapy, and low-dose steroids only in case of ATG. Peripheral blood was obtained from 30 patients (13 at time of rejection vs.17 controls) for mixed lymphocyte cultures (MLC) and flow cytometric analysis of immune cells. Alemtuzumab levels were measured with ELISA on 3 time points in 20 patients (10 patients with and 10 w/o acute rejection <6 months).
Results: After three years of follow-up acute rejection rate was significantly lower in patients who received alemtuzumab compared to ATG induction therapy (26.0% vs. 43.5%; aHR 0.38, 95% CI 0.16-0.91, p=0.029).

Figure 1.



Most acute rejections (84.2%) in the alemtuzumab group occurred after the first 3 months, while almost all acute rejections with ATG (94.6%) occurred within the first month. Patients with a late acute rejection in the alemtuzumab group, showed no differences in B and T cell repopulation or detectable plasma alemtuzumab levels (log rank 0.486) as compared to those w/o rejection. Of note, in the alemtuzumab group 13 of 19 acute rejection episodes were preceded by empiric reductions in maintenance immunosuppression (Tac and/or MMF) due to viral infections, leukopenia or gastrointestinal symptoms.
Conclusion: Alemtuzumab induction with steroid-free maintenance resulted in significant lower early acute rejection rates. Late rejections were associated with Tac and/or MMF dose reductions following adverse events. This suggests that empiric dose reductions carry an increased risk for late rejection in SPKT recipients, especially with steroid-free maintenance.

621.5

The clinical relevance of one-way zero HLA mismatches among post-pancreas transplant acute graft-versus host disease population

Yoshihide Nanno, Hirochika Toyama, Sadaki Asari, Tadahiro Goto, Sachio Terai, Hisoka Kinoshita, Kaori Kuramitsu, Taku Matsumoto, Motofumi Tanaka, Masahiro Kido, Tetsuo Ajiki, Takumi Fukumoto, Yonson Ku.
Hepato-Biliary-Pancreatic Surgery, Kobe University Graduate School of Medicine, Kobe, Japan.

Objective: Acute graft-versus-host disease (aGVHD) in pancreas transplantation is quite rare (less than 1%) but a lethal complication[1]. A human leukocyte antigen (HLA)-matched donor is identical for allogeneic transplantation because of the low risk of immune complications such as graft rejection. When the HLA mismatches present only in the graft-versus-host direction (one-way zero HLA mismatch), it is associated with a higher incidence of aGVHD. We experienced the first post-pancreas transplant aGVHD case in Japan, whose HLA was one-way zero HLA mismatched[2]. However, the clinical relevance of one-way zero HLA mismatches among pancreas transplant population is yet unclear. This is a review of aGVHD cases in pancreas transplantation with particular emphasis on one-way zero HLA mismatches.
Data Source and Data Extraction: We queried PubMed in search for articles describing aGVHD in pancreas transplantation. The reported cases of recent 20 years were reviewed. We summarized the data on baseline characteristics, presence of one-way zero HLA mismatches, type of treatment, and mortality of aGVHD in pancreas transplant.
Results: Table 1 shows the summary of total 14 cases reviewed[1]–[10].

Table 1. Clinical characteristics of patients with aGVHD following pancreas transplantation

	Reference	Type	Age Gender	One-way 0 mismatch	Onset (POD)	Histological diagnosis	Treatment	Outcome (POD)
1	Kimball (1997)	SPK	27M	–	9	+	–	Death of MOF (22)
2	Gulbahce (2003)	SPK	58NA	–	NA	–	NA	Death of sepsis (76)
3		DDP	32NA	–	NA	+	Graftectomy	Death of sepsis (NA)
4	Weinstein (2006)	DDP	27F	–	25	+	PSL, IgG, CyA	Death of MOF (72)
5		SPK	38M	+	21	+	PSL, IgG, CyA, Graftectomy	Death of MOF (55)
6	Weng (2008)	DDP	51M	+	106	+	PSL	Death of MOF (145)
7	Osband (2010)	SPK	45M	NA	15	+	PSL, ATG, FK	Death of sepsis (52)
8	Sharma (2012)	DDP	14M	–	120	+	PSL, MSC	Death of sepsis (165)
9	Asari (2014)	SPK	37M	+	10	+	PSL	Death of sepsis (156)
10	Chang (2014)	DDP	32M	NA	37	–	PSL, FK, MMF	Recovery
11		SPK	61M	NA	61	–	PSL, FK	Recovery
12		DDP	49M	NA	470	–	PSL, SRL, ATG, DZM, ROM	Recovery
13	Guy (2014)	DDP	38M	–	27	–	PSL, FK, ROM	Recovery
14	Rossi (2014)	SPK	29F	–	12	+	PSL, FK, MMF, HD	Recovery

A diagnosis of aGVHD was supported by histopathology in nine cases, and by clinical presentation in five cases. Histocompatibility data for HLA typing were obtained in 10 cases (71%), and three of which (30%) were one-way zero HLA mismatched cases. The HLA mismatches of graft-versus-host direction were present in at least two loci in the three cases.

Table 2. HLA antigens for the reported one-way zero HLA mismatch cases

Reference	Patient	A	B	Cw	DR	DQ
Weinstein (2006)	Recipient	2, 30	13, 39	–	7, 8	–
	Donor	2, 30	13	–	7	–
Weng (2008)	Recipient	1, 24	8, 62	7, 9	4, 17	2, 8
	Donor	1, 24	8	7	17	2
Asari (2014)	Recipient	11, 24	35, 52	–	4, 15	–
	Donor	24	52	–	4, 15	–

The time from transplantation to onset of symptoms varied from 9–120 days, and they inclined to present gastrointestinal symptoms followed by skin rash, pancytopenia, and liver dysfunction. The time to onset, type of symptoms, or time to death was similar between the one-way zero HLA mismatched and non-one-way zero HLA mismatched cases. A high-dose steroid therapy was performed in 13 of 14 cases (93%). The other patient died without any treatment. The mortality of one-way zero HLA mismatched cases was 100% (3 of 3), and that of non-one-way zero HLA mismatched cases was 71% (5 of 7). Our case (case #9 in table 1) obtained remission with high-dose corticosteroid therapy, but experienced relapse in the course of steroid tapering, which was resulted in death with sepsis. This may indicate that pancreas transplantation from one-way zero HLA mismatch donor is a risk of aGVHD onset, and also of aGVHD resistance to therapy.
Conclusion: Accumulation of aGVHD cases in pancreas transplantation contributed in better understanding of the clinical relevance of one-way zero HLA mismatches. More patient with aGVHD had one-way zero HLA mismatches and poor prognosis. The application of pancreas transplantation from one-way zero HLA mismatched donors might require greater consideration.
The authors declare that they have no conflict of interest
References:

[1] Gulbahce HE, et al. Graft-vs-host disease after solid organ transplant. *Am J Clin Pathol*. 2003; 119: 568-573.
[2] Asari S, et al. cute graft-versus-host disease following simultaneous pancreas-kidney transplantation: report of a case. *Surg Today*. 2015; 45: 1567-1571.
[3] Kimball P, et al. Lethal graft-versus-host disease after simultaneous kidney-pancreas transplantation. *Transplantation*. 1997; 15: 1685-1688.
[4] Weinstein A, et al. Acute graft-versus-host disease in pancreas transplantation: a comparison of two case presentations and a review of the literature. *Transplantation*. 2006; 82: 127-131.
[5] Weng FL, et al. Fatal graft-versus-host disease presenting as fever of unknown origin in a pancreas-after-kidney transplant recipient. *Am J Transplant*. 2008; 8: 881-883.
[6] Osband AJ, et al. Treatment of acute graft-vs-host disease after simultaneous pancreas-kidney transplantation: a case report. *Transplant Proc*. 2010; 42: 3894-3897.
[7] Sharma A, et al. Graft-versus-host disease after solid organ transplantation: A single center experience and review of literature. *Ann Transplant*. 2012. 17: 133-139.
[8] Chang JW, et al. Successful treatment for graft-versus-host disease after pancreas transplantation. *Clin Transplant*. 2014; 28: 217-222.
[9] Guy S, et al. Successful treatment of acute severe graft-versus-host-disease in a pancreas-after-kidney transplant recipient: case report. *Transplant Proc*. 2014; 46: 2446-2449.
[10] Rossi AP, et al. Graft-versus-host disease after simultaneous pancreas-kidney transplantation: a case report and review of the literature. *Am J Transplant*. 2014; 14: 2651-2656.

621.6

De-novo donor-specific anti-HLA antibodies in islet cell transplant recipients in Australia

S. M. Hue[1], D. J. Torpy[5,6], G. Bennett[2], T. W. Kay[3,6], H. E. Thomas[3,6], D. Goodman[3,6], T. Loudovaris[3,6], P. J. O'Connell[4,6], T. Radford[5,6], C. J. Drogemuller[5,6], P. T. Coates[5,6].
[1]Department of Medicine, Pamela Youde Nethersole Eastern Hospital, Hong Kong, Hong Kong; [2] Australian Red Cross Blood Service , Adelaide, Australia; [3]St Vincent's Hospital, Melbourne, Australia; [4]Westmead Hospital, Sydney, Australia; [5]Central Northern Adelaide Renal and Transplantation Service, Royal Adelaide Hospital, Adelaide, Australia; [6]Australian Islet Transplantation Consortium, Australia.

Background: Presence of donor-specific antibodies (DSAs) is increasingly recognized as detrimental factor in many organ transplants and is associated with graft rejection. Their role in islet cell transplant is unclear.
Materials and Methods: We conducted a retrospective study. The HLA antibodies status was assessed via Australian National Organ Matching System (NOMS) on 4/1/2016. Complement-dependent cytotoxicity and Luminex assays were used to identify the presence and specificity of anti-HLA antibodies. Median Fluorescence Intensity (MFI) >2000 was considered as positive. Clinical data was primarily assessed via Islet Recipient Database of Australian Islet Consortium.
Results and Discussion: From October 2002 to December 2015, 48 patients (33 females and 15 males; age range 33-66) received a total of 90 allogenic islet infusions in Australia. They had a mean age of 50±9.4 years old and a BMI of 22.3±3.2 kg/m^2. 3 patients received islets from double donors in one single infusion. 4 patients were excluded from subsequent analysis because of no post-transplant DSA data. At a median follow-up of 83 weeks after the first transplant (range: 7-563 weeks), 41% (18/44) of patients developed de-novo DSAs. 9 patients had HLA class I DSAs alone; 5 patients had HLA class II DSAs alone; 4 patients had both class I and II DSAs. De-novo DSAs were first detectable at a median time of 34.5 weeks (interquartile range: 6-105 weeks). 56% (10/18) of patients developed de-novo DSAs within one year after their first transplants. In the last follow up screening, DSAs usually persisted. Only 2 patients became negative whose antibodies were anti-HLA class II initially.
DSA-positive and -negative islet recipients were similar in age, gender, BMI, duration of diabetes and anti-GAD antibody status. DSA-positive group had higher pre-transplant HbA1c but it was not statistically significant (p=0.12). Both groups exposed to similar number of donors, received similar islet equivalents/kg and had similar length of follow up. A non-significant tendency of lower HbA1c and higher c-peptide levels was apparent in DSA-negative islet recipients (figure1).

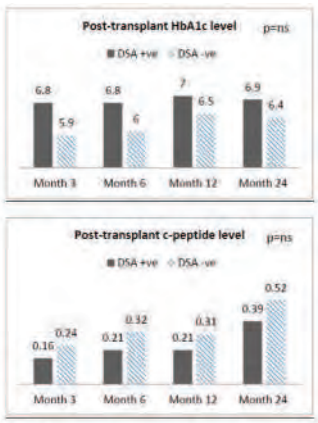


Figure 1: Post transplant HbA1c (%) & c-peptide level (nmol/L)

However, some patients in DSA-positive group actually had not yet developed DSAs at the specific time-point. Therefore, further analysis was performed. Recipients who had developed de-novo DSAs within 12 months were compared to those had not. HbA1c was 7.9% and 6.6% (p=0.23) while C-peptide was 0.08 nmol/L and 0.31 nmol/L (p=0.02, i.e. statistically significant) in DSA-positive group and DSA-negative group respectively (figure2). Similar analysis was performed at 24th month which continued to yield a significant difference in c-peptide level (0.23 nmol/L versus 0.55 nmol/L; p=0.03).

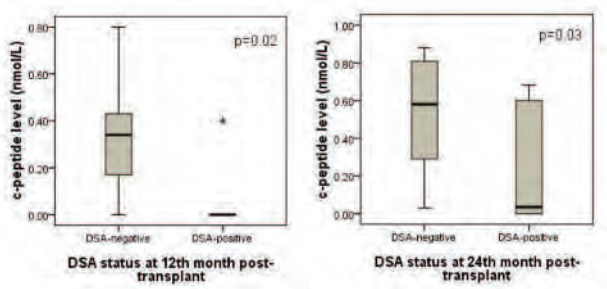


Figure 2: c-peptide level at 12th month and 24th month post-transplant

Conclusion: Significant proportion of islet recipients developed de-novo DSAs over time. Those who do not develop de-novo DSAs have higher post-transplant c-peptide level, indicating the absence of DSAs may associate with better graft function.
Australian Islet Transplantation Consortium

621.7

Application of treatment with plasmapheresis and immunoglobulins for high level of HLA donor specific antibodies in islet allograft recipients

Sabrina Matosz[1], Julia Solomina[1], Susana Marino[5], Jerome Weidner[5], Celeste Thomas[2], Zehra Tekin[1], Karolina Golab[1], Lindsay Schenck[1], Justyna Golebiewska[3], Maciej Sledzinski[6], Sabarinathan Ramachandran[1], W. James Chon[2], Natalia Marek-Trzonkowska[4], Piotr Witkowski[1].
[1]Surgery, University of Chicago, Chicago, United States; [2]Medicine, University of Chicago, Chicago, United States; [3]Nephrology, Transplantology, Internal Medicine, Medical University of Gdansk, Gdansk, Poland; [4]Family Medicine, Medical University of Gdansk, Gdansk, Poland; [5]Pathology, University of Chicago, Chicago, United States; [6]General Surgery, Endocrinology, and Transplantation, Medical University of Gdansk, Gdansk, Poland.

Background: Development of HLA donor specific antibodies (DSA) after organ or islet transplantation has been a poor prognostic factor for the graft survival[1]. Here, we present successful treatment of high level of DSA applying plasmapheresis and immunoglobulin, implemented for the first time in islet allograft recipients. Two patients who developed de novo DSA with high panel of reactive HLA antibodies (PRA) became insulin independent after the treatment and a subsequent islet infusion.
Material and Method: Two patients with PRA zero were transplanted intraportally with islet allograft in amount of 8,363 islet equivalents/kg of body weight (IEQ/kg) and 5,900 IEQ/kg, respectively. They received anti-thymocyte globulin as induction and tacrolimus and anti-metabolite (myfortic) as a maintenance immunosuppressive therapy. During the second islet transplant, basiliximab was used for the induction instead of anti-thymocyte globulin.
Results: On day 7 after the transplant, the first patient developed acute rejection with Panel of Reactive Antibodies (PRA) of 89% and high DSA (B57, B62, Bw4). He lost his serum c-peptide in the next few days despite treatment with steroids (3x 500mg IV of methylprednisolone). Plasmapheresis was initiated with subsequent low dose (100mg/kg) of immunoglobulin (IVIG). Five cycles every other day were given followed by high dose of IVIG- 2x1g/kg IV. Afterwards, PRA declined to zero and remained so afterwards. Patient was maintained on low dose of immunosuppression until subsequent islet transplant (5,800IEQ), which allowed him to wean off insulin completely with A1c still of 5.9, eleven months later.
Second patient developed PRA of 95% with high DSA (DQ4, DQ6) found on day 75 with still good islet function requiring only 15u of insulin. Course of plasmapheresis with high dose IVIG and anti- CD20 antibody allowed for complete resolution of PRA, maintaining stable partial islet graft function and for subsequent successful islet transplantation. Patient has been off insulin for the last 15 months without recurrence of PRA with A1c of 5.7. Of note, both patients developed anti-GAD antibody at high titer, which raised and declined in parallel to PRA.
Conclusion: De novo PRA/DSA after islet transplant can be successfully treated utilizing plasmapheresis and IVIG. Subsequent islet transplant from properly matched donor can improve overall outcome leading to the insulin independence and stable islet function.
References:
[1] Brooks AM1, Carter V2, Liew A, Marshall H, Aldibbiat A, Sheerin NS, Manas DM, White SA, Shaw JA. De Novo Donor-Specific HLA Antibodies Are Associated With Rapid Loss of Graft Function Following Islet Transplantation in Type 1 Diabetes. Am J Transplant. 2015 Dec;15(12):3239-46. Epub 2015 Jul 30.

621.8

Type one diabetes deceased organ donor isolated human islet-infiltrating CD4+ T cells recognize a deamidated proinsulin epitope presented by HLA-DQ8

Vimukti Pathiraja, Elenora Tresoldi, Michelle So, Stuart Mannering. Department of Medicine , University of Melbourne, Melbourne, Australia.

Type 1 diabetes (T1D) is caused by T-cell mediated destruction of the pancreatic insulin-producing beta cells. The HLA genes, HLA-DQ8 and HLA-DQ2 confer the highest risk of developing T1D implicating CD4+ T-cell responses in the development of T1D. Recently we reported that many human islet-infiltrating CD4+ T cells recognize epitopes derived the C-peptide of human proinsulin. And those T cell may responsible in pathogenesis in destroying beta cells and causing T1D.
Here we sought to determine if islet infiltrating CD4+ T cells recognized proinsulin epitopes formed by posttranslational modification (PTM). Specifically we tested proinsulin peptides with glutamine (Q) substituted for glutamic acid (E) and arginine substitute with citrulline. These modifications were chosen because they are central to the pathogenesis of the related autoimmune diseases: coeliac disease and rheumatoid arthritis. Islet-infiltrating CD4+ T-cell clones were tested for their capacity to respond to these peptides by IFNg ELISA.
One of 53 CD4+ clones recognized an epitope from the C-peptide of human proinsulin that incorporated glutamic acid (E) in place of glutamine (Q) (PI-Q54E) at position 54. This clone's response was restricted by HLA-DQ8 (DQA1*03:01, DQB1*03:02). We analyzed CD4+ T-cell responses in the peripheral blood mononuclear cells from subjects with recent onset T1D using a CFSE-based proliferation assay. This showed that 50% (6/12) of T1D subjects had detectable responses to (PI-Q54E), while none (0/5) of the healthy, HLA-DQ8+ subjects responded to this epitope. Further, we were able to isolate CD4+ T cell clone, which recognized C-peptide region of human proinsulin from a newly diagnosed T1D patient's peripheral blood, which confirms the findings from deceased organ donor islet-infiltrating T cell.
This is the first analysis of the PTM epitope specificity of human islet-infiltrating CD4+ T cell clones. Our work reveals that C-peptide and deamidated C-peptide specific, HLA-DQ8 restricted, CD4+ T-cells infiltrate human islets and suggest that these cells play a central role in the pathogenesis of human T1D. We anticipate that epitopes recognized by human islet-infiltrating CD4+ T cells will be strong candidates for developing effective antigen-specific pre-transplant screening toll as well as therapies for T1D.
References:
[1] Diabetes. 2015 Jan;64(1):172-82. doi: 10.2337/db14-0858. Epub 2014 Aug 25.

621.9

Fibroblast Growth Factor-21 in clinical islet transplantation

Raphael Meier, Nadja Niclauss, Philippe Morel, Sandrine Demuylder-Mischler, Mohamed Asrih, Domenico Bosco, François Jornayvaz, Thierry Berney.
Visceral and Transplant Surgery, Geneva University Hospital and Medical School, Geneva, Switzerland.

Background: Fibroblast Growth Factor-21 (FGF-21) is a metabolic regulator that increases islet insulin content and glucose-induced insulin secretion in diabetic animals. The aim of this study was to determine the correlation between donor FGF-21 levels and islet isolation and transplantation outcomes.
Methods: We have measured serum FGF-21 levels in 189 pancreas donor sera immediately before organ procurement. We had follow-up data in recipients of 67 islet transplantation procedures at 1, 6, and 12 months. We arbitrarily divided donors in two groups according to FGF-21 levels, with the median value as cut-off (FGF-21≥1500 pg/ml: N=33; FGF-21<1500 pg/ml: N=34).
Results: Donor FGF-21 levels were independent of age, gender, body mass index, cause of death or duration of ICU stay. At the end of the isolation procedure, higher donor FGF-21 level was associated with higher islet equivalent number (IEQ) in the mantled islet layers (i.e. endocrine/exocrine ratio of 10 to 50%) median 64'889 vs. 26'542 IEQ, p=0.003. Pancreas weight before and after digestion, islet size, total IEQ before and after purification were independent from FGF-21 levels. Recipients of islets from donor with FGF-21 ≥1500 pg/ml vs. <1500 pg/ml had insulin-independent rate of 21.9% vs. 20.6 at 1 month), 40.0% vs. 21.4% at 6 months, and 28.0% vs. 19.2% at 12 months (p value not significant for all time points).
Conclusion: FGF-21 levels correlated with higher IEQ in the mantled islet layers. Donors with the highest FGF-21 levels showed a trend toward higher insulin-independence rates at 6 and 12 months post transplantation. In conclusion, high FGF-21 levels may correlate with strong endocrine/exocrine cohesion and high islet functional reserve.

622.1

Outcomes with pre transplant bariatric surgery in renal transplant recipients

Girish Mour[1], Dave Taber[2], Zemin Su[3], Patrick Mauldin[3], Jingwen Zhang[3], Titte Srinivas[1].
[1]Medicine, MUSC, Charleston, United States; [2]Surgery, MUSC, Charleston, United States; [3]General Internal Medicine and Geriatrics, MUSC, Charleston, SC, United States.

Background: Most transplant centers have a thresold for BMI for transplantation. Many transplant centers use bariatric surgery (BS) to manage weight pre-transplantation (Pre-Tx) to make acceptable for transplantation. We don't know if this approach leads to increased complications post transplant. We hypothesized that grafts may be compromised by variable drug absorption, rejection and oxalate nephropathy. We examined the relationships between BS, tacrolimus (Tac) exposure, rejection (AR) and 1 yr graft loss (GL).
Methods: Through a large-scale quality improvement project, we developed a predictive risk model to assess the association between Pre-Tx BS and 1-yr GL then added Tac exposure measures (Tac Mean C0, FKM; Time to Tac C0 = 8-12 ng/mL and SD of Tac C0 0-90 days), and acute Banff scores to the model. We included adult only solitary kidney transplant recipients transplanted 2007 - 2015. We used Natural language processing to extract Banff lesion scores from the EMR. We excluded 1) patients without a recorded BMI at transplant, and 2) patients who did not have a GL recorded nor any other data during the first year post-transplant. We didn't have Pre-Tx BS BMI for recipients who underwent BS and neither did we know the weight loss achieved after BS.The primary covariates were: 1) patients with BS prior to transplant, 2) patients with BMI ≤ 35 without BS, and 3) patients with BMI >35 without BS (reference). IBM SPSS Modeler and Essentials for R were used for analysis. The Backward Selection Process was used at the 20% level for variable selection, and statistical significance was determined at the 5% level.
Results: A total of 1167 patients were evaluated. 5% experienced 1 yr GL. There were 31 patients who received BS prior to transplant, 974 patients with BMI ≤ 35 without BS, and 162 patients with BMI >35 without BS (reference). Patients with BS prior to transplant trended 3.41 times more likely to have 1-year GL than those with BMI > 35 without BS (reference) (p=0.134). Acute rejection and lower FKM were risk factors for GL (Table 1). The predictive model AUC was 0.80 (95% CI: 0.74-0.86) (Figure1) giving strong model accuracy. Other risk factors were KDRI and DGF which is not surprising.
Conclusion: Patients with bariatric surgery have a higher risk of graft loss risk as compared to patients with BMI > 35 without BS. In our model, achieving FKM levels and lower AR rates didn't lower the risk of 1 yr GL in BS. We don't know the exact reasons for graft loss. Also, we didn't have data on the type of BS. Our results still suggest that optimizing renal graft outcomes in BS patients will require a multipronged approach to optimize tacrolimus exposure, minimize rejection and minimize oxalate exposure.

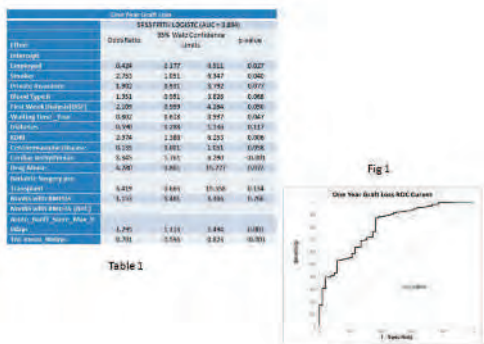


Table 1

Fig 1

622.2

Post-transplant diabetes mellitus after kidney transplantation in Japanese patients: The Japan Academic Consortium of Kidney Transplantation study

Kazuya Omoto[1,2], Masayoshi Okumi[2], Kohei Unagami[2], Miyuki Furusawa[2], Toshihito Hirai[2], Tomokazu Shimizu[2], Hideki Ishida[2], Kazunari Tanabe[2].
[1]Department of Urology, Todachuo General Hospital, Saitama, Japan; [2]Department of Urology, Tokyo Women's Medical University, Tokyo, Japan.

Background: Post-transplant diabetes mellitus (PTDM) is a major complication after kidney transplantation. However, PTDM has not been well studied in Japan.
Objectives: The aims of the present study were to: 1) clarify the PTDM incidence rate; 2) determine its potential risk factors; and 3) develop risk stratification matrix for the development of PTDM by exposure of risk factors among Japanese living kidney transplant recipients.
Patients: We analyzed 887 Japanese nondiabetic adult recipients who had undergone living kidney transplantation and received tacrolimus-based immunosuppression from 1996 to 2013 and were followed up for a mean of 4.3 years.
Results: Sixty-five patients developed PTDM during the follow-up. The incidence rate of PTDM was 7.7% (95% confidence interval [CI]: 5.9-9.5%) at 5 years. Recipient age more than 45 years (hazard ratio [HR] =4.81, 95%CI: 2.78-8.34, *P*<0.001), obesity (HR=3.23, 95%CI:1.80-5.78, P<0.001), TAC trough level >9 ng/mL (HR=2.24, 95%CI:1.28-3.93, *P*=0.005), and mycophenolate mofetil (HR=0.43, 95%CI: 0.24-0.78, *P*=0.005) were associated predictors for PTDM. According to risk stratification, recipients without any risk conditions had a 0.7% PTDM incidence rate (95%CI, 0.1-1.3%); conversely, those in patients with all risk factors were 62.9% (95%CI, 16.5-83.5%) 6 months after transplant.
Conclusions: PTDM is a common complication after kidney transplantation in Japan as well as other western countries. However, our results indicate appropriate tacrolimus-based immunosuppressive regimen has potential to decrease the incidence of PTDM.
Katsunori Shimada, PhD. (STATZ Institute, Inc., Tokyo, Japan); Astellas Pharma Inc. (Tokyo, Japan)

622.3

Sodium/Glucose Cotransporter 2 Inhibitor for the treatment of diabetes in kidney transplant patients

Jin Kong[1], Joon H.Jeong[2], Won Choi[3], Sung S.Son[1], Hyuk Y.Kwon[1], Eun J.Whang[3].
[1]Nephrology, Hanseo Hospital, Busan, Korea; [2]Surgery, Hanseo Hospital, Busan, Korea; [3]Laboratory Medicine, Hanseo Hospital, Busan, Korea.

Sodium/Glucose Cotransporter 2(SGLT2) inhibitors are newly introduced antidiabetic agents that reduce blood glucose by inhibiting reabsorption of glucose in renal proximal tubule. Because of the concern of acute renal dysfunction largely due to volume depletion associated with osmotic diuresis, these drugs are currently not recommended for patients with moderate to advanced chronic kidney disease. But experimental evidences indicate that SGLT2 inhibitors may be renoprotective in the long term by mitigating renal hypertrophy and inflammation, and also by reducing glomerular hyperfiltration independent of blood glucose change. Efficacy and safety profile of these drugs in kidney transplant(KT) patients is so far unknown.
Twenty KT patients were treated with dapagliflozin 5mg/day. Three patients had type 1 DM and 4 had NODAT. Thirteen patients were on insulin with or without oral agents. Baseline serum creatinine was 1.2±0.5(0.6~2.4)mg/dl.
Baseline HbA1c was 7.8±1.3%, which decreased significantly at 3(7.4±1.1%, p=0.011) and 6(7.4±1.0%) months. Body weight decreased significantly from 69.7±22.1 to 68.2±22.0(p=0.000). One patient could stop insulin and another 3 patients could reduce ≥20% dose of insulin. eGFR did not change significantly(70.5±22.1ml/min at baseline, 68.7±29.9 at 3 months, and 69.1±28.8 at 6 months). Office blood pressure also was not changed significantly but 8 of 20 treated patients had a decrease in number and/or dose of anti-hypertensive drugs. No significant change in urine albumin-creatinine ratio was observed at 6 months. Six patients stopped dapagliflozin due to acute cystitis in 2, weight loss in 1 and lack of efficacy in 3.
SGLT2 inhibitor is effective in reducing hyperglycemia in KT patients, and adverse reactions were manageable. Long term benefits and safety profile should be evaluated in larger number of KT patients.

622.4

Abnormal glucose metabolism in dialysis patients and its correlation with the development of new onset diabetes after renal transplantation

Manish Rathi[1], Namrata Rao S.[1], Ashish Sharma[2], Harbir S.Kohli[1], Krishan L.Gupta[1], Vinay Sakhuja[1].
[1]Nephrology, Post Graduate Institute of Medical Education & Research, Chandigarh, India; [2]Transplant Surgery, Post Graduate Institute of Medical Education & Research, Chandigarh, India.

Introduction: New onset diabetes after transplantation (NODAT) is a common and serious metabolic complication after renal transplantation. While a number of risk factors have been proposed, the role of pretransplant glucose metabolism parameters in the development of NODAT has not been studied in detail. It is not clear whether all patients with pretransplant hyperglycemia progress to NODAT, or whether an abnormal glucose metabolism is part of the natural history of NODAT. This study was carried out to correlate pretransplant Oral glucose tolerance test (OGTT), Hemoglobin A1c (HbA1C) and Homeostatic model assessment (HOMA) derived glucose metabolism parameters with the development of NODAT.
Material and Methods: In this prospective observational study, patients awaiting living donor transplant workup were included. Those with pre-existing diabetes mellitus, requirement of pre-transplant steroids and cadaver transplants were excluded. The study participants underwent measurement of anthropometric variables, and fasting blood samples were drawn for plasma glucose, insulin, C-peptide and HbA1c. This was followed by 2-hour OGTT. Insulin resistance and beta cell function were derived using HOMA calculator version 2.2.3. All these tests were done 1 week prior to the date of transplant. In the post transplant period, patients were followed with serial plasma glucose monitoring for establishing the diagnosis of NODAT according to the International Consensus guidelines. Statistical analysis was done using SPSS version 16.0.
Results and Discussion: A total of 72 patients were evaluated in the pretransplant period and followed up post transplantation. Twenty one patients (29%) developed NODAT. Of the total of 72 patients, 27 (37%) patients had prediabetes prior to transplantation; four with impaired fasting glucose and 23 with impaired glucose tolerance. Pretransplant fasting plasma glucose, 2-hour plasma glucose after OGTT, HbA1c and HOMA derived insulin resistance (HOMA IR) correlated significantly with the development of NODAT ($p<0.05$). On logistic regression analysis, pretransplant HbA1c retained significant predictive value (odds ratio 27.04) in predicting the development of NODAT. The incidence of NODAT in our study was comparable to published literature. Most patients with prediabetes in our study had impaired glucose tolerance, while very few had an impaired fasting glucose. Fasting insulin, C-peptide and β cell function (%B) were higher in patients who subsequently developed NODAT. It is known that a hyperbolic relationship exists between insulin sensitivity and secretion, ie., as the insulin sensitivity goes down, β cell function increases. But with severe insulin resistance, the β cell function cannot keep up, to maintain normal glucose values. So, a higher β cell function in the pretransplant period might signify the islet cell response to peripheral insulin resistance.
Conclusions: Incidence of prediabetes is high in patients with end stage renal failure, identified in a majority of cases only by OGTT. Pretransplant HbA1c, in conjunction with HOMA derived parameters, may be novel tools to identify the future risk of NODAT, and may enable the tailoring of immunosuppression accordingly.

622.5

Epidemiology and risk factors for infection after living donor liver transplantation

Cybele Lara Abad[1], Brian D.Lahr[2], Raymund R.Razonable[3].
[1]Division of Infectious Diseases, Department of Medicine, Mayo Clinic, Rochester, United States; [2]Department of Health Sciences Research (Division of Biomedical Statistics and Informatics), Mayo Clinic, Rochester, United States; [3]Department of Health Sciences Research, The William J. Von Liebig Center for Transplantation, Mayo Clinic, Rochester, United States.

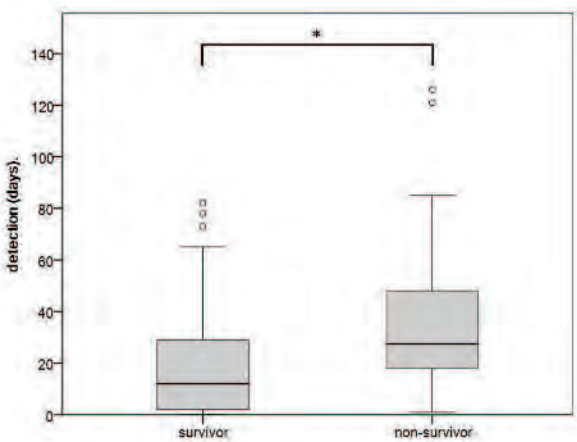
Introduction: Infections are a leading cause of morbidity and mortality among liver transplant patients[1],[2].The risk of bacterial and fungal infections can be as high as 50% in the first year, with most infections occurring in the first one to two months after liver transplantation[3]-[8]. This risk seems to be higher in those who receive transplants from living donors 9 although this has not been well examined. This study aims to describe the incidence of infection and determine risk factors for infection in a large cohort of adult living donor liver transplant (LDLT) recipients.
Methods: We retrospectively reviewed 223 adults who underwent LDLT at the Mayo Clinic, Rochester from January 1, 2000 through August 31, 2015. We gathered clinical data including pre-operative, operative, and post-operative variables associated with infections up to 1 year after transplant. The association of candidate risk factors with infection was analyzed with univariable and multivariable Cox regression.
Results: In our cohort of 223 adult LDLT recipients the majority were Caucasian (n=211, 95%) and male (n=144, 65%), with a median age of 55 years. The most common reason for transplantation was primary sclerosing cholangitis (38%). About half of the cohort (53%) did not have any major comorbid illness; others had underlying inflammatory bowel disease (28%) or diabetes (15%). Median MELD score at the time of transplantation was 14. Only 33 LDLT recipients (15%) had history of recurrent infections prior to transplantation, and 15 (6%) had active infections within the week before transplant date. At one year follow-up, a total of 122 patients (Kaplan-Meier rate, 56%) developed infection, with the majority occurring within 30 days of transplant (69%). Of these, 103 (84%) recipients had a non-opportunistic infection alone, 6 (5%) had an opportunistic infection, and 13 (11%) had both. Of the 116 patients with a non-opportunistic infection, 102 had microbiologic evidence and 14 were syndromic. Microbiology data showed that more than half of bacterial infections (54/100) were hepatobiliary in origin. Enterococcus sp. was the most frequently isolated organism (29/54). Multivariable analysis shows that Roux-en- Y biliary reconstruction and increasing age, MELD score, packed RBC transfusion, and number of hepatic vein anastomoses were independently associated with higher risk of all infections.
Conclusion: LDLT recipients are at high risk for bacterial infections within the first year of transplantation, especially during the first 30 days. The infections are predominantly hepatobiliary in origin. The type of biliary anastomoses, venous anastomosis, MELD score, age, and receipt of red blood cell transfusions are associated with higher risk of infection after LDLT.
References:
[1] Kusne S, Dummer JS, Singh N, et al. Medicine (Baltimore) 1988;67:132-43.
[2] Losada I, Cuervas-Mons V, Millan I, Damaso D.Enferm Infecc Microbiol Clin 2002;20:422-30.
[3] Avkan-Oguz V, Ozkardesler S, Unek T, et al.Transplant Proc 2013;45:993-7.
[4] Bodro M, Sabe N, Tubau F, et al.Transplantation 2013;96:843-9.
[5] Li C, Wen TF, Mi K, Wang C, Yan LN, Li B.World J Gastroenterol 2012;18:1975-80.
[6] Said A, Safdar N, Lucey MR, et al. Am J Transplant 2004;4:574-82.
[7] Rubin RH.Curr Clin Top Infect Dis 2002;22:125-54.
[8] Saner FH, Olde Damink SW, Pavlakovic G, et al. Transplantation 2008;85:1564-8.

622.6

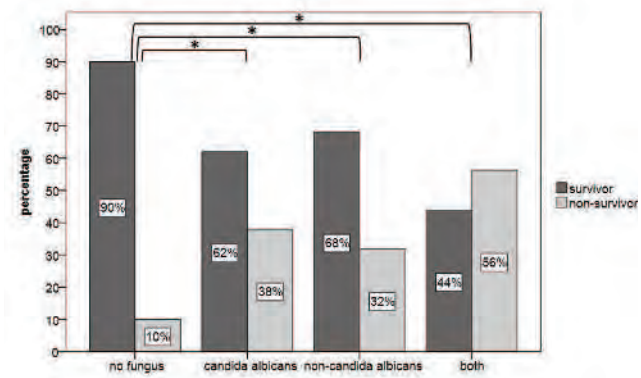
Postoperative fungal infection in liver transplant recipients: Indicator but not the cause of overall mortality

Katharina M Schmidt[1], Annette Pross[1], Florian Zeman[2], Sven ALang[1], Thomas Bein[1], Hans JSchlitt[1], Ivan Gocze[1].
[1]Department of Surgery, University Medical Center Regensburg, Regensburg, Germany; [2]Center for Clinical Studies, University Medical Center Regensburg, Regensburg, Germany.

Introduction: Infections, particularly fungal disease (FD), remain a major factor associated with poor prognosis in liver transplant recipients. Despite risk factors for FD development have been identified, fungal prophylaxis in high-risk patients results in reduction of proven fungal infections, but has no effect on overall mortality. Therefore, it still remains to be elucidated, whether FD itself causes elevated mortality, especially in high MELD patients with various other clinical risk factors.
Methods: All adult liver transplant recipients between 2008 and 2013 at the University Hospital Regensburg were evaluated for this retrospective study. Out of 237 screened patients, all 69 (29%) patients with a microbiologically proven fungal infection were selected for our primary analyses. Further 70 patients without fungal infection matched by MELD score were selected as control group for univariate 6-months survival analyses. Predictors for 6-months survival were analyzed by uni- and multivariable logistic regression models.
Results: Out of the 69 patients with fungal infection, candida albicans (CA) was detected in 29 patients (42%), candida non-albicans (CnA) in 22 patients (32%) and both candida species (CA+CnA) in 16 (23%) patients. Aspergillus was identified in 2 (3%) patients. Median MELD score was 31 and median time for the first positive microbiological fungal finding was day 12 in survivors (n=41, 59%) and day 28 in non-survivors (n=28, 41%) (p=0.013) [Fig 1].



Clinical variables such as abdominal source of fungal infection, alcoholic liver cirrhosis, packing of the liver intraoperatively, azole drugs or fungal colonization <3 months before transplantation and mechanical ventilation over 72 hours after transplantation were predictors of mortality in univariate analyses. Fungal infection (CA, CnA or CA+CnA) was no significant risk factor for mortality in neither uni- nor multivariable analysis. In the multivariable model high volume blood transfusions, antibiotic therapy >14 days and dialysis remained as independent predictors for 6-months mortality (p=002, p=0.009, p=0.004). In comparison to the control group (n=70, median MELD=29), patients with CA, CnA and CA+CnA showed significantly increased risk for 6-months mortality (OR=5.5, 4.2, 11.6; p=0.002, 0.018, <0.001) [Fig 2].



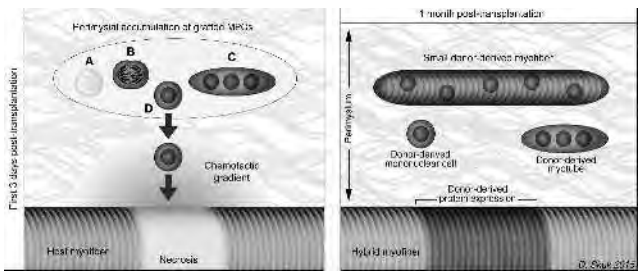
Conclusions: Although microbiologically proven fungal infection is associated with increased mortality in liver transplant recipients, the presence of FD is not prognostic with regard to patient outcome. This finding may explain the beneficial effect of antifungal prophylaxis on reduction of candida infections without improvement in overall mortality. Reduction of perioperative bleeding, judicious antibiotic use and nephroprotective strategies may represent the key elements for improving overall outcome in high MELD patients who develop postoperative fungal infection.

622.7

An international survey of infections after total artificial heart transplantation

Heather Chambers[1], Peggy Pelish[2], Fang Qiu[3], Diana F.Florescu[1,4]. [1]Transplant Infectious Diseases, University of Nebraska Medical Center, Omaha, NE, United States; [2]College of Nursing, University of Nebraska Medical Center, Omaha, NE, United States; [3]Department of Biostatistics, University of Nebraska Medical Center, Omaha, NE, United States; [4]Transplant Surgery Division, University of Nebraska Medical Center, Omaha, NE, United States.

Background: Infections in mechanical assist devices are the most serious complications leading to increased morbidity and mortality and decreased quality of life. There are no large studies on Total Artificial Heart transplants (TAH-t) infections. The aim of our survey was to assess the infections in TAH-t recipients and the outcomes after TAH-t among different programs.
Methods: An electronic survey was sent to 78 programs that implant Syncardia TAH. Proportions were calculated for categorical variables; means and SDs were calculated for continuous variables.
Results: Forty-four percent of TAH-t programs responded to the survey, 85% North American centers and 15% European centers. Academic centers comprised 79.3%.
Infections were reported to occur post-operatively in 52.2% centers. During the first month after TAH implantation, infections were: driveline site 27.2%, urinary tract infections 18.2%, pulmonary infections 45.5%, mediastinal infections 18.2%, and abdominal infections 9%. After the first month, infections were: bacteremia 40.9%, urinary tract infections 13.6%, pulmonary infections 18.2%, driveline infections 22.7%, and mediastinal infections 4.5%. The organisms isolated are presented in the Table. The most common organisms seen within the first month were Candida spp. (22.7%), Pseudomonas aeruginosa (13.6%), Escherichia coli (9%), followed by 4.5% of Staphlococcus spp., Enterococcus spp., Enterobacter, Serratia, and CMV. The most common organisms after the first month were Staphlococcus spp. (40.9%), Candida spp. (18.2%), followed by 1% of Enterococcus, E. coli, and Pseudomonas.
Sixty-five percent of the centers reported mortality; the mean rate of death post-TAH transplant due to infection was 14.5% (SD, 22.3). The mean rate of patients surviving until an orthotopic heart transplant was 58.6% (SD, 27.7).



Conclusions: TAH-t have an increased risk of infections caused by a wide-variety of microorganisms. In the first month, Gram-negative bacteria and Candida infections were more common, while Gram-positive infections increased after 30 days. The high rate of Gram-negative bacterial and fungal infections might be explained by prolonged hospitalization of these patients. Preventing these infections is key to decreased morbidity and mortality in this population.

References:

[1] Physician consortium for performance improvement: heart failure performance measurement set by American College of Cardiology Foundation/American Heart Association/American Medical Association. Updated May 15, 2012
[2] Yancy, C. W., Jessup, M., Bozkurt, B., Butler, J., Casey, D. E.,Jr, Drazner, M. H., . . . American Heart Association Task Force on Practice Guidelines. (2013). 2013 ACCF/AHA guideline for the management of heart failure: A report of the American College of Cardiology Foundation/American Heart Association task force on practice guidelines. Journal of the American College of Cardiology, 62(16), e147-239
[3] Ahmed, W., Esmailian, F., & Hernandez Conte, A. (2013). Con: The total artificial heart-is it an appropriate replacement to existing biventricular assist devices? Journal of Cardiothoracic and Vascular Anesthesia, doi:10.1053/j.jvca.2013.07.005; 10.1053/j.jvca.2013.07.005
[4] Kirklin, J. K., Naftel, D. C., Kormos, R. L., Stevenson, L. W., Pagani, F. D., Miller, M. A., . . . Young, J. B. (2013). Fifth INTERMACS annual report: Risk factor analysis from more than 6,000 mechanical circulatory support patients. The Journal of Heart and Lung Transplantation : The Official Publication of the International Society for Heart Transplantation, 32(2), 141-156. doi:10.1016/j.healun.2012.12.004; 10.1016/j.healun.2012.12.004
[5] Holman, W. L., Park, S. J., Long, J. W., Weinberg, A., Gupta, L., Tierney, A. R., . . . REMATCH Investigators. (2004). Infection in permanent circulatory support: Experience from the REMATCH trial. The Journal of Heart and Lung Transplantation : The Official Publication of the International Society for Heart Transplantation, 23(12), 1359-1365. doi:10.1016/j.healun.2003.09.025
[6] Baddour, L. M., Bettmann, M. A., Bolger, A. F., Epstein, A. E., Ferrieri, P., Gerber, M. A., . . . AHA. (2003). Nonvalvular cardiovascular device-related infections. Circulation, 108(16), 2015-2031. doi:10.1161/01.CIR.0000093201.57771.47
[7] Schulman, A. R., Martens, T. P., Russo, M. J., Christos, P. J., Gordon, R. J., Lowy, F. D., . . . Naka, Y. (2009). Effect of left ventricular assist device infection on post-transplant outcomes. The Journal of Heart and Lung Transplantation : The Official Publication of the International Society for Heart Transplantation, 28(3), 237-242. doi:10.1016/j.healun.2008.12.007; 10.1016/j.healun.2008.12.007
[8] Nienaber, J. J., Kusne, S., Riaz, T., Walker, R. C., Baddour, L. M., Wright, A. J., . . . Mayo Cardiovascular Infections Study Group. (2013). Clinical manifestations and management of left ventricular assist device-associated infections. Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America, 57(10), 1438-1448. doi:10.1093/cid/cit536; 10.1093/cid/cit536
[9] Baddour, L. M., Bettmann, M. A., Bolger, A. F., Epstein, A. E., Ferrieri, P., Gerber, M. A., . . . Taubert, K. A. (2004). Nonvalvular cardiovascular device-related infections. Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America, 38(8), 1128-1130. doi:10.1086/383060
[10] Bomholt, T., Moser, C., Sander, K., Boesgaard, S., Kober, L., Olsen, P. S., . . . Gustafsson, F. (2011). Driveline infections in patients supported with a HeartMate II: Incidence, aetiology and outcome. Scandinavian Cardiovascular Journal : SCJ, 45(5), 273-278. doi:10.3109/14017431.2011.577236; 10.3109/14017431.2011.577236
[11] Acharya, M. N., Som, R., & Tsui, S. (2012). What is the optimum antibiotic prophylaxis in patients undergoing implantation of a left ventricular assist device? Interactive Cardiovascular and Thoracic Surgery, 14(2), 209-214. doi:10.1093/icvts/ivr054; 10.1093/icvts/ivr054
[12] Maniar, S., Kondareddy, S., & Topkara, V. K. (2011). Left ventricular assist device-related infections: Past, present and future. Expert Review of Medical Devices, 8(5), 627-634. doi:10.1586/erd.11.36; 10.1586/erd.11.36
[13] Morris, R. J. (2008). Total artificial heart—concepts and clinical use. Seminars in Thoracic and Cardiovascular Surgery, 20(3), 247-254. doi:10.1053/j.semtcvs.2008.08.006; 10.1053/j.semtcvs.2008.08.006
[14] Conger, J. L., Inman, R. W., Tamez, D., Frazier, O. H., & Radovancevic, B. (2000). Infection and thrombosis in total artificial heart technology: Past and future challenges—a historical review. ASAIO Journal (American Society for Artificial Internal Organs :

1992), 46(6), S22-7.
[15] Copeland, J. G. (2013). SynCardia total artificial heart: Update and future. Texas Heart Institute Journal / from the Texas Heart Institute of St.Luke's Episcopal Hospital, Texas Children's Hospital, 40(5), 587-588.
[16] Kirsch, M. E., Nguyen, A., Mastroianni, C., Pozzi, M., Leger, P., Nicolescu, M., . . . Leprince, P. (2013). SynCardia temporary total artificial heart as bridge to transplantation: Current results at la pitie hospital. The Annals of Thoracic Surgery, 95(5), 1640-1646. doi:10.1016/j.athoracsur.2013.02.036; 10.1016/j.athoracsur.2013.02.036
[17] Copeland, J. G., Copeland, H., Gustafson, M., Mineburg, N., Covington, D., Smith, R. G., & Friedman, M. (2012). Experience with more than 100 total artificial heart implants. The Journal of Thoracic and Cardiovascular Surgery, 143(3), 727-734. doi:10.1016/j.jtcvs.2011.12.002; 10.1016/j.jtcvs.2011.12.002
[18] Walker, P. C., DePestel, D. D., Miles, N. A., & Malani, P. N. (2011). Surgical infection prophylaxis for left ventricular assist device implantation. Journal of Cardiac Surgery, 26(4), 440-443. doi:10.1111/j.1540-8191.2011.01262.x; 10.1111/j.1540-8191.2011.01262.x
[19] Koval, C. E., Rakita, R., & AST Infectious Diseases Community of Practice. (2013). Ventricular assist device related infections and solid organ transplantation. American Journal of Transplantation : Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons, 13 Suppl 4, 348-354. doi:10.1111/ajt.12126; 10.1111/ajt.12126
[20] Trampuz, A., & Zimmerli, W. (2006). Antimicrobial agents in orthopaedic surgery. Drugs, 66 (8), 1089-1105. Doi: 012-6667/06/0008-1089/$44.95/0
[21] Califano, S., Pagani, F.D. & Nalani, P.N. (2012). Left ventricular assist device—Associated infections. Infect Dis Clin N Am, 26, 77-87. Doi: 10.1016/j.idc.2011.09.008
[22] Aslam, S., Hernadez, M., Thornby, J., Zeluff, B., & Darouiche, R.O. (2010). Risk factors and outcomes of fungal ventricular-assist device infections. Clin Infect Dis, 50 (5), 664-671. Doi: 10.1086/650454
[23] Gordon, S.M., Schmitt, S.K., Jacobs, M., Smedira, N.M., Goormastic, M., Banbury, M.K. et al. (2001). Nosocomial bloodstream infections in patients with implantable left ventricular assist devices. Ann Thorac Surg, 72, 725-730.
[24] Choi, E.Y., Park, D., Kim, H.J., & Park, J. (2015). Efficacy of chlorhexadine bathing for reducing healthcare associated bloodstream infections: A meta-analysis. Annals of Intensive Care, 5 (1): 31. Doi: 10.1186/s13613-015-0073-9
[25] Thompson, P., & Houston, S. (2013). Decreasing methicillin-resistant staphylococcus aureus surgical site infections with chlorhexidine and mupirocin. American Journal of Infection Control, 41(7), 629-633. doi:10.1016/j.ajic.2012.09.003 [doi]

622.8

Risk factors for clinical urinary tract infections in kidney transplant recipients with asymptomatic bacteriuria

Samar Medani, Marc Dorais, Alexandre Tavares-Brum, Habib Mawad, Alain Duclos, Claude Lemieux, Azemi Barama, Heloise Cardinal.
University of Montreal Hospital Research Centre, University of Montreal, Montreal, Canada.

Background: There is no clear consensus on whether asymptomatic bacteriuria (ABU) should be treated in kidney transplant recipients (KTRs). We sought to identify patient or episode related characteristics associated with subsequent symptomatic urinary tract infection (UTI) and with antibiotic prescribing in KTRs with ABU.
Methods: We retrospectively recorded all episodes of ABU, cystitis and pyelonephritis in KTRs transplanted at our centre between January 1st 2008 and June 1st 2013. Bacteruria was defined as ≥104 colony-forming units (CFU) /ml as 23% of symptomatic UTIs occurred with colony counts ≥104-105 CFU/ml. Through logistic regression models using generalized estimating equations to account for within patient correlations, we assessed i) factors associated with occurrence of a symptomatic UTI within 3 months of an episode of ABU; ii) factors associated with antibiotic treatment of ABU.
Results: Among 318 study participants, there were 622 episodes of ABU in 156 patients. Symptomatic UTI within 3 months occurred following 87(14 %) of ABU episodes. Factors associated with an increased likelihood of a symptomatic UTI following ABU were younger age (OR: 1.38 per 10-year decrease in age; 95% CI: 1.12-1.70), previous symptomatic UTI (OR: 5.24; 95% CI: 2.18-12.59), the presence of leukocyturia or haematuria (OR: 3.38; 95% CI: 1.86-6.17), isolation of a ciprofloxacin resistant or extended-spectrum beta-lactamase (ESBL) producing strain (OR: 2.21; 95% CI: 1.03-4.75), and isolation of gram negative bacteria (GNB) (OR: 2.44; 95 % CI: 1.12-5.31). Antibiotics were prescribed in 24% of ABU episodes. The presence of leukocyturia or haematuria (odds ratio (OR): 2.02, 95% confidence interval (CI): 1.27-3.22), urinary nitrites (OR: 2.54, 95% CI: 1.08-5.99), GNB (OR: 2.08, 95% CI: 1.11-3.85), and bacterial count >105 CFU/ml (OR: 5.55 versus 104-105 CFU /ml, 95% CI: 3.44-8.33) were associated with an increased likelihood of receiving antibiotic treatment for ABU. We could not demonstrate an association between antibiotic treatment and protection from symptomatic UTI (OR: 0.64; 95 % CI: 0.27-1.51).
Conclusion: Younger age, leucocyturia or haematuria, gram negative and quinolone-resistant or ESBL producing strains, and a history of previous cystitis or pyelonephritis are predictors of progression of ABU to symptomatic UTI that can help in guiding the management of KTRs with ABU and in the design of future therapeutic trials.

622.9

Beta cell function and insulin resistance after the conversion from tacrolimus twice daily to extended-release tacrolimus once daily in stable renal transplant recipients

Prajej Ruangkanchanasetr, Natthi Sanohdontree, Thanom Supaporn, Sirilak Leawseng, Bancha Satirapoj.
Medicine, Phramongkutklao Hospital and College of Medicine, Bangkok, Thailand.

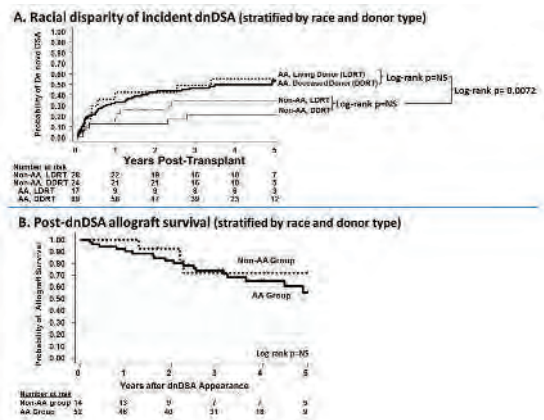
Background: Major metabolic side effect of tacrolimus (TAC) is posttransplantation diabetes mellitus (PTDM) which can cause allograft dysfunction and patients' morbidity and mortality. The objective of the study was to determine if the conversion from tacrolimus twice daily (TAC-BID) to extended-release tacrolimus once daily (TAC-OD) in stable renal transplant recipients (RTRs) had any effect on insulin sensitivity, insulin resistance, the patients' preference and the expense.
Material and Methods: Twenty-eight RTRs were recruited and conversed from TAC-BID to TAC-OD in the same dose. The clinical and laboratory parameters especially fasting plasma glucose and insulin were monitored at the baseline and after the conversion up to 16 weeks. Insulin sensitivity and resistance were determined by HOMA-β and HOMA-IR, respectively. The patients' satisfaction was evaluated by using visual analog scale ratings. The expense of medication was compared before and after the conversion. For continuous variables, results are presented as mean + SD or median (IQR; percentile 25, percentile 75).
Results: Conversion decreased TAC trough levels at 16 weeks (6.43±2.27 vs 5.23±1.3 ng/mL, p=0.01) and required the increased dosage to maintain the TAC trough level of 4-7 ng/mL (3.63±1.93 vs 3.86±1.87 mg/day, p=0.085). No significant change in the HOMA-β and HOMA-IR was found in all 28 RTRs. However, insulin sensitivity by HOMA-β increased from 60 (37.33, 109.71) to 78.5 (44.3, 108.4) (p=0.02) in 15 patients who had the conversion within 4 years after renal transplantation. By using the visual analog scale ratings, the RTRs reported that the conversion was more satisfactory and the cost of treatment was comparable. No patient developed acute rejection or any significant adverse effects during the study.
Conclusion: In the short-term follow up, conversion from TAC-BID to TAC-OD is safe in stable RTRs and might be beneficial in term of improved insulin sensitivity in the early years after renal transplantation. The conversion caused comparable cost and was preferable by RTRs.
Trial registration: The study was registered in the clinical trial as Thai Clinical Trials Registry Trial Number TCTR20151005001.
Phramongkutklao Hospital and College of Medicine

623.1

Racial differences in incident de novo anti-HLA alloantibodies among primary renal transplant recipients

Matthew Everly[1], Lorita M.Rebellato[2], Kimberly P.Briley[2], Anh Nguyen[1], Paul Bolin[2], Scott A.Kendrick[3], Claire Morgan[2], Angela Q.Maldonado[4], Carl E.Haisch[2].
[1]Terasaki Foundation Laboratory, Los Angeles, CA, United States; [2]East Carolina University, Greenville, NC, United States; [3]Eastern Nephrology Associates, Greenville, NC, United States; [4]Vidant Medical Center, Greenville, NC, United States.

African-American/Black-American (AA) renal transplant recipients have been reported to have a higher rate of acute rejection and higher rates of allograft loss. One plausible reason for this is that this cohort develops a de novo DSA (dnDSA) at a rate higher than non-AA patients. In our study, we aimed to assess whether transplant recipients' of African American race had similar dnDSA incidence rates, dnDSA risk factors, and dnDSA related outcomes and non-AA patients.
Methods: We performed a single center retrospective analysis of 158 HLA mismatched patients receiving a primary transplant between 1/06 to 12/10. All patients underwent frequent HLA IgG antibody monitoring by single antigen beads pre-transplant, post-transplant at 1,3,6,9,12 months, and annually, thereafter. All patients were DSA negative at time of transplantation.
Results: 106/158 transplant patients were AA. Of the 106 AA patients 49% developed dnDSA by 5 years compared to 27% in the non-AA group (p=0.0072, Fig A). Among AA patients, donor type did not differentiate risk for dnDSA. Of all other potential risk factors in AA patients, HLA-DQ mismatch (HR 3.3, 95%CI 1.2-9.3), non-adherence (HR 2.6, 95%CI 1.4-4.8), and BK viremia (pre-dnDSA, HR 2.3, 95%CI 1.2-4.4) were the most common clinical and demographic dnDSA antecedents. In AA patients, choice of tacrolimus (as calcineurin inhibitor), thymoglobulin induction, pre-dnDSA acute cellular rejection were found to lead to a slightly lower risk of dnDSA formation in AA patients, but not statistically significant. In the non-AA group, we were unable to establish a clear set of factors to serve as possible antecedents. Even though more AA patients developed dnDSA and subsequently experienced graft failure, the rate of post-dnDSA graft failure did not differ between AA and non-AA patients (Fig B). The actual 3 year post-dnDSA allograft failure was 30% for both groups.



Conclusion: This analysis showed that the risk of de novo DSA is higher in AA than non-AA patients. As a result, AA primary transplant recipients are at a higher risk of failure, especially if they are DQ mismatched or are at risk for BK viremia or nonadherence.

623.2

IgG dilutions, subclasses, C1q, and IgM: What are the important characteristics of de novo DSA?

Matthew Everly[1], Lorita M.Rebellato[2], Carl E.Haisch[2], Kimberly P.Briley[2], Paul Bolin[2], Scott A.Kendrick[3], Claire Morgan[2], Angela Q.Maldonado[4], Anh Nguyen[1].
[1]Terasaki Foundation Laboratory, Los Angeles, CA, United States; [2]East Carolina University, Greenville, NC, United States; [3]Eastern Nephrology Associates, Greenville, NC, United States; [4]Vidant Medical Center, Greenville, NC, United States.

Secondary antibody testing of IgG (subclasses, C1q, etc) in de novo DSA (dnDSA) positive patients may have clinical relevance. Herein, we performed longitudinal testing of a multitude of secondary tests (IgG3, IgG4, C1q, IgG dilutions, IgM) in 41 consecutive dnDSA positive patients to see which of these test discern clinical risk and when is the most valuable time to test with these.
Methods: All patients received their first transplant between 2006 to 2010, had 3 years of post-dnDSA follow-up, and were pre-txp IgG DSA(-). All patients were tested at the following time points: at the time of DSA onset, at 6 months post dnDSA, and once between 12-24 months post-dnDSA. All samples were tested for IgG at a 1:3 dilution, IgG subclasses (IgG3 and IgG4), and IgG C1q. Samples at dnDSA onset were also tested at IgG at a 1:10 dilution and for IgM.
Results: At the time of dnDSA onset, IgG subclass and C1q testing did not distinguish those at increased risk of graft loss (Fig). A dilution of 1:10 at dnDSA onset was correlated with risk of eventual graft loss showing a increase risk of 4.6 times (over DSA IgG 1:10 negative patients). Looking into testing beyond dnDSA onset, patients having C1q+ dnDSA at 6 months and those with IgG3 DSA at 12 months post-dnDSA were at the highest risk of graft loss.
The overall trends in the progression of positivity in these patients are the following:
1. At the time of dnDSA onset, patients are primarily IgG+/IgG3-/IgG4-/C1q- (59%) and secondarily IgG4+ (IgG3-/C1q- , 22% of cases).
2. Withing 6 months from dnDSA onset there is a shift toward IgG3 and C1q positivity (37% of cases).
3. For those converting to IgG3+ or C1q+ (n=15), 40% were IgG3+/C1q+, 47% are IgG3+/C1q-, and 13% IgG3-/C1q+.
4. 67% of patients developing IgG3+ and C1q+ dnDSA by 6 months post-dnDSA eventually experience allograft failure.

Risk associated with each secondary DSA testing at multiple time point testing

Testing **at time of dnDSA onset (n=41 patients)**		
Test	**Number of patients positive At selected time point (%)**	**Graft Loss, n=11 (OR, 95% CI)**
IgG 1:10 dilution+	19 (46%)	4.6 (1.01-21.0)
IgG3+	6 (15%)	3.4 (0.8-20.1)
IgG4+	12 (29%)	0.9 (0.2-4.1)
C1q+	4 (10%)	3.1 (0.4-25.3)
IgM+	20 (50%)	2.2 (0.5-9.0)
Testing **at 6 months post-dnDSA onset**		
Test	**Number of patients positive At selected time point (%)**	**Graft Loss, n=11 (OR, 95% CI)**
IgG3+	13 (38%)	3.6 (0.8-17.0)
IgG4+	11 (32%)	3.0 (0.6-14.1)
C1q+	8 (24%)	16.5 (2.4-112.8)
Testing **at 12-24 months post-dnDSA onset**		
Test	**Number of patients positive At selected time point (%)**	**Graft Loss, n=11 (OR, 95% CI)**
IgG3+	10 (27%)	17.9 (2.9-109.3)
IgG4+	12 (33%)	1.5 (0.3-6.8)
C1q+	10 (27%)	8.3 (1.6-43.1)

Conclusions: A dilution of 1:10 may be useful in initial DSA testing to reduce the number of positive less influential dnDSA detected. Using IgG3 and C1q testing can greatly improve the prediction of graft loss in dnDSA positive patients. These tests will likely need to be tested over time.

623.3

Microarray assessment of kidney transplant indication biopsies in real time: First results of the INTERCOMEX Study

Philip Halloran[1,2], Jeff Reeve[2], Katelynn Madill-Thomsen[2].
[1]Medicine, University of Alberta, Edmonton, AB, Canada; [2]Alberta Transplant Applied Genomics Centre, Edmonton, AB, Canada.

Central molecular tissue assessment is used in oncology to guide management but its potential in transplantation is unknown. A prospective trial of real time central molecular tissue assessment was performed using 526 indication kidney biopsies collected in European and North American centers between 2014 and 2015 (Clinicaltrials.gov#NCT01299168). Biopsies from 0-27 years post-transplant were collected and stabilized in RNAlater, shipped at ambient temperature to a central laboratory, and analyzed on Affymetrix microarrays (processing time 29 hours). An automated report (independent of histology or DSA results) was generated. The molecular microscope (MMDx) system of algorithms identified T cell-mediated rejection (TCMR), antibody-mediated rejection (ABMR), all rejection, acute kidney injury (AKI), and irreversible injury (atrophy-fibrosis). 501 biopsies had complete data for analysis by January 2016. Results were compared to histology and clinical assessment, and we sought feedback from participating clinicians. Histological rejection diagnoses were ABMR (18%), TCMR (6%) and mixed (1%). As in previous studies, TCMR was extremely rare after 10 years post-transplant, but ABMR was common. A non-adherence/under-immunosuppression classifier was positive in 22 biopsies (4%), typically between 1-5 years in biopsies with molecular rejection, mostly TCMR/mixed. The molecular phenotype agreed with the conventional phenotype of TCMR (accuracy 90%, sensitivity 63%, specificity 92%) and ABMR (accuracy 78%, sensitivity 64%, specificity 83%), but with some notable disagreements.

Histologic and molecular classification of INTERCOMEX biopsies (n=501)

Histology Diagnosis	Number of biopsies	Median molecular (MMDx) Score				
		ABMR	TCMR	Rejection	AKI	Fibrosis
ABMR	89 (18%)	***0.54***	0.02	***0.54***	0.72	0.37
ABMR suspected	16 (3%)	***0.15***	0.008	***0.17***	0.75	0.56
Acute kidney injury	52 (10%)	0.04	0.009	0.07	***0.78***	0.10
BK nephropathy	8 (1.5%)	0.17	0.16	0.43	1.58	0.66
Borderline	25 (5%)	0.07	0.009	0.06	0.23	0.37
Diabetic Nephropathy	6 (1.2%)	0.28	0.006	0.11	0.90	0.88
Glomerulonephritis	18 (3.5%)	0.15	0.01	0.17	0.47	0.52
"IFTA"	69 (14%)	0.09	0.008	0.07	0.60	***0.39***
Mixed	4 (1%)	0.34	0.29	0.89	0.54	0.31
Relatively normal	172 (34%)	0.08	0.006	0.06	0.13	0.18
Other	5 (1%)	0.04	0.002	0.10	0.48	0.22
TCMR	31 (6%)	0.13	***0.19***	***0.51***	0.88	0.43
Transplant glomerulopathy	6 (1.2%)	0.17	0.01	0.24	0.66	0.69

This comparison between local conventional and central MMDx testing uncovered unexpected variation in histology/molecular concordance from center to center, despite all nominally using the Banff Classification system. There were also differences between histological and clinical interpretations within centers. Although histology at each center operated under consensus guidelines, inconsistency in guideline interpretation between pathologists resulted in diagnoses deviations and visible trends between centers.

Average Molecular scores in biopsies diagnosed as either ABMR/Mixed/TG or TCMR/Mixed				Accuracy	
Site ID	Total Number of biopsies from site	ABMR	TCMR	ABMR	TCMR
1	26	0.72	No TCMRs	0.89	0.92
2	30	0.20	0.87	0.8	0.97
3	46	0.56	0.71	0.74	0.86
4	47	0.27	0.20	0.77	0.87
5	47	0.60	0.32	0.68	0.89
6	96	0.60	0.22	0.72	0.92
7	85	0.39	0.03	0.75	0.94
8	17	0.58	No TCMRs	0.77	0.65
9	80	0.39	0.43	0.61	0.83
10	27	0.32	0.99	0.78	1
Average	-	**0.46**	**0.47**	**0.75**	**0.88**

In feedback forms to date, clinicians indicated that 128/149 MMDX reports (86%) agreed with clinical judgment regardless of histological interpretation; 58/69 MMDx reports (84%) added confidence for management decisions; and 26/79 MMDx reports (33%) would alter therapy and/or investigations. In conclusion, real-time molecular tissue assessment offers a new dimension to precision in biopsy interpretation, and can provide a standard to guide more consistent conventional assessment among centers.

623.4

Early accelerated rejection – an emerging distinct entity in antibody incompatible renal transplantation which can be treated

Anna Adamusiak[1], Miriam Manook[1], Olivia Shaw[2], Robert Vaughan[2], Nicos Kessaris[1], Irmen Generali[1], Anthony Dorning[1], Nizam Mamode[1].
[1]Department of Transplantation, Guy's Hospital, London, United Kingdom; [2]Clinical Transplantation Laboratory, Guy's Hospital, London, United Kingdom.

Antibody mediated rejection (AMR) is a significant obstacle to successful renal transplantation. The most common mechanism of AMR is the binding of antibodies specific to antigens expressed on endothelial cells in the kidney followed by complement activation. Patients undergoing ABO or positive cross match HLA incompatible renal transplantation are at risk of AMR occurring early after transplantation (early accelerated rejection; EAR) which may result in graft loss. Eculizumab is a humanised monoclonal antibody that specifically targets and blocks action of complement and has been successfully used in early AMR.
The aim of our study was to assess the incidence and characteristics of EAR in antibody incompatible renal transplants (AIT).
EAR was defined as sudden deterioration in graft function within 2 weeks after transplant presenting with oligoanuria which often requires re-commencing on dialysis. Within the cohort of 169 AIT performed at our centre between 2009 and 2015 we identified 10 patients (6%) who experienced EAR. In some of these cases eculizumab was used as a salvage therapy. The summary of cases is given in the table.

Gender & age	AIT	Biopsy	ABO titre	DSA	Ecu	Graft survival	Patient survival
42F	ABOi DSA+	TMA	no rise	rise	yes	yes	yes
35F	ABOi	TMA	no rise	no rise	no	no	yes
49F	ABOi	TMA	no rise	no rise	yes	yes	yes
54F	HLAi	TMA	n/a	rise	yes	yes	yes
19M	ABOi	TMA	rise	no rise	yes	no	yes
18F	ABOi	TMA	no rise	no rise	yes	no	no
66F	HLAi	TMA	n/a	rise	yes	yes	yes
44F	HLAi	TMA	n/a	rise	yes	no	no
56F	HLAi	none	n/a	rise	yes	no	no
26F	HLAi	TMA	n/a	rise	yes	yes	yes

We compared patients who developed EAR (AIT EAR) with these who didn't (AIT non-EAR). There was no statistically significant difference in age between EAR and non-EAR group (41 vs. 45, $p = 0.52$) but the majority of patients in EAR group were female (90% vs. 47%, $p = 0.009$). For ABO AIT there was no statistically significant difference in baseline antibody titres but HLA AIT EAR patients had statistically significant higher baseline donor specific antibody (DSA) levels (46481 MFI vs. 17801 MFI, $p = 0.028$).
We postulate that EAR is a distinct entity, not yet well characterised which can rapidly progress to graft loss despite standard anti-rejection treatment. A short course of eculizumab is very effective in some cases but not in others. Female patients and patients with high levels of DSA are at higher risk of developing EAR but ABO antibody baseline levels are irrelevant.

623.5

Rapid removal of anti-HLA antibodies in immunized patients. Two dose finding studies of the IgG degrading enzyme IdeS

Tomas Lorant[1], Lena Winstedt[2], Mats Bengtsson[3], Torsten Eich[3], Torbjörn Lundgren[4], Britt-Marie Eriksson[5], Lars Bäckman[1], Lars Wennberg[4], Erik Larsson[6], Christian Kjellman[2], Gunnar Tufveson[1].
[1]Department of Surgical Sciences; Transplantation Surgery, Uppsala University, Uppsala, Sweden; [2]Hansa Medical AB, Lund, Sweden; [3]Department of Immunology, Genetics and Pathology; Clinical Immunology, Uppsala University, Uppsala, Sweden; [4]Department of Clinical Science, Intervention and Technology; Transplantation Surgery, Karolinska Institute, Stockholm, Sweden; [5]Department of Medical Sciences; Infectious Diseases, Uppsala University, Uppsala, Sweden; [6]Department of Immunology, Genetics and Pathology; Molecular and Morphological Pathology, Uppsala University, Uppsala, Sweden.

Background: Approximately one third of the patients waiting for kidney transplantation are sensitized to human leukocyte antigen (HLA) which may deny them from transplantation due to the risk of hyperacute rejection of the graft. The immunoglobulin G (IgG)-degrading enzyme of Streptococcus pyogenes (IdeS) has the characteristics of cleaving IgG.
Aim: 1) To evaluate the efficacy, safety, tolerability, and pharmacokinetics of intravenous IdeS after administration of ascending doses in sensitized patients with chronic kidney disease (CKD). 2) To transplant patients with anti HLA antibodies including donor specific antibodies (DSA) after IdeS treatment.
Materials: Two phase II single arm, ascending dose studies were conducted in Sweden. Patients with CKD and with antibodies against at least two HLA were included. At least one antibody should have a mean fluorescence intensity (MFI) of >3000 measured by a single antigen bead assay. The patients were given 0.12 mg/kg BW (n=3) or 0.25 mg/kg BW (n=4) of IdeS. Based on MFI data the protocol allowed a second dose within 48 hours. All subjects at 0.12 mg/kg and two patients at 0.25 mg/kg received a second dose. The primary objective was to find an IdeS dosing scheme, which in the majority of the patients would result in HLA antibody levels acceptable for transplantation, measured as an MFI of <1100 within 24 hours from dosing. The follow up period was 64 days.
In the second study so far 6 patients have been transplanted after desensitisation with a single dose of 0.25 or 0.5 mg/kg of IdeS. The longest follow up time is 8 months and the shortest 2 months.
Results: The MFI of HLA antibodies were significantly reduced in all patients treated with IdeS. At 0.25 mg/kg three out of four patients reached the primary endpoint. C1q-binding antibodies were almost eliminated within one hour. One patient with DSA in the first study was successfully transplanted after having received 0.12 + 0.12 mg/kg BW of IdeS. Positive cytotoxic and flow cytometry crossmatches against the donors were converted to negative by IdeS treatment. In the second study all kidney transplants (n=6) are functioning with no signs of antibody-mediated rejection (all creatinine values < 150 mol/L).
Conclusions: Treatment with IdeS significantly reduced the level of HLA antibodies and eliminated C1-binding antibodies. The treatment allowed transplantation in all patients attempted (n=7) so far and none has experienced rejection 2 to 18 months post transplantation.

623.6

Ultrastructural changes add valuable diagnostic and prognostic information in evaluation of antibody-mediated rejection (AMR) in renal allograft biopsies

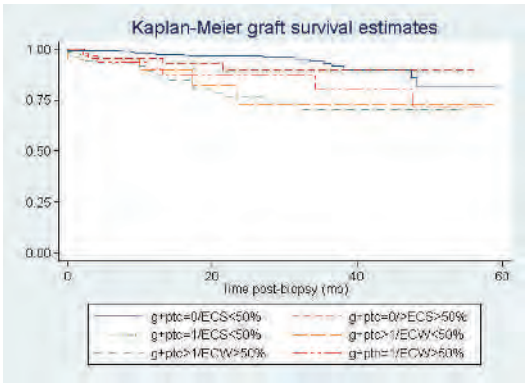
A. Haririan[1], C. J. B.Drachenberg[2], M. Chaudhry[2], R. Ugarte[1], H. Madariaga[1], B. Thomas[1], J.C. Papadimitriou[2].
[1]Medicine, University of Maryland, Baltimore, MD, United States; [2]Pathology, University of Maryland, Baltimore, MD, United States.

The role of electron microscopy (EM) in diagnosis of AMR is not well-defined. We sought to examine the EM findings in 796 biopsies from 622 adult recipients performed between July 2010 and Oct 2014. Median time to biopsy was 12.6 mo from transplantation [IQR:4.2-32.5]. The light microscopic (LM) and EM biopsy findings and DSA daa are summerized in Figure 1.

EM findings	None	10-25%	26-50%	>50%
GECS (%)*	38.7	24.2	8.1	29.1
Podocyte effacement (%)	41.2	35.6	10.3	12.9
	0	1-3	4-5	>5
PTCBMML (%)	57.6	23.9	10.4	8.1
	None		Present	
SEE/GBMR (%)	75.5		24.5	
ED deposits (%)	94.2		5.8	

Banff score	0	1	2	3
g (%)	73	15.1	8.3	3.7
Cg (%)	83.0	10.2	4.4	2.4
Ptc (%)	76.1	15.3	7.7	0.9
C4d (%)	60.9	18.6	15.6	4.9
	None	Class 1	Class 2	Class 1/2
DSA (%)	51.4	8.3	28.3	12.1

Extent of glomerular endothelial cell-swelling (GECS) was associated with DSA class (OR: 2.7, P<0.001 [CI:1.7-4.5], 3.5, P<0.001 [CI:2.5-4.7], 6.3, P<0.001 [CI:4.1-9.7] for class I, II, I&II, respectively, g score (OR: 6.0, P<0.001 [CI;4.1-8.9], 19.1, P<0.001 [10.3-35.4], and 31.5, P<0.001 [10.7-92.2], respectively for g1-3) and cg score (OR: 8.7, P<0.001 [CI:5.2-14.4], 14.7, P<0.001 [6.4-34.2], 10.1, P<0.001 [3.6-28.3], respectively for cg1-3). So was subebdothelial_expansion/GBM reduplication (SEE/GBMR) OR: 3.1, P<0.001 [CI:1.7-5.7], 3.5, P<0.001 [2.3-5.2], 5.6, P<0.001 [3.5-9.3], for DSA class I, II, I&II, respectively, OR: 6.4, P<0.001 [CI:4.1-9.9], 10.5, P<0.001 [6.1-18.2], 21.5, P<0.001 [CI:8.9-52.1], for g1-3, respectively, and OR: 15.0, P<0.001 [CI:8.8-25.7], 16.2, P<0.001 [7.4-35.6], 6.2, P<0.001 [2.5-15.7], for cg1-3, respectively.
EM parameters in the first biopsy that were associated with graft survival included GECS≥50% (HR:3.1, P=0.001, CI:1.6-5.8) and podocyte effacement≥50% (HR:2.7, P=0.003, CI:1.4-5.3), SEE/GBMR (HR:2.4, P=0.001; CI:1.4-4.1), and PTC BM multilayers (ML)>3 (HR:3.3, P<0.001, CI:1.9-5.6). PTCBMML>3 was independently predictive of graft failure (HR:2.2, P=0.02; CI:1.1-4.2), and extensive GECS had borderline association (HR:1.8, P=0.07, CI:0.95-3.4); both were strongly associated with the number of DSA specificities (OR:3.5, P<0.001, CI:2.5-4.9; HR:4.4, P<0.001, CI:3.3-6, respectively). With g+ptc score 1, presence of GECS≥50% was associated with worse graft survival (HR: 3.0, P=0.02 [1.2-7.6].



We observed that 11.1% of the biopsies without DSA and 14.9% with DSA had no LM features of AMR but had extensive GECS in EM; SEE/GBMR was observed in 7.6% and 8.4% of these biopsies, respectively.

The results of this study show that EM features are strongly associated with DSA and LM features of AMR and are strong predictors of graft outcome. With g+ptc score 1, extensive GECS is predictive of worse outcome similar to g+ptc score ≥ 2. In the absence or incomplete presence of LM features of AMR, EM could help in recognizing antibody-mediated graft injury, including in those cases with no identifiable HLA DSA.

623.7

Effect of complement inhibition therapy (eculizumab) in patients with C1q-binding donor-specific anti-HLA antibodies: A molecular appraisal

Carmen Lefaucheur[1], Luis Hidalgo[2], Jeff Reeve[2], Denis Viglietti[1], Clément Gosset[1], Olivier Aubert[3], Camilo Ulloa[3], Christophe Legendre[3], Denis Glotz[1], Philip Halloran[2], Alexandre Loupy[1]. [1]Saint-Louis Hospital, Paris, France; [2]University of Alberta, Edmonton, AB, Canada; [3]Necker Hospital, Paris, France.

Complement-binding anti-HLA DSA have demonstrated higher rejection rate and decreased allograft outcome. We investigated the effect of terminal complement inhibition (Eculizumab) in a cohort of patients with C1q-binding DSA at the time of transplantation.

We enrolled 2 groups of patients, all with C1q-binding DSA at the time of transplantation between 2011 and 2013: i) 12 patients (study group) received Eculizumab in the prevention of AMR according to a phase 2 clinical trial (NCT01567085); ii) A matched control group of 12 patients receiving standard of care (PP x4 and IVIG 2g/kg BW). Surveillance kidney allograft biopsies were performed at 14 days and 12 months post-transplant in all patients. In both groups the allograft injury phenotype was assessed by histopathology and gene expression. DSA characteristics, GFR and proteinuria were also determined at those time points.

Baseline characteristics were similar in both groups in term of donor, recipient, transplant and DSA characteristics. At Day-14, patients receiving Eculizumab had lower histological Banff scores for peritubular capillaritis (p=0.0074), interstitial inflammation (p=0.0133) and tubulitis (p=0.0055). Molecular allograft gene expression revealed lower AMR activity reflected by decreased endothelial DSA-selective transcripts (DSAST, 3.97 fold-change, p<0.001) and ABMR Molecular Score (1.81 fold-change p=0.0061). This lower activity was related to lower expression of NK transcripts (NKb, 5.25 fold-change, p<0.0001), macrophage transcripts (QCMAT, 1.67 fold-change, p=0.04), IFNG production and inducing transcripts (GRIT, 2.65 fold-change, p<0.001) and acute kidney injury transcripts (IRRATS, 1.53 fold-change, p=0.01) as compared with patients receiving standard of care. At 1-year (after 9 months of treatment wash out), the histologic scores (g, ptc, cg, C4d, i, t, v, IFTA and cv) and molecular scores for ABMR, GRIT, QCMAT and IRRATS were similar in both groups with the exception of NKb (1.8 fold-change, p=0.02) and DSAST (2 fold-change, p=0.01). At 1 year, eGFR and DSAmax MFI were similar in both groups while there was a trend lo lower proteinuria among the Eculizumab treated group (p=0.0562).

Eculizumab prophylaxis in patients with C1q-binding DSA reduces early AMR activity with major effect on NK burden with limited effect after treatment discontinuation.

623.8

C1-inhibitor in acute antibody-mediated rejection non-responsive to conventional therapy in kidney transplant recipients: A pilot study

Carmen Lefaucheur[1], Denis Viglietti[1], Clément Gosset[1], Alexandre Loupy[2], Adriana Zeevi[3], Denis Glotz[1].
[1]Nephrology and Kidney Transplantation, Saint-Louis Hospital, Paris, France; [2]Kidney Transplantation, Necker Hospital, Paris, France; [3]University of Pittsburgh Medical Center, Pittsburgh, PA, United States.

The use of complement inhibitors in the treatment of acute antibody-mediated rejection (AMR) has not been thoroughly evaluated. We performed a prospective, single-arm, pilot study to assess the efficacy and safety of C1-inhibitor (CI-INH) Berinert added to high-dose intravenous immunoglobulin (IVIG) for the treatment of acute AMR that is non-responsive to conventional therapy.
Kidney recipients with acute AMR that was non-responsive to conventional standardized treatment (plasmaphereses [x4], high-dose IVIG [2 g/kg] repeated every 3 weeks for 3 rounds and rituximab [375 mg per square meter of body-surface area]) were prospectively enrolled between April 1, 2013, and July 1, 2014 (N=6). They received C1-INH (20 U/kg twice weekly) and high-dose IVIG (2 g/kg bw every 3 weeks) for 6 months. C1-INH patients were compared with a historical control group treated with high-dose IVIG alone (N=21). The primary endpoint was allograft function (eGFR) changes between the groups at 6 months after inclusion. Secondary end points included allograft histology, donor-specific anti-HLA antibody (DSA) characteristics and adverse events in the C1-INH group, as evaluated at 6 months after inclusion.
The C1-INH group showed a significant improvement in eGFR compared with the control group:
+16.6±9.9% vs. -13.0±33.1% (p=0.01). The mean eGFR at the end of the study was of 45.2±21.3 mL/min/1.73 m2 in the C1-INH group vs. 31.7±14.6 in the control group (p=0.08). All patients in the C1-INH group showed an improvement in eGFR between inclusion and study end from 38.7±17.9 mL/min/1.73 m2 to 45.2±21.3 mL/min/1.73 m2 (p=0.03). There was no change in the histological features in patients in the C1-INH group between the biopsies at inclusion and those obtained at the end of the study, except for a significant decrease in the C4d deposition rate (83% vs 17%, respectively, p=0.04). There was a significant change in anti-HLA DSA C1q-binding status from 6/6 (100%) positive at enrolment to 1/6 (17%) positive at the end of the study (p=0.02). One deep venous thrombosis of a lower limb occurred during follow-up.
C1-inhibitor added to high-dose IVIG may improve allograft function in kidney recipients with non-responsive acute AMR.

623.9

Composite score to define the risk of allograft loss after standard-of-care treatment of antibody-mediated rejection in kidney transplantation

Denis Viglietti[1], Alexandre Loupy[2], Jean-Paul Duong Van Huyen[3], Jérôme Verine[4], Adriana Zeevi[5], Christophe Legendre[2], Denis Glotz[1], Carmen Lefaucheur[1].
[1]Nephrology and Kidney Transplantation, Saint-Louis Hospital, Paris, France; [2]Kidney Transplantation, Necker Hospital, Paris, France; [3]Pathology, Necker Hospital, Paris, France; [4]Pathology, Saint-Louis Hospital, Paris, France; [5]University of Pittsburgh Medical Center, Pittsburgh, PA, United States.

The development of a reliable surrogate endpoint for allograft survival after AMR treatment is a current unmet need in clinical transplantation (FDA AMR Workshop). We investigated the clinical, histological and immunological determinants of allograft survival in patients with active AMR receiving standard-of-care treatment in a prospective observational study.
We prospectively enrolled consecutive kidney transplant recipients with biopsy-proven active AMR diagnosed between 2007 and 2013 in two Paris transplant centers. All study patients received standardized treatment including plasmaphereses (x4), high-dose intravenous immune globulins (2 g/kg) repeated every 3 weeks for 3 rounds and rituximab (375 mg per square meter of body-surface area). Patients were systematically assessed at the time of diagnosis and 3 months post-treatment for clinical data (eGFR and proteinuria), histological characteristics (allograft biopsy) and circulating anti-HLA DSA characteristics (specificity, HLA class, mean fluorescence intensity [MFI] and C1q-binding capacity).
We included 291 patients with biopsy-proven acute or chronic active AMR who received standard-of-care treatment. The 5-year allograft survival after AMR diagnosis was 69.5% (95% CI: 62.7-75.2). Post-treatment independent determinants of allograft loss included eGFR (HR=0.97, 95% CI: 0.96-0.98, p<0.001), microvascular inflammation (g+ptc) score (HR=1.2, 95% CI: 1.0-1.3, p=0.035), allograft glomerulopathy (cg) score (HR=1.4, 95% CI: 1.1-1.8, p=0.004) and complement-binding capacity of DSA (HR=5.2, 95% CI: 3.3-8.3, p<0.001). On the basis of these predictors, we built a composite risk score for allograft loss after AMR treatment that showed a good predictive capacity: c-statistic, 0.77 (1000 bootstrap 95% CI: 0.71-0.84). For centers that do not use C1q-binding assay, we replaced C1q-binding assessment by the MFI of the highest rank anti-HLA DSA. The predictive capacity of the MFI-based score was 0.74 (95% CI: 0.68-0.81).
A systematic clinical, histological and immunological evaluation of the response to treatment at 3-month post-AMR allows to identify patients at high risk of allograft loss. We defined a post-AMR treatment composite score with a good performance to predict allograft loss. Further studies should validate this score on different independent cohorts.

624.1

Refractory vascular rejection in a hand allograft in the presence of antibodies against angiotensin II (type 1) receptor

Karen M. Dwyer[1,2,3], Robert Carroll[8,9], Prue Hill[4], Samantha Bateman[1], Chris Baker[5], Robyn G. Langham[1,2], Wayne Morrison[6,7].
[1]Nephrology, St.Vincent's Hospital Melbourne, Fitzroy, Australia; [2]Medicine, University of Melbourne, Melbourne, Australia; [3]School of Medicine, Deakin University, Geelong, Australia; [4]Pathology, St.Vincent's Hospital Melbourne, Fitzroy, Australia; [5]Dermatology, St.Vincent's Hospital Melbourne, Fitzroy, Australia; [6]Plastic Surgery, St.Vincent's Hospital Melbourne, Fitzroy, Australia; [7]Microsurgery, Bernard O'Brien Institute, Fitzroy, Australia; [8]Nephrology, Royal Adelaide Hospital, Adelaide, Australia; [9]Medicine, University of Adelaide, Adelaide, Australia.

The identification of antibodies targeting the angiotensin II type 1 receptor (AT1R) as the cause of refractory vascular rejection in kidney transplant recipients without donor specific antibodies (DSA) has changed transplant monitoring and treatment practices. Understanding the pattern of rejection in hand transplantation is still evolving: cell mediated rejection is well described, however reports of antibody mediated rejection in the presence of DSA are only just emerging. Herein we report the first case of refractory vascular rejection in a hand allograft without DSAs but with antibodies against AT1R.
The recipient was 69 year old and 4.5 years post hand transplantation when he presented with a palmar rash. Immunosuppression consisted of tacrolimus (level 4 following a recent reduction in dose from 1.5mg to 1.0mg bd); myfortic 540mg bd and prednisolone 5mg d. The recipient reported recent work fixing a fence which involved repetitive opening and closing of his hand whilst using fencing pliers. He did not wear a glove.
The initial biopsy revealed dermal perivascular CD4+ T-cell infiltrate with endothelialitis and fibrin thrombi consistent with Grade II T-cell-mediated rejection. He was treated with pulse intravenous methylprednisolone and topical steroid. Persistence of the rash mandated a repeat biopsy which showed ongoing rejection with transmural lymphocytic vasculitis of skin arterioles. Two low level DSA (A24 MFI 1750, DP3 MFI 1299) and one potential DSA (DQ6 MFI 656) were detected. He was subsequently treated with a week long course of ATGAM. A further biopsy showed persistence of the vascular changes of T-cell mediated rejection.
A lack of response to this therapy led to screening for antibodies to AT1R on historical pre-transplant and serial post-transplant serum samples. Antibody titre pre-transplant was 14.7 (10-17 deemed at risk); post-transplant sequential titres were 8.2 (5 months post-transplant), 6.7 (2.5 years post-transplant), 4.1 (at the time of rejection). He was commenced on candesartan and dose increased to 32mg and underwent alternate daily plasma exchange followed by low dose IVIg for 2 weeks. Six weeks later the histological changes had resolved as had the rash.
In conclusion, we report the first case of refractory vascular rejection in a hand allograft potentially precipitated by mechanical trauma in the setting of subtherapeutic immunosuppression and antibodies to AT1R.

624.2

Ultra-high resolution non-contrast imaging for chronic rejection monitoring and procedural planning in reconstructive transplantation

Shailesh B. Raval[1], Tiejun Zhao[3], Darren M. Smith[2], Cynthia Britton[4], Narayanan Krishnamurhty[1], Tales Santini[1], Sossena Wood[1], Tamer S. Ibrahim[1], Vijay S. Gorantla[2].
[1]Bioengineering, University of Pittsburgh, Pittsburgh, PA, United States; [2]Plastic Surgery, University of Pittsburgh, Pittsburgh, PA, United States; [3]MR Research Collaboration, Siemens Medical System, Pittsburgh, PA, United States; [4]Radiology, University of Pittsburgh, Pittsburgh, PA, United States.

Introduction: Chronicrejection(CR) in both solid organ transplantation(SOT) and reconstructive transplantation(RT) is associated with progressive, insidious intimal hyperplasia(IH) resulting in occlusion (obliterative arteriopathy), ischemia and graft loss. It is an emerging threat in RT with 4 hand transplants and 1 face transplant lost. The incidence and severity of IH correlates with cellular and humoral parameters as well as the immunosuppression load. Early detection is key to prevent CR graft loss. Skin biopsies can detect acute rejection (AR) but miss CR changes. Sequential vascular mapping with CT angiography is fraught with radiation/contrast risks and intravascular imaging is invasive and cause graft ischemia. For the first time, we developed a non-invasive, reliable and reproducible, non-radiation, contrast-free, ultra-high resolution (UHR) 3D vascular MRI imaging strategy for preoperative (surgical planning) and perioperative (graft viability) and post-transplant (CR monitoring) applications in RT.
Methods: 3D high resolution (T1VIBE) and T2DESS images of craniofacial(CF) and upper extremity(UE) soft tissues was using customized head[1] and extremity coil on a UHR 7T-MRI (Siemens) in normal IRB consented volunteers to map size, anatomical location and signal intensity of macro/microvasculature and other soft tissues including nerve tractography.
Results: T1VIBE images revealed exquisite anatomical details (A-D) but also accurate delineation of soft tissues (muscles, nerves, ligaments, tendons, fat). Diffusion tensor and diffusion spectrum fiber tractography revealed UHR of nerve fascicular anatomy(G, H, I). Our non-contrast technique allowed UHR luminal and vessel wall segmentation of micro and macrovasculature in the CF (M, N, O: arteries in face) and UE tissues (S-W: UE arteries and venae comitantes). Volume rendering and post-processing allowed successful 3D reconstruction and segmenting micro/macrovasculature of CF and UE without skeletonization or dilation.

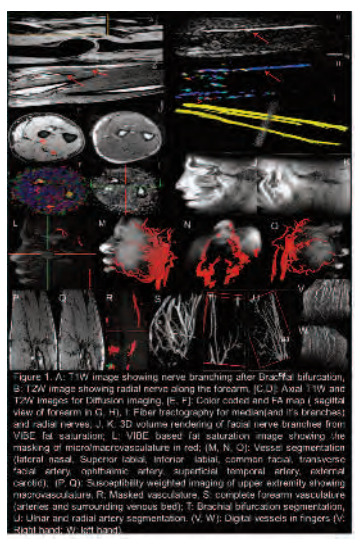


Figure 1. A: T1W image showing nerve branching after Brachial bifurcation, B: T2W image showing radial nerve along the forearm, (C,D): Axial T1W and T2W images for Diffusion imaging, (E, F): Color coded and FA map (sagittal view of forearm in G, H), I: Fiber tractography for median(and it's branches) and radial nerves, J, K: 3D volume rendering of facial nerve branches from VIBE fat saturation; L: VIBE based fat saturation image showing the masking of micro/macrovasculature in red; (M, N, O): Vessel segmentation (lateral nasal, Superior labial, inferior labial, common facial, transverse facial artery, opthalmic artery, superficial temporal artery, external carotid). (P, Q): Susceptibility weighted imaging of upper extremity showing macrovasculature, R: Masked vasculature, S: complete forearm vasculature (arteries and surrounding venous bed); T: Brachial bifurcation segmentation, U: Ulnar and radial artery segmentation. (V, W): Digital vessels in fingers (V: Right hand; W: left hand).

Conclusion: Current state of the art imaging in RT includes conventional imaging (3D CT,1.5/3T MRI,CT-angio, intravascular ultrasound,plain radiography) and stereolithography for surgical planning. The limitations of these imaging strategies are that they involve radiation, renal- toxic-contrast or are of sub-optimal resolution to map microvessels/other structures[2]. Our prior studies involved cutting-edge multimodality imaging in RT[3]. Our approach is renal-toxic-contrast and radiation-free, increasing its safety in RT(CForUE) or even SOT (especially renal transplant) applications for sequential non-invasive graft monitoring of CR. These could include intimal, luminal and flow parameters reflective of CR related vascular changes that may result in ischemic graft attrition or loss in RT or in SOT. Nervetractography can monitor neuroregeneration after transection, repair or transplant related nerve outcomes. In addition UHRimaging can be used for spatial orientation and precise localization of structures such as vessels, nerves, tendons, muscle, and bone for patient screening, selection, procedural planning and sequential monitoring of macro/microvascular parameters.

References:

[1] Zhao Y, Zhao T, Raval S, Krishnamurthy N, Zheng H, harris CT, Handler W, Chronik BA, Ibrahim TS. Dual optimization method of radiofrequency and quasistatic field simulations for reduction of eddy currents generated on 7T radiofrequency coil shielding. MRM 74(5), 1461–1469, Nov 2015

[2] Kumamaru K, Sisk G, Steigner M, Pomahac B, Rybicki F, Schultz K, Mitsouras D, Enterline D, Bueno E. Vascular Communications between Donor and Recipient Tissues One Year after Successful Full Face Transplantation. *RSNA* 2013 Chicago, IL.

[3] Smith D, Losee J, Gorantla V, Visual Data Integration for Imaging-based Planning in Human Craniofacial Transplantation. *RSNA* 2011 Chicago, IL

624.3

Chronic rejection in vascularized composite allotransplantation

Emmanuel Morelon[1], Palmina Petruzzo[1], Jean kanitakis[2], Aram Gazarian[5], Sylvie Testelin[3], Bernard Devauchelle[3], Olivier Thaunat[1], Fanny Buron[1], Valérie Dubois[4], Lionel Badet[1].
[1]Transplantation, Hospices Civils de Lyon, Lyon, France; [2]Dermatology, Hospices Civils de Lyon, Lyon, France; [3]Maxillofacial surgery, CHU Amiens, Amiens, France; [4]Laboratoire HLA, EFD Rhône Alpes, Lyon, France; [5]Hand surgery, Clinique du Parc, Lyon, France.

Background: Clinical and pathological features of chronic rejection (CR) in vascularized composite allotransplantation (VCA) are still poorly defined. Three cases of CR in VCA are here reported.
Methods: The first case is a man who received a face allograft in 2009 and developed during the follow-up a EBV+ B-cell lymphoma followed by hepatic EBV-associated post-transplant smooth muscle tumors, for which the immunosuppression (IS) was greatly reduced. His IS protocol included ATG, tacrolimus, MMF and steroids. He consequently developed several episodes of acute rejection (AR) treated by steroids. Donor specific HLA antibodies (DSA) remained always negative.
The second case is a woman who received a facial graft in 2005. Her IS treatment included ATG, tacrolimus, MMF and steroid. Since the first post-transplant year there was a switch from tacrolimus to sirolimus. She developed class II DSA in 2013, without concomitant acute skin rejection episode.
The third case is a man who received a bilateral upper extremity transplantation in 2003. His IS included ATG, tacrolimus, MMF, steroid. The functional recovery was excellent and only two episodes of AR occurred in the first year. He developed DSA in 2009 which then disappeared in 2013. The patient was not compliant to the IS in this period.
Results: In the first case since the second post-transplant year the allografted facial skin became progressively sclerotic and presented pigmented macules on a background of hypopigmentation and telangiectases, realizing a poikilodermatous aspect. Skin biopsies showed epidermal atrophy, basal cell vacuolization and diffuse dermal sclerosis, in the absence of large vessels involvement. In the second case nine months after DSA occurrence she developed several skin rejection episodes and the sentinel skin graft necrosis, due to graft vasculopathy. In the third case ten years after the transplantation the patient developed other two episodes of AR and then digital necrosis due to thrombosis of the digital arteries requiring phalange amputations.
Discussion: In the first case the CR process was characterized by skin graft sclerosis (only dermal capillaries showed thickened walls) which might be the results of a T-cell mediated anti-graft response, while in the other two cases it was dominated by graft vasculopathy.
Conclusions: CR leading to graft loss may develop also in VCA. The humoral arm of the immune response might contribute to the development of chronic allograft vasculopathy as observed in solid organ transplantation.

624.4

Dynamics and correlates of skin dendritic cells with distinctive immune response profiles in vascularized composite allografts

Wensheng Zhang, Chiaki Komatsu, Firuz Feturi, Maxine Miller, Jeffrey Walch, Alicia Mathers, Angus Thomson, Kia Washington, Vijay Gorantla, Mario Solari.
Department of Plastic Surgery, Thomas E Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA, United States.

Introduction: Vascularized composite allografts are composed of tissues that express varying degrees of immunogenicity, notably large amounts of highly antigenic skin. Multiple acute rejection episodes in the majority of human vascularized composite allotransplantation (VCA) routinely target skin while sparing other tissue types, such as muscle. Skin dendritic cells (DCs) are thought to play critical roles in the initiation and regulation of skin immunity. However, their contribution to the unique alloimmune response of VCA is largely unknown. This study seeks to characterize the spatiotemporal dynamics of distinct subsets of skin DCs at different stages of rejection after VCA and determine the relevance of skin DCs to the skewed T-cell alloresponse mounted against VCA.
Materials and Methods: Orthotopic hind-limb transplantation, vascular skin-muscle-bone transplantation, and full-thickness skin transplantation were performed from Brown Norway rats to MHC-mismatched Lewis rats. Without any immunosuppressive treatment, recipients were inspected daily for clinical signs of rejection on the transplanted limbs and flaps using a visual scoring system. Animals were sacrificed at different time points post-transplantation. Allograft skin, adjacent recipient skin, and draining lymph nodes were harvested and processed for flow cytometric analysis of phenotype of DC subsets. Total RNA or proteins were extracted from skin samples of different groups. T-cell associated gene expression was assessed by real-time PCR and a panel of 23 cytokines were measured by Luminex assay.
Results: 1) Skin resident lymphocytes and distinct subsets of skin migratory DCs: Langerhans cells, dermal DCs, and langerin+ dermal DCs, in the transplanted limb skin were identified and enumerated. 2) Migratory skin DC subsets in both limb allograft and recipient limb showed different patterns of change with increasing severity of rejection. 3) The expression of gene IFN-gama, Gzmb, IL-6, TGF-beta1, GATA3, and Foxp3 in skin of allografts and recipients exhibited dynamic changes and temporal correlation with the quantity of skin DCs during the process of rejection. 4) Principle Component Analysis on the cytokine concentrations in skin of different forms of VCA indicated a distinct cytokine network and the relevance with skin immune cells regulation.
Discussion: Dynamic expression of Th- and Treg- associated genes and pro-inflammatory cytokines in allograft and recipient skin reflected the T cell polarization. The correlative trends between skin DC subsets and T cell-mediated alloimmune response suggest a complex cutaneous immune cell network modulated by skin DCs in VCA.
Conclusions: Our study characterized the dynamics of skin DC subsets and evidenced their contribution to the unique skewing of T-cell response in VCA. This represents a promising step towards discovering targeted immunomodulation in VCA.
This work was supported by American Association of Plastic Surgeons Academic Scholarship
References:
[1] 1. Morelon E, Kanitakis J, Petruzzo P. Immunological issues in clinical composite tissue allotransplantation: where do we stand today? Transplantation. 2012; 93: 855-859.
[2] 2. Igyarto BZ, et al. Skin-resident murine dendritic cell subsets promote distinct and opposing antigen-specific T helper cell responses. Immunity. 2011; 35: 260-272.
[3] 3. Romani N, Brunner PM, Stingl G. Changing views of the role of Langerhans cells. J Invest Dermatol. 2012; 132: 872-881.

624.5

Novel approaches to assessment of facial nerve regeneration in a swine hemi-facial composite tissue flap model: A physiological and histology study

Anita Mohan[1], Marissa Suchyta[1], Saad Alsubaie[1], James Schmelzer[3], Karim Bakri[1], Basel Sharaf[1], Chunfeng Zhao[2], Eric Sorenson[3], Phillip Low[3], Peter Dyck[3], Samir Mardini[1].
[1]Plastic Surgery, Mayo Clinic, Rochester, MN, United States; [2]Orthopedic Surgery, Mayo Clinic, Rochester, MN, United States; [3]Neurology, Mayo Clinic, Rochester, MN, United States.

Purpose: The aim of this project was to create a large-animal model of facial nerve regeneration in the context of face transplantation. Current facial nerve regeneration studies concentrate on small animal models, which do not recapitulate the distance and time axons need to travel in human.
Methods: Six 10kg domestic pigs were used. Facial nerve anastomosis of the buccal, posterior auricular, and cervical branches of the facial nerve were performed in conjunction with a hemi-facial composite tissue flap. The marginal mandibular nerve was preserved as a control. Nerve function was measured quantitatively using both compound muscle action potentials and electroneurograms and subjectively for functional muscle recovery. At euthanasia four months post-surgery, facial nerves were collected, cross-sectioned, and stained with tolouidine blue. Utilizing a novel algorithm, the nerves' axonal and fiber density, myelination ratio, and diameter were calculated along the length of the regenerating nerve.
Results: The hemi-facial composite tissue flap surgery was successful in all animals. ENG and CMAP studies demonstrated results consistent with regeneration along the anastomosed nerves, including decreasing amplitude in neurophysiological readings distal to the anastomosis site. A novel algorithm analyzed histological facial nerve cross-sections, determining their fiber density, myelination, and diameter. Furthermore, an anatomical topography map of pig facial anatomy was generated.
Conclusion: This novel swine experimental model of facial nerve regeneration can be utilized to inform post-surgical care, including immunosuppression protocols to improve facial regeneration, in the context of face transplant. In addition, the model has research applications relevant to facial reanimation patients.

624.6

The international registry on hand and composite tissue allotransplantation (IRHCTT)

Palmina Petruzzo[1], Marco Lanzetta[2], Jean Michel Dubernard[1].
[1]Dept of Transplantation, Edouard Herriot Hospital, Lyon, France; [2]Hand Surgery Dept., Italian Institute of Hand Surgery, Monza, Italy; [3]Dept of Transplantation, Edouard Herriot Hospital, Lyon, France.

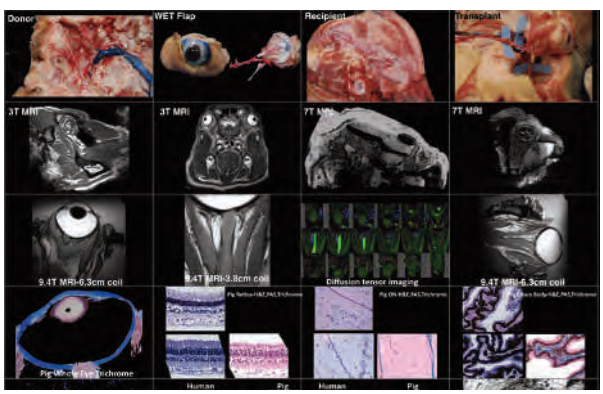
Background: The primary purpose of the IRHCTT is to collect information on voluntary basis, allowing to know what happens in this new field of transplantation. At present it includes upper extremity and face allotransplantations.
Methods: Since September 1998 25 unilateral and 30 bilateral transplants, for a total of 55 patients have been reported. In the majority of cases the level of amputation was distal, but there were also 9 arm transplantations. Since November 2005 28 cases of partial or total face allotransplantations have been reported. In the majority of cases the deficit included cheek, nose, chin, lips and perioral area. Among them there were also two cases of simultaneous face and bilateral hand allotransplantations, one case of bilateral hand allotransplantations following a kidney transplantation and one case of kidney transplantation after upper extremity allotransplantation. In both types of transplantation the immunosuppressive therapy included tacrolimus, mycophenolate mofetil, sirolimus and steroids; polyclonal or monoclonal antibodies were used for induction.
Results: Patient survival in upper extremity transplantation was 96.37%: 1 patient died after simultaneous face and bilateral hand transplantation and another one after bilateral arm transplantation; while in face transplantation it was 89.29% (three patients died including the case of simultaneous face and bilateral hand transplantation). Graft survival in upper extremity transplantation was 81.82%: in 5 cases graft loss occurred in the first period after transplantation (poor vascularization or infectious complications) and in other 5 patients during the follow-up (chronic rejection/graft vasculopathy). Graft survival in face transplantation was 96.43% (one face graft was removed for unknown cause).
Seventy-six percent of recipients experienced at least one episode of acute rejection within the first post-transplant year. Six cases of chronic rejection in upper extremity and one in face transplantation have been reported. Complications included, as in solid organ transplantation, opportunistic infections, metabolic complications and malignancies.
Hand-grafted patients developed protective sensibility, 90% of them tactile sensibility and 82.3% developed also a partial discriminative sensibility. There was also a partial recovery of the intrinsic muscles in the majority of the recipients. Sensitivity and motor recovery enabling patients to perform most daily activities. Face-grafted patients improved their aesthetic aspect and they were able to perform some activities such as eating, drinking and speaking which were impossible before the transplantation.
Conclusions: Upper extremity and face allotransplantations are successful procedures, however careful evaluation of patients before and after transplantation are indispensable.
All the teams participating to the IRHCTT

624.7

Translational considerations in whole eyeball transplantation - porcine model

Vasil Erbas[1], Huseyin Sahin[1], Sinan Oksuz[2], Liwei Dong[1], Maxine Miller[1], Golnar Shojaati[1], Matthias Waldner[1], Gadi Wollstein[3], Kevin Chan[6], Ian Sigal[3], Nataliya Kostereva[1], Shailesh Raval[7], Tamer Ibrahim[7], Jeffrey L. Goldberg[4], Larry Benowitz[5], Joel S.Schuman[3], Fatih Zor[2], Wensheng Zhang[1], Mario Solari[1], Kia M.Washington[1], Vijay S. Gorantla[1].
[1]Plastic Surgery, University of Pittsburgh, Pittsburgh, PA, United States; [2]Plastic Surgery, Gulhane Askeri Tip Akademisi, Ankara, Turkey; [3]Ophthalmology, University of Pittsburgh, Pittsburgh, PA, United States; [4]School of Medicine, Stanford University, Palo Alto, CA, United States; [5]Harvard Medical School, Harvard Medical School, Boston, MA, United States; [6]McGowan Institute of Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA, United States; [7]Bioengineering, University of Pittsburgh, Pittsburgh, PA, United States.

Purpose: Whole eyeball transplantation (WET) is the holy grail of vision restoration and is conceptually the most challenging of vascularized composite allografts (VCA). The swine eye is analogous to the human eye and is the ideal model for human WET. Our goal was to develop the protocols (surgical planning/procedures/post operative imaging /evaluations) as a foundation for a robust, large animal, preclinical WET model.
Methods: WET techniques were optimized in 17 fresh tissue swine dissections. An eyeball-periorbital VCA subunit with extra ocular muscles, and optic nerve (ON) was raised superolaterally and anastomosed to the recipient external ophthalmic artery (EOA) after exenteration. Methylene blue perfusion and microfil vascular mapping of central retinal artery (CRA)/vortex veins/ciliary plexus was done. Orbital contents and ON were imaged with DCE-DTI-MRI [T1/T2 MRI at 3T/7T/9.4 Tesla]. Advanced protocols for histopathology, immunohistochemistry, epoxy-embedding, corrosion casting, optical coherence tomography (OCT)/tonometry/fundoscopy/ERG were optimized and surgical techniques for ON crush, cut and coaptation established.
Results: Like humans, the swine retina is holangiotic and the ON has a lamina cribrosa. However, the CRA is absent with a large EOA. OCT/MRI allowed real-time, high definition, non-invasive, in situ, micron-scale, cross-sectional visualization of structure/topography of ocular structures.
Conclusion: Our study is the critical first step towards a swine WET model optimized for viability, retinal survival, ON regeneration and reintegration while documenting key immune responses, and enabling key neuro-immuno-therapeutic interventions.



624.8

Analysis of acute skin rejection in non-human primate models of face and hand allotransplantation

Zhi Yang Ng[1,2], Matthew W. Defazio[2], Harrison Powell[2], David A. Leonard[1,2], Zachary W. Heroux[2], Alexandre G. Lellouch[1,2], Curtis L. Cetrulo[1,2], Josef M. Kurtz[2,3].
[1]Plastic and Reconstructive Surgery, Massachusetts General Hospital, Boston, MA, United States; [2]Vascularized Composite Allotransplantation Laboratory, Center for Transplantation Sciences, Boston, MA, United States; [3]Biology, Emmanuel College, Boston, MA, United States.

Introduction: The incidence of acute rejection (AR) of the skin within the first year after hand or face transplantation is approximately 85%, with up to 56% of patients experiencing multiple episodes[1]. Significant immunosuppresive treatment is required to prevent allograft loss, and recent murine studies have shown that repeated AR episodes can lead to eventual VCA dysfunction and loss[2]. However, the mechanisms underlying variability in AR presentation remain poorly defined.
Materials and Methods: 6 cynomolgus monkeys received either an orthotopic hand (n=2) or heterotopic face VCA from MHC-mismatched donors. The induction and maintenance regimen included anti-thymocyte globulin with post-operative triple immunosuppression – tacrolimus, mycophenolate mofetil, methylprednisolone – for up to 120 days. Protocol biopsies of VCA and host skin were performed at 30-day intervals for flow cytometric analysis of resident skin leukocyte populations based on CD3, CD4, CD8, and CD207 staining; VCA-resident cells were differentiated by H38 status (mouse antihuman HLA class I monoclonal antibody that cross reacts with cynomolgus monkeys) for donor or recipient derivation. Clinical AR was treated by steroids with further biopsies taken for histopathology; corresponding anti-donor responses were evaluated by mixed lymphocyte reaction (MLR) and the presence of allo-antibodies.
Results: By day 30 post-VCA, more than 80% of skin-resident T lymphocytes (CD4+, CD8+) within VCA dermis were of recipient origin, demonstrating rapid immigration of various lineages into the VCA. In the epidermis, Langerhans cells were approximately 75% donor-origin during AR, and decreased to 17% after treatment. Of note, these observations coincided with the first episode of AR in fully mismatched recipients. However, no AR developed in haplomatched animals despite the same immunosuppressive regimen and comparable leukocyte numbers in VCA dermis and epidermis by flow cytometry. All but one episode of AR were successfully treated. No allo-antibodies were detected in and anti-donor responses by MLR were insignificant. Histological grading of AR was Banff I to II with corresponding higher ratios of CD8:CD4 T cells.
Discussion: Clinical, histological, and immunologic analyses in this study parallel findings that have been reported in human VCA cases. AR can usually be successfully treated with steroids and avoided with strict maintenance of target levels of immunosuppression, as per the clinical experience in VCA.
Conclusion: Here we show a clinically-appropriate model for studying AR in VCA. Our results suggest that further understanding of the relative importance of MHC differences in transplant pairs may lead to differences in outcomes for VCA recipients maintained under standard immunosuppressive regimens. In turn, the potential to avoid or negate AR may have implications on both long-term management and the likelihood of success in future tolerance induction strategies.

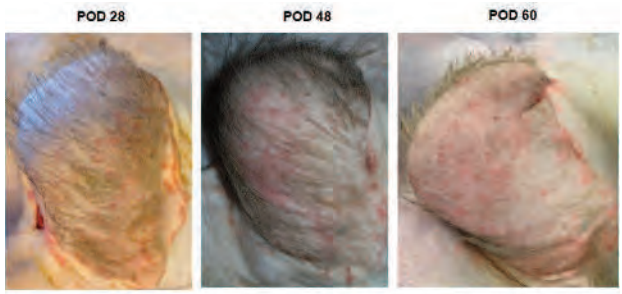


Figure 1. Maculopapular erythematous rash of varying color intensities demonstrate clinical acute rejection of the skin in a full MHC-mismatched recipient of a heterotopically transplanted facial allograft.

References:
[1] Petruzzo et al. The International Registry on Hand and Composite Tissue Transplantation. *Transplantation*. 2010;90:1590-1594.
[2] Unadkat et al. Functional outcomes following multiple acute rejections in experimental vascularized composite allotransplantation. *Plast Reconstr Surg*. 2013;131:720e-30e.

624.9

Intra-graft injection of tacrolimus may modulate local immune response promoting long-term acceptance of vascularized composite tissue allotransplantation.

Adriano Taddeo[1,2], Julie Denoyelle[1], Radu Olariu[1,2], Dzhuliya V.Dzhonova[1], Franck Leclère[1,2], Thusitha Gajanayake[1,2], Robert Rieben[1], Esther Vögelin[1,2].
[1]Department of Clinical Research, University of Bern, Bern, Switzerland; [2]Plastic, Reconstructive and Hand Surgery, Inselspital, Bern, Switzerland.

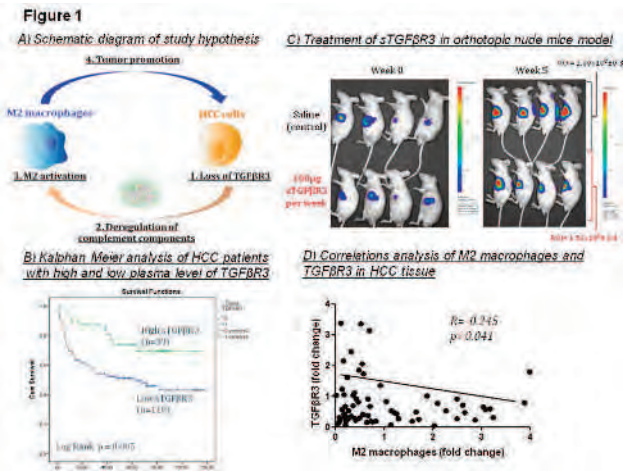
Background and Hypothesis: Side effects associated with systemic immunosuppression remain a pace-limiting obstacle to widespread adoption of vascularized composite allotransplantation (VCA). Unlike most solid organ transplantations, VCA offer unique possibilities to monitor rejection and deliver immunosuppressive drugs directly into the graft, in particular into the skin, the most immunogenic components in the VCA. We hypothesized that local delivered immunosuppressant may modulate the regional immune response and inflammatory milieu influencing the rejection process.
Methods and Results: In order to analyze the therapeutic and immunomodulatory efficacy of locally delivered immunosuppression we performed Brow Norway-to-Lewis rat hind-limb transplantations. The day after the operation the rats were treated with a single subcutaneous injection of 7 mg tacrolimus into the transplanted limb. The treatment significantly prolonged graft survival as compared to untreated animals (n=12 median survival 152.5 days; n=9 median survival 11 days, respectively). Interestingly, 50% of the rats survived for an average of 70.5 days and the other 50% remained rejection-free for more that 200 days without any further intervention. This dichotomy was associated neither with differential levels of systemic or intra-graft tacrolimus, nor with induction of tolerance (analyzed by mixed lymphocyte reaction, or secondary challenge with skin-graft). Notably, a better preservation of circulating T_{reg} after therapy and a long-lasting macro-chimerism were observed in the long-term surviving rats. Analysis of tissue-resident immune cells is currently on going, with the aim to unveil the specific effects of tacrolimus on the regional immune system.
Conclusions: Our data show that locally administered immunosuppression may induce an immunomodulatory milieu within the graft able to promote the long-term acceptance of VCA. This study suggests that the therapeutic modulation of the regional immunity may influence the outcome of the transplantation and underline that tissue-specific immunoresponse warrants further investigation in VCA

625.1

Dysregulation of transforming growth factor beta receptor III induces tumor promoting macrophages via complement C3a in Hepatocellular Carcinoma recurrence after liver transplantation

Wai Ho Oscar Yeung, Chun Mau Lo, Kevin Tak Pun Ng, Kwan Man . Department of Surgery, The University of Hong Kong, Hong Kong, Hong Kong.

Introduction: Hepatocellular Carcinoma (HCC) is one of the dominant malignancies worldwide and emerges to be the major cause for liver transplantation. Our group previously reported that a subpopulation of macrophages (M2) enhance HCC invasiveness through the secretion the chemokine CCL22 in response to tumorous cells[1]. Currently how malignant cells educate the particular immune population towards tumor promoting phenotypes is unknown. Recently, we found that TGFβR3 is down-regulated in HCC patients who expressed high level of M2 macrophages in our preliminary study. Here, we aimed to investigate the clinical significance of TGFβR3 in HCC and its roles on macrophage activation.



Materials and Methods: Clinical specimens were obtained from 150 HCC patients who underwent tumor resection and liver transplantation. The expression level of tissue and plasma soluble TGFβR3 (sTGFβR3) and complement components C3a/C5a were determined by q-PCR and ELISA respectively followed by the association analyses with clinical pathological parameters. To explore their therapeutic potential, sTGFβR3 were injected into orthotopic nude mice bearing HCC tumor. In vitro, HCC cells were forced over-expressed with TGFβR3 for functional studies and its roles on macrophage activation were also investigated.
Results and Discussion: Significant low expression level of TGFβR3 were found in both HCC tissue and plasma compared to healthy controls ($P<0.01$). Importantly, patients with low level of plasma TGFβR3 (<12ng/ml) developed late tumor stage, higher recurrence rate and decreased disease free survival period ($P<0.05$, Fig. 1B). Injection of sTGFβR3 into HCC tumor bearing mice reduced tumor size by 1.8 fold compared to control (Fig. 1C). By silencing TGFβR3 in hepatocyte cells, up-regulation of complement components C3a was observed. Treatment of these cell supernatants as well as C3a shifted the tumor promoting M2 macrophages to pro-inflammatory and tumoricidal M1 phenotypes. Further clinical analysis confirmed the close correlation among TGFβR3, C3a and M2 macrophages in HCC patients (TGFβR3 & M2 macrophages: $R=-0.245$, $P=0.041$, Fig. 1D) (TGFβR3 & C3a: $R=0.249$, $P<0.001$).
Conclusion: Here we reported that loss of TGFβR3 associated with poor survival outcome and disease progression in HCC patients. Its potential roles as prognostic and therapeutic target were also revealed.

A novel molecular mechanism for M2 macrophages activation by HCC cells via TGFβR3 and the complement component C3a signaling was presented.

References:

[1] Yeung WHO, Lo CM, Ling CC, Qi X, Geng W, Li CX, et al. Alternatively activated (M2) macrophages promote tumour growth and invasiveness in hepatocellular carcinoma. J Hepatol. 2015 Mar;62(3):607-16.

625.2

Up-regulation of GSTA2 at early-phase after liver transplantation increases the risk of late-phase hepatocellular carcinoma recurrence

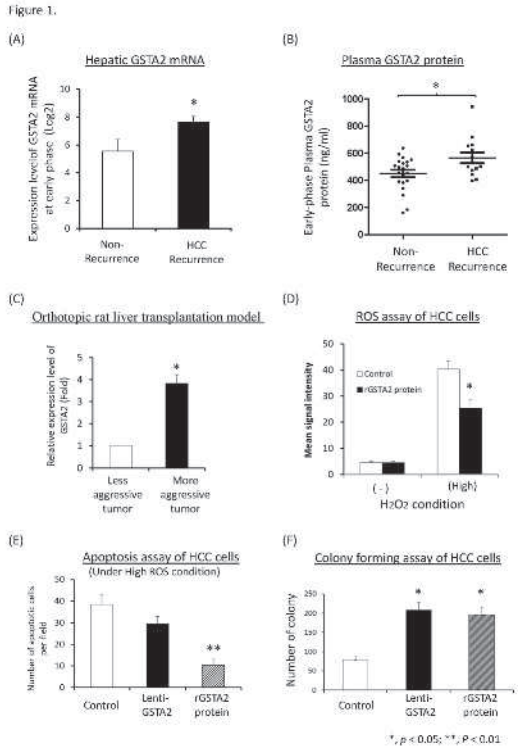
Kevin Tak-Pan Ng, Lynn Yin-Fan Lam, Chang Xian Li, Xiang Qi, Oscar Wai-Ho Yeung, Hui Liu, Jiang Liu, Chung Mau Lo, Kwan Man.
Department of Surgery, The University of Hong Kong, Hong Kong , People's Republic of China.

Introduction: Liver transplantation is the best surgical treatment for patients with early hepatocellular carcinoma (HCC), but post-liver transplantation HCC recurrence remains a major obstacle. Our previous studies have demonstrated that elevated hepatic injury at early-phase after liver transplantation promotes late-phase HCC progression and invasion[1]. We aimed to identify and characterize potential molecules responsible for early-phase hepatic injury and late-phase HCC recurrence after liver transplantation.

Materials and Methods: Liver tissues and bloods were collected from recipients at early-phase during liver transplantation (2-hour after reperfusion). RNA-sequencing analysis was performed to compare the transcription profile of early-phase liver tissues between the recipients with (N=6) and without (N=5) HCC recurrence after transplantation. The sequencing data were mapped to the human reference genes using high-throughput alignment software. Differential genes were identified by statistical analysis. Quantitative real-time RT-PCR was performed to validate the expression of glutathione S-transferase A2 (GSTA2) mRNA in 58 HCC recipients and in an orthotopic rat liver transplantation model. The plasma level of GSTA2 protein was analyzed by ELISA assay. The role of GSTA2 in HCC recurrence after liver transplantation was explored through a series of functional assays.

Results: By applying RNA-sequencing technology, we identified 7 up-regulated and 31 down-regulated genes in the recipients with HCC recurrence compared to the recipients without HCC recurrence. GSTA2 was the most up-regulated gene in patients with HCC recurrence. Validation study showed that upregulation of hepatic *GSTA2* mRNA at early-phase after liver transplantation was significantly associated with late-phase HCC recurrence of recipients (Figure 1A). The expression level of hepatic *GSTA2* mRNA at early-phase was significantly correlated with the expression of *TNFa* mRNA. Plasma level of GSTA2 protein was significantly increased at early-phase after transplantation and correlated with serum AST and ALT levels. Importantly, higher level of plasma GSTA2 was significantly associated with the occurrence of late-phase HCC recurrence (Figure 1B). In our orthotopic rat liver transplantation model, we found that the expression level of *GSTA2* mRNA was positively correlated with aggressive tumor phenotype after liver transplantation (Figure 1C). Functional study revealed that up-regulation of GSTA2 could confer advantage for HCC cells to compensate H_2O_2-induced high reactive oxygen species (ROS) stress (Figure 1D). Moreover, up-regulation of GSTA2 could protect HCC cells against high ROS-induced apoptosis and death (Figure 1E). In addition, up-regulation of GSTA2 could promote the proliferation and invasiveness of HCC cells (Figure 1F).

Conclusion: In conclusion, up-regulation of hepatic and plasma GSTA2 at early-phase after liver transplantation indicates not only an increased early-phase hepatic injury and but also a higher risk of late-phase HCC recurrence after liver transplantation. Targeting suppression of GSTA2 is possibly a novel strategy to reduce the likelihood of HCC recurrence after liver transplantation.



References:

[1] Man K, Lo CM, Xiao JW, Ng KT, Sun BS, Ng IO, Cheng Q, Sun CK and Fan ST. The significance of acute phase small-for-size graft injury on tumor growth and invasiveness after liver transplantation. Annals of Surgery 2008, 247:1049-1057.

625.3

Patients with 18F-FDG non-avid HCC on PET beyond Up-to-seven criteria have an excellent outcome following liver transplantation

Arno Kornberg[1], Ulrike Witt[1], Jennifer Kornberg[2], Helmut Friess[1], Katharina Thrum[3].

[1]Surgery, Technical University, Munich, Germany; [2]Anesthesiology, LMU, Munich, Germany; [3]Pathology, Helios Klinikum , Berliny, Germany.

Introduction: 18F-FDG positron emission tomography (PET) was shown predict outcome in liver transplant patients with hepatocellular carcinoma (HCC). The aim of this trial was to analyze the prognostic value of pre-LT 18F-FDG assessment in patients with HCC meeting and exceeding the (extended) Up-to-seven selection criteria.

Methods: A total of 107 liver transplant patients with HCC were included. In all of them pretransplant 18F-FDG PET was used for identifying patients with PET + (increased FDG-uptake) and PET – (no increased FDG-uptake) HCC. The impact of PET status and other established clinical (age of donor and recipient, AFP-level, number and size of tumour nodules, locoregional treatment) and histopathologic (microvascular invasion, lymphovascular invasion, tumor grading) variables on risk of HCC recurrence and outcome was analyzed in uni- and multivariate analysis.

Results: Based on final pretransplant radiographic staging, 77 patients had tumors meeting (UTS In) and 30 patients HCC exceeding (UTS Out) the Up-to-seven criteria (sum of max. nodule size and number HCC nodules not exceeding 7). Overall 5-year recurrence-free survival rates were significantly higher in UTS In patients (82.9%) compared to UTS Out recipients (56.4%; log rank = 0.011). Posttransplant tumor recurrence rate was 16.9% in the UTS In group, but 40% in the UTS Out subset (P = 0.011). In the subgroup of patients with UTS In tumors (n = 77), number of HCC nodules > 3 (HR = 28) and presence of microvascular tumor invasion (HR = 48.5) were the only independent predictors of HCC recurrence. In the subset of UTS Out recipients (n = 30), however, only a positive PET status was identified to independently predict post-LT HCC relapse (HR = 22; P = 0.008). In this special subgroup, 5-year recurrence-free survival rate was 92.3% in patients with 18F-FDG-non-avid HCC, but only 28.2% in those with 18F-FDG-avid HCC (log rank = 0.002).

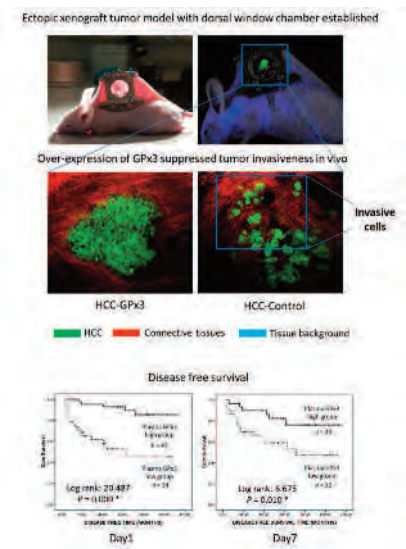
Conclusion: The implementation of the (clinical) Up-to-seven criteria for standard might result in excellent survival following LT for HCC. However, there is even excellent outcome beyond these selection limits in patients with favorable tumor metabolism. This special subset of patients may be identified by pretransplant 18F-FDG uptake assessment on PET.

625.4

The clinical significance and potential therapeutic role of GPx3 in tumor recurrence after liver transplantation

Xiang Qi, Kevin Tak-Pan Ng, Yan Shao, Chang Chun Ling, Chang Xian Li, Yeun Yeun Ma, Chung Mau Lo, Kwan Man.
Surgery, The University of Hong Kong, Hong Kong, Hong Kong.

Objective: Both of our clinical and animal studies showed that small-for-size liver graft may provide favorable environment for tumor recurrence after transplantation. Recently, we also demonstrated that GPx3, an anti-oxidant, not only attenuates oxidative stress, but also suppresses liver tumor growth[1]. Here, we aimed to characterize the clinical significance and explore the functional role of GPx3 in HCC recurrence after liver transplantation.
Methods: To explore the association between GPx3 expression and HCC invasiveness, a rat orthotopic liver transplantation model with tumor development was established. To investigate the clinical relevance of GPx3, 105 HCC patients who have undergone liver transplantation were recruited. The suppressive role of GPx3 in HCC cells was studied using wound healing, Matrigel invasion assay and lung metastasis model. The real-time intravital imaging system was applied to directly visualize the tumor cells invasion in a living animal. The underlying mechanism was further explored.
Results: GPx3 was identified as a down-regulated protein in small-for-size liver graft and significantly associated with invasive phenotype of tumor growth in a rat model. Plasma GPx3 was significantly lower in small-for-size graft group post-transplantation (day1: 33 vs 1147; day3: 3209 vs 4459; day7: 303 vs 2506; mU/mL, P<0.05) in rat model. Clinically, the plasma GPx3 was significantly lower in the recipients with HCC recurrence post-transplantation (day1: 4.16 vs 8.99 μg/mL, P<0.001; day7: 3.86 vs 9.99 μg/mL, P<0.001). Furthermore, lower plasma GPx3 was identified as an independent predictor (HR=4.528, P=0.046) for poor overall survival post-transplantation. Over-expression of GPx3 significantly suppressed migration, invasiveness and metastasis of HCC cells. Real-time intravital imaging showed that GPx3 significantly suppressed HCC invasiveness in a live animal. GPx3 suppressed the tumor invasiveness through inhibition of JNK-cJun-MMP2 pathway. The negative correlation between GPx3 and MMP2 expression was further validated in both animal model and clinical samples.
Conclusion: GPx3 may possess prognostic and therapeutic value for HCC patients after liver transplantation.



References:
[1] Qi, et al. *Oncotarget*. 2014

625.5

Influence of donor age and cold ischemia on recurrence of hepatocellular carcinoma after liver transplantation

Dilip Moonka[1], Shunji Nagai[2], George Divine[3], Reena Salgia[1].
[1]Gastroenterology and Hepatology, Henry Ford Health System, Detroit, MI, United States; [2]Transplant Institute, Henry Ford Health System, Detroit, MI, United States; [3]Public Health Sciences, Henry Ford Health System, Detroit, MI, United States.

Introduction: The role of donor age on recurrence of hepatocellular carcinoma (HCC) after liver transplant (LT) is not clear. In the current analysis, we evaluate the impact of donor age and cold ischemia time on HCC recurrence after LT.
Methods: We evaluated 303 consecutive LT patients at our institution with HCC. Nine were excluded because of findings of cholangiocarcinoma, 11 were excluded because of death within three months and one because of positive HCC margins at LT leaving 282 patients. . Patients were evaluated for a variety of factors for association with time to HCC recurrence using Kaplan-Meier estimates and log-rank tests. Multivariate modeling was done using Cox regression analysis.
Results and Discussion: In the 282 patients, there were 41 HCC recurrences (14.5%) occurring at a median of 17 months and a mean of 22.2 months and a range of 22 to 98 months. On univariate analysis, factors associated with HCC recurrence were cold ischemia (P < 0.001), donor age (P=0.025), tumor burden within Milan criteria on explant (P < 0.001), maximum alpha-fetoprotein (AFP) (P=0.005) and AFP at time of LT (P < 0.001), poorly differentiated histology (P < 0.001), vascular invasion (P < 0.001) and percent necrosis (P=0.017) in those undergoing pre-LT treatment.
Patients with HCC recurrence had a donor age of 47.0 ± 16.7 and those without had a donor age of 41.2 ± 16.2 (P=0.038). Patients with recurrence had a cold ischemia time in hours of 6.8 ± 2.1 vs 5.6 ± 1.7 in those without (P=0.001). For both donor age and cold ischemia, the Youden's J statistic was used to determine an optimal cutoff to discriminate between patients with and without recurrence. The 209 patients with donors less than 55 years old had 1, 3 and 5 year tumor free survival of 93.7%, 90.2% and 87.3% vs 91.5%, 78.4% and 70.3% in the 73 patients with donors over 55 (P=0.009). The 119 patients with cold ischemia less than 5.5 hours had 1, 3 and 5 year tumor free survival of 95.8%, 92.6% and 91.0% vs 91.2%, 82.7% and 76.6% in the 150 patients over 5.5 hours (P=0.006). The 37 patients with donors over 55 and cold ischemia over 5.5 hours had 1, 3 and 5 year tumor free survival of 88.7%, 67.0% and 57.4% vs 95.4%, 92.5%, and 92.5% for the 88 patients with both lower donor age and shorter cold ischemia.
In a multivariate analysis controlling for tumor burden, histology and vascular invasion: donor age (P=0.007), cold ischemia (P=0.005), Milan criteria on explant (P=0.002), maximum AFP (P=0.016), AFP at OLT (P=0.004), poorly differentiated histology (P < 0.001) and vascular invasion (P=0.002) were associated with time to tumor recurrence
Conclusions: Both lower donor age and shorter cold ischemia time were independently associated with improved tumor free survival after liver transplant. If confirmed in larger cohorts, these represent potentially modifiable factors in liver transplant patients with liver cancer.

625.6

Liver transplantation outcomes in patients with primary liver cancer

Douglas Slakey, Zaid Al-Qurayshi, David Pointer, Mary Killackey, Anil Paramesh, Emad Kandil.
Surgery, Tulane University, New Orleans, LA, United States.

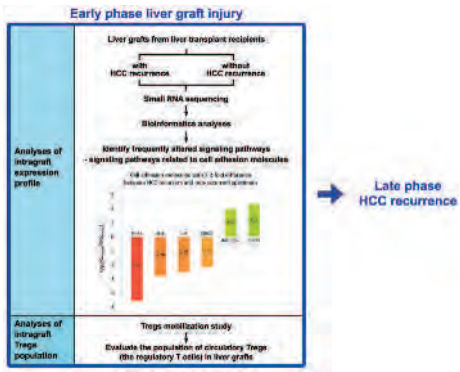
Introduction: In 1996 the Milan criteria were published which, along with the 2002 MELD exceptions, helped set standards for liver transplantation with primary hepatocellular cancer (HCC). Patient and center specific data has furthered confidence in transplantation as a treatment for patients with HCC. However, little is known about the evaluation of these outcomes using non-transplant data sets. In this analysis we aim to examine the trends, complications, and outcomes using the United States National Inpatient Sample database.
Methods: A cross-sectional analysis using the National Inpatient Sample database, 2003 – 2010. The study population included adult (≥ 18 years) inpatients who underwent liver transplantation indicated for either primary liver cancer (cases) or other nonmalignant conditions (controls).
Results and Discussion: A total of 1,767 (27.4%) cases, and 4,708 (72.6%) controls were included. Patients with liver cancer who underwent transplantation were more likely to be >60 years old, male, and non-White ($p<0.05$ each). There was a significant increase in the number of patients with cancer receiving liver transplants during the study period ($p=0.002$). Mortality risk was significant lower in patients with liver cancer (3.3% vs. 5.5%, $p<0.001$). Patients with liver cancer had a lower risk of general postoperative complications [OR: 0.52, 95%CI: (0.44, 0.62), $p<0.001$], and specific complications [OR: 0.59, 95%CI: (0.53, 0.65), $p<0.001$]. Additionally, transplantation in patients with liver cancer is associated with shorter hospital stay (14.1±1.0 vs. 22.8±1.6 days, $p<0.001$) and lower cost of health services ($98,822.97±2,838.97 vs. $127,329.74±4,837.58, $p<0.001$).
Conclusions: Liver transplantation in patients with a history of primary liver cancer has increased in the United States and is associated with more favorable immediate clinical outcomes as compared to patients with nonmalignant diseases, including lower length of hospitalization and lower cost of transplantation.

625.7

Integrative analyses of acute phase liver graft injury on late phase tumor recurrence after liver transplantation

Nikki Lee[1], Ruibang Luo[2], Haiyang Wu[2], Chang-Xian Li[1], Kevin Ng[1], Tak-Wah Lam[2], Chung-Mau Lo[1], Kwan Man[1].
[1]Surgery, The University of Hong Kong, Hong Kong, Hong Kong; [2]Computer Science, The University of Hong Kong, Hong Kong, Hong Kong.

Introduction: Liver transplantation is the best treatment for a selected group of patients with hepatocellular carcinoma (HCC). However, the long-term benefit of such treatment is greatly hampered by HCC recurrence after transplantation. Our previous studies have hallmarked liver graft injury is an early phase event leading to late phase HCC recurrence after transplantation. To re-examine early phase liver graft injury at the molecular and cellular level and in an integrated way, RNA sequencing using early phase liver graft biopsies in parallel with post-transplant circulatory Tregs mobilization study were performed. We aim to capture frequent alterations occurred during early phase liver graft injury that may account for late phase tumor recurrence in recipients.
Materials and Methods: Six liver graft biopsies from the recipients at 2 hours after reperfusion with late phase tumor recurrence were sequenced and compared with five non-recurrent samples. The top signaling pathways selected from comparing the RNA expression profiles between these two groups were further validated in a clinical cohort with 120 HCC recipients. The correlation among tumor recurrence, gene signaling pathways and Tregs mobilization were concurrently studied in these liver transplant recipients with HCC and orthotopic rat liver transplantation model.
Results and Discussion: Comparison of RNA expression profiles between these two groups pointed to several pathways with frequent alterations in our studied condition, in which pathways related to cell adhesion molecules were amongst the top involved pathways in acute phase liver graft injury. Subsequent confirmation using quantitative polymerase chain reaction has further implicated the diversified effects of two cell adhesion molecules HFE and CD274 and their altered expressions in early phase liver graft injury. Clinically, patients received grafts with graft weight ratio (GWR) <60% had higher HCC recurrence after transplantation than those with GWR≥60% graft. In those recipients with GWR<60% graft who are prone to HCC recurrence, their liver grafts had more circulating Tregs, and contained higher intragraft levels of studied cell adhesion molecules. Our results showed a tight correlation between the event of tumor recurrence after liver transplantation and the involvement of signaling pathways related to cell adhesion molecules and Tregs mobilization.
Conclusion: This whole transcriptome strategy coupled with Tregs mobilization study unravels the landscape representing intragraft alterations, which can lead to the identification of key cellular events involved in early phase liver graft injury that may lead to late phase tumor recurrence after liver transplantation.



625.8

A novel prognostic index in patients with hepatocellular cancer waiting for liver transplantation: Time–Radiological-response–Alpha-fetoprotein–INflammation (TRAIN) Score

Quirino Lai[1], Daniele Nicolini[2], Pierre Goffette[1], Samuele Iesari[1], Andrea Agostini[2], Andrea Giovagnoni[2], Marco Vivarelli[2], Jan Lerut[1].
[1]UCL, S. Luc Hospital, Brussels, Belgium; [2]Unit of Hepatobiliary surgery and Transplantation, Azienda Ospedaliero-Universitaria "Ospedali Riuniti Torrette, Ancona, Italy.

Objective: The role of radiological response to loco-regional therapies, alpha-fetoprotein modification, inflammatory markers and length of waiting time (WT) have been recently shown as important selection criteria for the risk of intention-to-treat (ITT)-death and recurrence in patients with hepatocellular cancer (HCC) waiting for liver transplantation (LT). A novel and easy prognostic score based on these, pre-operatively available, variables has been obtained from a long-WT training set and then validated in a short-WT set.
Methods: The training set was composed by 179 HCC patients listed for LT during the period Jan2000-Dec2012 from UCL Brussels; the validation set by 110 patients listed during the period Jan2005-Dec2014 from Ancona.
Results: The training set had a longer median WT compared to the validation set (5.1vs.2.7 months). The training set had more drop-outs from the wait list (19.0vs.13.6%), whilst the validation set had more recurrences (12.6vs.8.3%). The proposed Time–Radiological-response–Alpha-fetoprotein–INflammation (TRAIN) score was the best predictor of microvascular invasion, a well-known risk factor for recurrence. A TRAIN score≥1.0 excellently stratified both the investigated populations in terms of ITT- and recurrence survivals.
Conclusions: The proposed TRAIN score is an easy selection tool based only on pre-LT obtainable variables. This score enables to refine the selection process in two different scenarios of long as well as short WT. In case of a longer WT, the score better predicts the risk of death during the waiting time; in case of a short WT constellation, the score better identifies high-risk patients for post-LT.

625.9

Degree of tumor necrosis from pre-transplant loco-regional therapy is associated with tumor free survival after liver transplantation

Dilip Moonka[4], Veena Shah[3], Lagnajita Datta[3], Ramya Gadde[3], George Divine[2], Atsushi Yoshida[1], Syed-Mohammed Jafri[4], Reena Salgia[4].
[1]Transplant Institute, Henry Ford Health System, Detroit, MI, United States; [2]Public Health Services, Henry Ford Health System, Detroit, MI, United States; [3]Surgical Pathology, Henry Ford Health System, Detroit, MI, United States; [4]Gastroenterology and Hepatology, Henry Ford Health System, Detroit, MI, United States.

Introduction: Previous studies have looked at the impact of tumor necrosis from loco-regional therapy (LRT) prior to liver transplant (LT) on the rate of recurrence of hepatocellular carcinoma (HCC) after transplant. These studies have shown mixed results. We describe our results in 181 LT patients with HCC at our center.
Methods: We evaluated 260 consecutive LT patients at our institution with known HCC. Patients were excluded for cholangiocarcinoma (9), death within three months (11), residual cancer after LT (1), no LRT (47) or unavailable necrosis data (11). This left 181 patients of whom 30 had HCC recurrence. Patients were evaluated for a variety of factors for association with time to HCC recurrence using Kaplan-Meier estimates and log-rank tests. Multivariate modeling was done using Cox regression analysis.
Results and Discussion: Of 181 LT patients, 152 patients had one treatment, 25 had two and four patients had three treatments. There were 70 ablations, 80 chemoembolization, 47 bland embolization and 10 yttrium (Y-90) embolization. Thirty patients had HCC recurrence at a mean of 24.0 months with a range of 3.4 to 97.6 months. On univariate analysis, factors associated with time to HCC recurrence were percent necrosis (P=0.016), longer cold ischemia time (P=0.008), older donor age (P=0.050), tumor exceeding Milan criteria on explant (P<0.001), maximum AFP (P=0.015), poorly differentiated histology (P=0.027) and vascular invasion (P=0.007). The type of LRT was not associated with time to recurrence.
Patients with HCC recurrence had a mean percent necrosis of 52.7 ± 32.7% compared to 67.7 ± 33.5% for patients without recurrence (P=0.023). A cutoff of 85% tumor necrosis gave the maximal Youden's J statistic for discriminating groups most likely to recur from those less likely to do so. 77 patients had > 85% necrosis and their 1, 3 and 5 year tumor free survival was 94.7%, 94.7% and 92.9%. For the 114 patients with < 85% necrosis, 1, 3 and 5 year tumor free survival was 91.3%, 80.1% and 71.6%. The difference between the two curves is significant at P=0.002.
On multivariate analysis, percent tumor necrosis was no longer a significant predictor of time to recurrence (P=0.117) when controlling for whether tumor burden met Milan criteria on explant. Of note, all patients with > 85% necrosis were within Milan criteria on explant. However, percent necrosis remained significant when controlling for pre-transplant variables of tumor within Milan criteria on pre-LT imaging and maximum AFP prior to LT (P=0.012). Cold ischemia time, maximum AFP, poorly differentiated histology, Milan criteria and vascular invasion remained significant in multivariate models.
Conclusions: In patients undergoing LRT, the percent necrosis is associated with tumor free recurrence with near complete necrosis of over 85% demonstrating the best efficacy. These results highlight the importance of effective LRT prior to transplant in patients with liver cancer.

626.1

Prospective randomized controlled study evaluating the impact of an electronic feedback system (EFS) on medication adherence in solid organ transplantation

Mark Lockwood, Lisa Potter, Brenna Kane, Adam Bodzin, Laura Lourenco, J. Michael Millis.
Surgery, University of Chicago Medicine, Chicago, IL, United States.

Purpose: To evaluate the impact of and electronic feedback system, SIMpill Medication Adherence System® on medication adherence in patients who have received a solid organ transplant.
Methods: A total of 89 solid organ transplant recipients were randomized to 1 of 4 groups; 2 intervention groups (I1 and I2) & 2 control groups (C1 and C2). Subjects in I1 or I2 received EFS [figure 1], a medication-dispensing device that communicates timing of openings or doses taken to a secure server. If an IM dose is missed a text reminder message is sent to the subject (I1). In addition to patient reminders, providers were notified if consecutive doses were missed over a 72 hour period (I2), enabling intervention. C1 received EFS device but feedback was not provided to the patient or provider. C2 did not receive an EFS device.
Results: A total of 43 subjects from two subgroups, I1 and C1, were selected for analysis with Mann-Whitney U tests. A majority of patients used the device for greater than 6 months (N=36). Median comparison of total doses taken, days with correct dosing, and doses taken within 2 hours of target time were significantly greater in I1 compared to C1 [table 1].
Conclusion: The use of EFS, such as the SIMpill Medication Adherence System®, may improve adherence in solid organ transplant recipients, may allow for identification of medication non-adherence, and may facilitate provider intervention for non-adherence.

[figure 1] SIMpill medication adherence system

Table 1.

Adherence Parameter	Interventional Group 2 (% Median)	Control (% Median)	p Value
Total Doses Taken	97	88	<0.001
Days with Correct Dosing	94	74	<0.001
Doses Taken within Two Hours of Target Time	65	52	<0.001

626.2

Randomized controlled study evaluating adherence monitoring with electronic feedback on reducing renal allograft rejection

Brenna Kane, Mark Lockwood, Adam Bodzin, Lisa Potter, Lauren Lourenco, J. Michael Millis.
Surgery, University of Chicago, Chicago, IL, United States.

Purpose: The negative impact of non-adherence to the immunosuppressant medication (IM) regimen on transplant outcomes is well recognized. In an attempt to improve outcomes, the SIMpill® System is currently being utilized in kidney transplant(KT) recipients in a 3-year study in which allograft rejection is being evaluated.
Methods: KT recipients(n=46) were randomized to 4 groups, 2 Intervention Groups(I1 & I2) or 2 Control Groups(C1 & C2). Subjects enrolled in I1, I2 and C1 received the SIMpill® Electronic Medication Adherence Monitoring System. C2 did not receive a device. This system is a medication-dispensing device that communicates and stores timing of openings, or doses taken to a secure server. The Intervention Group subjects received notification if a scheduled IM dose was missed via text message and/or email. In addition, in I2, if a scheduled dose was missed despite the reminder message, a study provider was notified, enabling intervention.

In C1 no one received any feedback, but their adherence is stored on the server for analysis.
Results: At one year, 46 KT recipients were enrolled with an average follow-up of 334 days (median=413.5[range 118-559]). 7 of the 9 rejections were ACR where 2 of the 9 were ABMR. 5 of the 7 ACR biopsies were categorized as Banff grade ≤ 2 while the other 2 were classified as borderline. 5 of the 9 rejection episodes resulted in hospital admission totaling 34 days. 1 of the 9 subjects with ACR experienced graft failure and died on post-transplant day 140.

Compliance and Outcomes			
	Intervention (n=20)	Control Group (n=26)	P-value
Patient-months	242.4	286.8	<0.005
Total Doses Taken (%)	90	84	NS
Days with Correct Dosing	87	74	<0.005
# of Biopsies	4	9	<0.001
BPR (% of Group)	0(0)	9(19.2)	0.021
LOS for treatment (days)	0	34	0.012

Conclusions: Preliminary 1-year results demonstrate that utilization of the SIMpill® with feedback on adherence shows a potential reduction in biopsies performed, and rejection episodes as well possibly reducing hospitalizations and cost related to rejection.

626.3

Importance of early medication adherence for long-term graft survival: A prospective, single-centre cohort study

Mirjam Tielen[1], Mirjam Laging[1], Denise K.Beck[1], Teun van Gelder[1,2], Michiel G.H.Betjes[1], Willem Weimar[1], Emma K.Massey[1].
[1]Nephrology and Transplantation, Erasmus MC, Rotterdam, Netherlands; [2]Pharmacology, Erasmus MC, Rotterdam, Netherlands.

Introduction: The rate of nonadherence to medication among renal transplant patients is reported to be as high as 20-37%. There is mixed evidence regarding the relationship between nonadherence and clinical outcomes with the current immunosuppressive regimes in use[1],[2]. To what extent is nonadherence in our kidney transplant population related to clinical outcomes 4 years after transplantation?
Materials and Methods: All consecutive kidney transplant recipients were invited to participate in this prospective study. A face-to-face interview took place 6 weeks (T1), 6 months (T2) and 18 months (T3) after transplantation. To measure nonadherence to immunosuppressive medication respondents completed the Basel Assessment of Adherence to Immunosuppressive Medications Scale (BAASIS©-interview). This scale is a self-report instrument that consists of 4 questions on the taking and timing of medication, drug holidays, reduction of the dose, and persistence over the past month. Patients who gave an affirmative answer to any of the questions were classified as nonadherent. We operationalized clinical outcomes as graft survival censored for death and graft rejection (yes/no) and measured these up to 4 years post-transplant.
Results: 113 renal transplant recipients (19-75 yrs) participated in the study from 2010-2011. Eighty-four patients remained in the study at 18 months post-transplant. The BAASIS©-interview revealed that 19/113 were classified as nonadherent at T1, 29/106 at T2 and 26/84 at T3. Patients categorized as nonadherent at T1 had a lower 4-years graft survival (p=0.012) while patients categorized as nonadherent at six months (T2) had a higher incidence of graft rejection during a 4-years' follow-up (p=0.021).
Discussion: Even very soon after kidney transplantation 17% of the patients reported a certain degree of non-adherence and this increased over time. We showed that in particular nonadherence immediately after transplantation till the first 6 months is a risk factor for graft rejection and even graft loss. The BAASIS-interview is a brief and affordable tool in the clinic to detect nonadherence early and flag up patients at risk for poorer clinical outcomes.
Conclusion: These results highlight the need for adherence promoting interventions early after kidney transplantation to promote improved long-term outcomes.
References:
[1] Prihodova, L., et al. Adherence in patients in the first year after kidney transplantation and its impact on graft loss and mortality: a cross-sectional and prospective study. J Adv Nurs 2014 70(12): 2871-2883.
[2] Denhaerynck, K., et al. Clinical consequences of non adherence to immunosuppressive medication in kidney transplant patients. Transpl Int 22 2009 (4): 441-446.

626.4

Interdisciplinary organ and tissue donor suitability assessment simulation training: "Sharing the responsibility for safe organs and tissues for transplantation in training and in practice"

Patrick R.Wood[1], Wendy-Jo Wilkinson Bowman[2], Sergio Manzano[1], Brian Roe[1], Anne Floden[4], Laura Teasley[5], Kevin Myer[1], Maria Stadtler[3].
[1]Organ and Tissue Donation & Recovery, LifeGift, Houston, TX, United States; [2]Research & Development, CAE Healthcare, Sarasota, FL, United States; [3]Education & Research Development, Methodica, Boulder, CO, United States; [4]Research, University of Boras, Boras, Sweden; [5]Self, Employed, Glendale, CA, United States.

Introduction: Organ and tissue donation organizations (OPOs) in the United States adhere to regulations and policies administered by the Food and Drug Administration and the Organ Procurement Transplant Network. To ensure the safety, quality, and viability of transplanted organs and tissues, a physical exam (PE) on all potential organ and/or tissue donors is mandated. Based on donor type either organ or tissue staff carry out the PE. More organ donors proceed to become tissue donors, but despite this overlap, OPO staff routinely train separately resulting in unilateral proficiency in this function. In the US no formal interdisciplinary, simulation-based PE training is at hand or documented that incorporates a cross-functional blended learning approach and assesses competency.
Method: A quasi-experimental course was collaboratively developed with LifeGift a Texas, USA –based OPO. A three-part "Introduction to PE" eLearning module series employing pre/post testing was completed by 86 organ and tissue staff to evaluate baseline knowledge parity. Afterwards, all staff participated in a one-day high-fidelity simulation-training day with competency assessment in all three regional offices. Consistency across training sessions was abided by utilizing the identical eLearning content, instructors, simulators, scenarios, and core competencies across all regions. A post-course Likert-scale survey was applied immediately following the live training, and at three-month post intervention.
Results: Baseline cognitive knowledge improved in all participants. In all three e Learning module topics, pre-test scores (≈ 59%) increased on post-testing (≈92%).The post-course survey (n=80) revealed a strong perceived educational value for interdisciplinary training ,individual confidence and competence level as reported via median Likert survey response of 3 = strongly agree. Three-month post-course follow-up (n=58), 88% of the participants reported to have retained 75-100% of the knowledge and skills. Among respondents, 14% reported performing the PE 100% more often as compared to before the training.
Conclusion: This blended-learning training course resulted in reported individual participant increased confidence to perform a physical exam on an organ and tissue donor. Post training evaluation demonstrated reported increase of numbers of PEs performed since the training. Reported participant evaluations demonstrate that consistency and interdisciplinary training ameliorates competency, application of standardized practice, and enhances the awareness of the importance of teamwork, and communication in the PE are to enhance reliability of safety in the organ and tissue donation and transplantation process.
All Organ and Tissue Donors and Their Families; LifeGift's Dedicated Staff

626.5

The HINT study: a cross-sectional survey of Australian and New Zealand transplant clinicians on hepatitis transmission risk in solid organ transplantation

Karen Waller[1], Kate Wyburn[1,2], Nicholas Shackel[1,2,3], Michael O'Leary[1,2,4], Patrick J.Kelly[1], Angela C.Webster[1,5].
[1]Sydney Medical School, University of Sydney, Camperdown, Australia; [2]Royal Prince Alfred Hospital, Camperdown, Australia; [3]Centenary Institute of Cancer Medicine and Cell Biology, Camperdown, Australia; [4]NSW Organ and Tissue Donation Service, Kogarah, Australia; [5]Centre for Transplant and Renal Research, Westmead Hospital, Westmead, Australia.

Introduction: Understanding donor and recipient hepatitis serology and transmission risk can be challenging. We aimed to survey understanding among the Australian and New Zealand medical transplant workforce of hepatitis risk and transplant decision-making, given abnormal donor and/or recipient hepatitis serology.
Materials and Methods: An anonymous, self-completed, cross-sectional survey was distributed via mailing lists to Australian and New Zealand nephrologists, transplant surgeons, intensive care physicians and transplant hepatologists. Participants answered 8 serology scenarios in random order (Hepatitis B, HBV:5; Hepatitis C, HCV:3), assessing donor and recipient hepatitis status, and transplant suitability. Results were analysed firstly by simple proportions. Transplant suitability responses were compared for concordance with both current guidelines (TSANZ Transplantation Society of Australia and New Zealand) or more progressive literature. Multiple logistic regression models were produced for transplantation suitability outcomes using a random effects model with backwards stepwise regression.
Results and Discussion: Of 110 respondents, most were male (63%) nephrologists (74%) aged 40-49. Vaccinated and naïve donors and recipients were well-identified (>90% respondents). However, only 35% respondents recognised HCV antibody-positive but NAT-negative donors as an infection risk. Only one scenario, with benign serology, had >90% respondents transplanting in accordance with TSANZ guidelines; often, stronger consensus was given to another option. For instance, given a HBV surface antigen positive donor, and vaccinated recipient, 44% concurred with guidelines that this was unsuitable for transplant; however >50% respondents would transplant (although some of these only after specific consent and/or prophylaxis). When both donor and recipient were HCV antibody-positive and NAT-negative, guidelines suggest unsuitability for transplant (7%); but most respondents would transplant (63% with specific informed consent; 26% suitable without special measures). Across scenarios, up to 10% respondents self-identified as unsure of transplant decisions. For HBV suitability responses only transplant patient burden (>20/month) was predictive of either concordance with guidelines (OR 1.70, p = 0.01) or progressive responses (OR 1.52, p = 0.04). For HCV scenarios, progressive responses were more likely to be chosen by clinicians based in New Zealand (OR 4.01, p = 0.01).

Table One: Proportions of respondents for each scenario identifying donor and recipient risk status (vaccination status with guidelines, and selecting transplant responses in concordance with current guidelines or progressive literature.

	Scenario	Respondents answering (%; correct response):			
		Donor Risk Status	Recipient Risk Status	Transplant Suitability: TSANZ Guidelines	Transplant Suitability: Progressive Choices
Hepatitis B					
5	D: HBsAb +ve HBcAb +ve (>100) R: HBsAb +ve HBsAg -ve HBsAb +ve	96 Vaccinated	100 Naive	86 Suitable	96 Suitable
1	D: HBsAb +ve HBcAb +ve (>100) R: HBsAb +ve HBsAg -ve HBsAb -ve	62 Exposed, low risk	88 Naive	20 Suitable, consent	40 Suitable, prophylaxis
2	D: HBsAb +ve HBsAg +ve HBsAb +ve R: HBsAb +ve HBsAg -ve HBsAb +ve (<10)	98 Exposed, high risk	93 Vaccinated	37 Unsuitable	75 Suitable, prophylaxis
3	D: HBsAb +ve HBsAg +ve HBsAb +ve HBV NAT +ve R: HBsAb +ve HBsAg -ve HBcAb +ve HBV NAT -ve	97 Exposed, high risk	89 Exposed, no value	61 Unsuitable	87 Unsuitable
6	D: HBsAb +ve HBsAg +ve HBsAb +ve R: HBsAb -ve HBsAg -ve HBsAb +ve	62 Exposed, high risk	100 Naive	81 Unsuitable	97 Unsuitable
Hepatitis C					
8	D: anti-HCV Ab +ve HCV NAT -ve R: anti-HCV Ab -ve	96 Exposed, risk	65 Naive	47 Unsuitable	96 Suitable, consent
7	D: anti-HCV Ab +ve HCV NAT +ve R: anti-HCV Ab +ve HCV NAT -ve	98 Exposed, risk	63 Exposed, no value	68 Unsuitable	98 Unsuitable
4	D: anti-HCV Ab +ve HCV NAT -ve R: anti-HCV Ab +ve HCV NAT -ve	94 Exposed, risk	60 Exposed, no value	7 Unsuitable	63 Suitable, consent

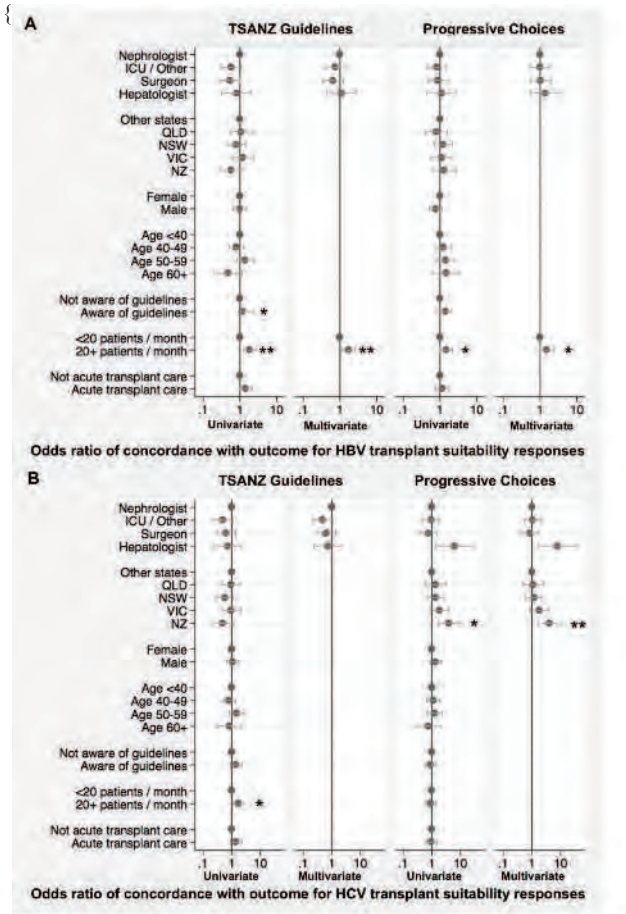


Figure One: Univariate and multivariate regression models produced to understand respondent factors influencing transplant suitability responses for (A) HBV and (B) HCV scenarios. Significant responses denoted by * p ≤ 0.05 ** p ≤ 0.01

Conclusions: Although interpretations of hepatitis serology were broadly consistent, transplant suitability decisions showed considerable variability and often discordance with guidelines. Best-practice management may outpace guidelines, guidelines may be ambiguous, or regional/personal preferences may drive practice. Contemporary pragmatic guidance to support doctors making high-impact decisions in a fast-paced environment is crucial.

626.6

Vascular access types in patients starting hemodialysis with failed kidney transplants: a retrospective analysis

Naveed Ul Haq[1], Mohammed Althaf[1], Mohamed Abdelsalam[1], Abdulrahman Khormi[1], Abdulrahman Khormi[1], Hassan Al Harbi[1], Dieter Broring[2], Lutfi Korbi[1], Hassan Aleid[2].
[1]Medicine, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia; [2]Kidney and Pancreas Transplantation, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia.

Introduction and Objectives: Native arteriovenous fistulae (AVF) are preferred while central venous catheters (CVC) are least suitable vascular access (VA) in patients with end stage renal disease (ESRD) requiring hemodialysis (HD). Despite clinical benefits of AVF, as much as 80% of patients start HD with a CVC. One of the factors thought to be responsible for this high CVC rate is late referral to nephrologist in the course of chronic kidney disease. We retrospectively analyzed the type of VA at the time of initiation of HD in renal transplant recipients closely followed by nephrologists who subsequently failed their renal allograft. If the early referral to nephrologist improves AVF use, these patients should have a higher prevalence of AVF at the time of initiation of HD.
Material and Methods: We reviewed charts of all patients at our center who failed their kidney transplants between January 2002 to April 2013. Study was approved by the local IRB. Data regarding planning of VA by nephrologist including patient demographics, documented discussion about dialysis with patient, glomerular filtration rate (GFR) 6 months before starting HD and at last clinic visit before HD started, time of referral for VA creation, ordering vascular mapping, and the subsequent type of VA at the start of dialysis was gathered and analyzed.
Results: Total of 83 patients failed their transplant after having a successful functioning allograft during study period. Data was inaccessible in 6 patients. The paper and electronic charts on remaining 77 patients were analyzed. 11 patients started peritoneal dialysis after failing transplant while 66 went on HD. Out of these 66, 32 had a previous functioning VA while 34 needed a VA. Demographics of these patients are shown in table 1. 11/34 patients (32%) had a GFR < 15 ml/min six months before initiation of HD. Average time from first documented GFR < 20ml/min to initiation of HD was 62 days (range 0-307 days). 21/34 (61%) had a documented GFR < 15ml/min on last clinic visit before HD initiation. Only 11/34 (32%) had documented renal replacement therapy discussion, 8/34 (24%) had VA creation referral, and only 7/34 (21%) had vein mapping for VA creation. 30/34 (88.3%) started HD with a CVC while 4/34 (11.3%) started HD with an AVF ($p<0.0001$).
Conclusion: Early referral to nephrologist by itself may not adequately improve VA care amongst patient with ESRD. Other more important factors need to be examined for improvement of VA care in these patients.
Table 1: Baseline characteristics of patients starting HD post failed transplant without prior functional vascular access
Total number of patients 34
Male 53% Female 47%
Age 40±18 years
Cause of ESRD: Diabetes Mellitus 18% Glomerular Nephritis 15% Unknown etiology 20% Others 47%
Other significant comorbidities: Diabetes Mellitus 38% Hypertension 76% Peripheral Vascular Disease 9% Coronary Artery Disease 15%
Renal Replacement modality pre transplant: Hemodialysis (HD) 82% Preemptive transplant 18%
Vascular access pre transplant for those on HD: Arteriovenous Fistula 53% Tunneled Catheter 29%
Transplant type: Cadaveric Renal Transplant 26% Living Related Renal Transplant 68% Living non-Related Renal Transplant 6%

626.7

Additional opportunities in Australia for transplanting organs from donors with brain malignancies? A retrospective cohort study of the NSW Organ and Tissue Donation Service organ donor register

Rebecca Hancock[2], Kate Wyburn[1,5], Imogen K Thomson[1], Michael O'Leary[1,3,6], Angela C Webster[1,2,4,7].
[1]Sydney Medical School, University of Sydney , Sydney, Australia; [2]School of Public Health, University of Sydney, Sydney, Australia; [3]NSW Organ and Tissue Donation Service, Sydney, Australia; [4]Centre for Kidney Research, The Children's Hospital at Westmead, Sydney, Australia; [5]Renal Unit, Royal Prince Alfred Hospital, Sydney, Australia; [6]Intensive Care Service, Royal Prince Alfred Hospital, Sydney, Australia; [7]Centre for Transplant and Renal Research, Westmead Hospital, Sydney, Australia.

Introduction: Donors with primary brain malignancies present an on-going challenge due to uncertainty around transmission risk, classification complexity and variability in guidelines among countries. We aimed to describe patterns of organ donation among referrals with primary brain malignancies in New South Wales, Australia, and to identify any untapped opportunities for donation.
Materials and Methods: We reviewed the Organ and Tissue Donation Service (OTDS) organ donor register, which records demographic and clinical information about all people in NSW referred for donation, from January 2010 to December 2015. We compared 'potential' donors (referred but did not proceed to donation), with 'intended' (donation abandoned before organ removal) and 'actual' donors, who had past and/or current primary brain malignancies, including those deemed not medically suitable due to cancer, in light of current evidence. Reasons for outcome variability were evaluated. Transmission risks were obtained using international best estimates. Wilcoxon's rank sum test was used to test age differences across donor outcome groups.
Results: Of 3,005 total donation referrals (2,337 potential, 668 intended/actual), 67 patients had primary brain malignancies,14 of whom donated (10 actual, 4 intended) and 53 who did not (23 excluded due to their brain malignancy, 17 for other medical reasons, and 13 for family consent was not obtained). The median age of those excluded due to primary brain malignancies (50 years) was significantly lower than those excluded for other medical reasons (69 years) ($p=.008$). Those who donated had lower grade tumours, while patients excluded due to primary brain malignancies were more likely to have higher grade tumours or unclear grading (refer to table). Medical suitability decisions were variable for astrocytoma, meningioma, and unspecified cerebral tumours. Some of that variability was explained by tumour grade. We identified 21 additional potential donor opportunities among referrals deemed not medically suitable due to primary brain malignancies. This included 3 people with brain malignancy of 'low' transmission risk (<2%) (2 astrocytoma, 1 meningioma) and 18 people with 'intermediate' transmission risk (2.2% with an upper 95% CI of 6.4%) malignancies (glioblastoma, thalamic germinoma, ependymoblastoma). Realisation of an additional potential 21 donors would increase intended/actual donor pool by 3.1%. Limitations in administrative data mean all considerations informing past decisions may not be clear.
Conclusion: Potential donors currently excluded due to primary brain malignancies are of younger relative age. In weighing the risk/benefit threshold for patients offered organs from donors with primary brain malignancies, further consideration may be warranted for some individuals to receive organs from such donors, including potentially from those with higher grade tumours.

Table: Type of primary brain malignancy by donation outcome, 2010-2015, in New South Wales Australia.

Type of primary brain malignancy	Tumour grading[1]	Estimated transmission risk (upper 95% CI)[2]	Donor referral outcome	
			Potential (NMS due to malignancy)	Actual and Intended
Acoustic neuroma	Low	<2%	3	2
Astrocytoma	Variable	<2%	3 (2)	5
Craniopharyngioma	Low	<2%	1	1
Ependymoblastoma	High	2.2% (6.4%)	1 (1)	0
Ganglioma	Low	<2%	1	0
Glioma/Glioblastoma/ Glioblastoma multiforme	High	2.2% (6.4%)	22 (16)	0
Medulloblastoma (past cancer, deemed cured)	High	N/A		1
Meningioma	Variable	<2%	11 (2)	3
Pituitary	Low	<2%	4	1
Thalamic germinoma	High	2.2% (6.4%)	1 (1)	0
Unspecified Cerebral	N/A	N/A	6 (1)	1
Total			53 (23)	14

1. Tumour grades obtained from OTDS registry records, with WHO Grading of Tumours of the Central Nervous System guideline used where grades were not recorded. 2. Estimated transmission risks were obtained from SaBTO Advisory Committee on the Safety of Blood, Tissues and Organs, Transplantation of Organs from Deceased Donors with Cancer or a History of Cancer, United Kingdom, 2014.

626.8

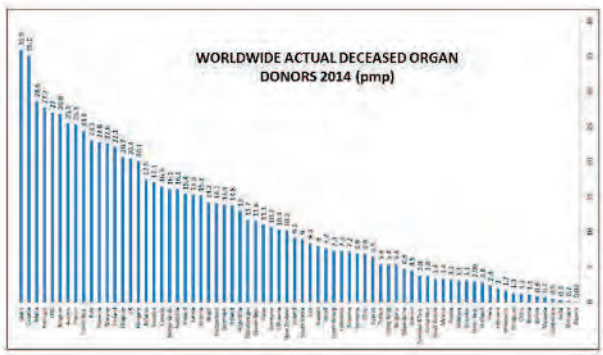
18 years of worldwide reporting in organ donation and transplantation IRODaT

Marti Manyalich[1,2], Maria P. Gomez[1], Gloria Paez[1], Chloë Ballesté-Delpierre[1], Ricard Valero[1,2].
[1]Donation and Transplantation Institute- DTI , Barcelona, Spain; [2]Clinic Hospital , Barcelona, Spain.

Introduction: The WHO´s Guiding Principles on Human Cell, Tissue and Organ Transplantation promotes the transparency of the organizations and performances "The development and maintenance of public national registries would support the grade of transparency in each country as well as will contribute to the governmental oversight and scientific research". Since 1998 the International Registry in Organ Donation and Transplantation – IRODaT have been compiling and reporting information regarding deceased/living organ donors and transplant worldwide every year. The data is accessible online in www.irodat.org.

Methods: The information registered follows the definition of "The Critical Pathway of Deceased Donation(1)", ensuring uniformity throughout the registry and aiding correct interpretation of the data by the scientific community. Each country is represented by an Official Reporter who registers the figures for donation and transplant activity in the previous year directly to the webpage. IRODaT counts with experts in donation and transplant who revise thoroughly the data. Specialized reports required for specific investigations, studies or for general consultation could be provided by contacting the registry. All data compiled is publishing in the Newsletter of the Registry accesses through the website.

Results: Through the last 18 years more than 55.000 records is been collected in IRODaT database from 93 countries. It has been used by Universities, Laboratories, National Authorities and Governmental bodies to analyse trends, tendencies and develop comparison among other. In 2014 an improvement of the actual deceased donor rates in some countries around the world was recorded. The most significant data were located in Finland and Denmark whit an increase of 5 and 3 points in its dpmp rate in one year respectively. The figure presents the worldwide deceased donor per million of population rate from 2014.



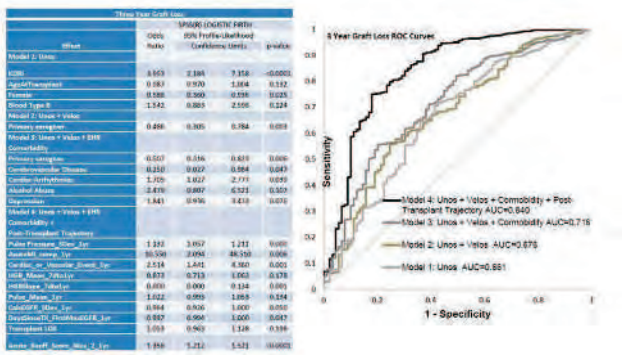
Conclusions: IRODaT is an open database able to provide statistics within a short timeframe, based on a worldwide network of experts involved in organ donation and transplantation. The data have proved to be of an extreme value to scientific programs, social and governmental bodies.

626.9

A big data approach to prediction of three-year graft loss risk in kidney transplants: structured and unstructured dynamic patient level data improve predictive efficacy

Titte Srinivas[1], Jingwen Zhang[1], Zemin Su[1], Justin Marsden[1], Haroon Anwar[3], Arun Tripathy[3], David Taber[2], William Moran[1], Patrick Mauldin[1].
[1]Medicine , Med. Univ. of S. Carolina, Charleston, SC, United States; [2]Surgery, Med. Univ. of S. Carolina, Charleston, SC, United States; [3]IBM Corporation, Armonk, NY, United States.

Background: Predictive models in kidney transplantation using national data (UNOS, SRTR) lack longitudinal patient level data, limiting accuracy. Adding patient level data capturing dynamic clinical evolution post-tx, may improve predictive accuracy for graft loss (GL) risk.
Methods: In a quality initiative, we built GL risk prediction models, using baseline and follow up data (0- 90-days post-tx; structured & unstructured) on adult solitary kidney transplant recipients transplanted 2007-15. Structured data were directly applied from electronic medical records (Electronic Health Record (EHR),Txp database (Velos) and UNOS).Natural Language Processing (IBM Watson) was applied to unstructured text to extract Banff scores and vitals. We built 4 models: Model 1) UNOS data; Model 2) UNOS & Velos (caregiver) data; Model 3) UNOS, Velos & EHR data (comorbidity); Model 4) UNOS, Velos, EHR & Post-tx trajectory data. We used IBM SPSS Modeler and Essentials for R in analyses. We used Backward Selection at the 20% level to select variables; statistical significance was determined at the 5% level.
Results: We included 890 patients in the GL model, with a 10% 3-yr GL rate. Model 1: KDRI significantly associated with increased 3-year GL (AUC ,0.66 ;95% CI: 0.60, 0.72). Model 2: With addition of Velos data we saw a favorable association on 3-year GL of the patient having a Primary Care Giver at Tx (AUC, 0.68 ;95% CI: 0.61, 0.74). Model 3 EHR data (Cardiac Arrhythmia) added significant positive association with 3-year GL(AUC,0.72;95% CI: 0.66, 0.77). Model 4 : Several post-transplant trajectory variables through year 1 post-tx added to model accuracy: Pulse Pressure Standard Deviation, Hemoglobin Slope (day7-365), Std Deviation of eGFR, Days from Tx to 1st Maximum eGFR, Acute MI, Cardiac or Vascular Events, and 1st year Acute Banff lesion scores (AUC ,0.84, 95% CI: 0.79, 0.89).



Conclusions: A Big Data approach to curating diverse data sources significantly adds accuracy to 3 year graft loss prediction models, capturing complexity and longitudinal evolution across several clinically mutable domains. This solution is executable daily through EHR workflows to optimize outcomes.

627.1

DNA-typed, MHC-inbred CLAWN miniature swine represent a highly-valuable species for the development of preclinical translational research in transplantation and regenerative medicine

Takahiro Murokawa[1], Hisashi Sahara[1], Takehiro Iwanaga[1], Mitsuhiro Sekijima[1], Yuichi Ariyoshi[1], Yurika Ichinari[1], Akira Shimizu[2], Kazuhiko Yamada[1].
[1]Division of Organ Replacement and Xenotransplantation Surgery, Center for Advanced Biomedical Science and Swine Research, Kagoshima University, Kagoshima, Japan; [2]Department of Analytic Human Pathology, Nippon Medical School, Tokyo, Japan.

Introduction: We have extensively studied transplantation tolerance of allogeneic organs in MHC-inbred MGH miniature swine. Recently, we developed a line of Japanese MHC-inbred CLAWN miniature swine. A potential difference between CLAWN miniature swine and MGH miniature swine is MHC typing; CLAWN miniature swine are typed using DNA-PCR, whereas sero-typing is employed in the MGH herd. Using this Japanese pig line, we established several models of organ transplantation such as orthotopic kidney and lung transplantation. Here, we present data indicating that CLAWN-swine models represent a highly-valuable preclinical model of transplantation.
Methods: We have previously demonstrated that 12-days of high-dose of tacrolimus administered via continuous IV infusion (35-50 ng/m) facilitates tolerance induction of fully MHC-mismatched kidneys and lungs in MGH miniature swine. Twelve CLAWN miniature swine received allogeneic kidneys (n=6) or lungs (n=6) with the same 12-days of tacrolimus, across fully MHC mismatched barriers. MHCs were defined using DNA typing as well as cellular assays. Additional four CLAWN miniature swine received MHC-matched lungs under the same immunosuppressive protocol to investigate the influence of MHC disparity on allograft survival. Graft function was monitored by blood chemistry, histology and X-rays. Immunologic responses were assessed by MLR, CML and allo-antibody (Ab) development using flow cytometry.
Results: All MHC-matched lung was uniformly accepted over 6 months. However, despite an identical tolerance-inducing protocol with 12 days of continuous (24 hours/day) tacrolimus and surgical technique to minimize study variability, all MHC-mismatched grafts in CLAWN miniature swine was rejected within 3 months. All recipients of kidneys developed acute rejection with severe mononuclear cell infiltrates and vasculitis soon after cessation of tacrolimus. Four rejected acutely and 2 rejected chronically. All MHC-mismatched lung was rejected within 63 days with diffuse mononuclear infiltrates associated with intra-alveolar hemorrhage and capillary congestion. This was consistent with severe cellular and Ab-mediated rejection.
Conclusions: Despite efforts to decrease study variability, tolerance was not induced to kidneys or lungs in CLAWN-miniature swine. These data indicate that DNA-typed, MHC-inbred CLAWN miniature swine represent a highly-valuable species for the development of preclinical models for the systematic study of transplantation and also regenerative medicine.

627.2

An intronic SNP of SHROOM3 promotes kidney inflammation and injury after ischemia-reperfusion injury in female mice

Min Hu[1], Bo Lu[2], Heather Burns[1], Qi Cao[1], Wayne Hawthorne[1], Shounan YI[1], Peter Cowan[2], Madhav Menon[3], Barbara Murphy[3], Stephen I.Alexander[4], Philip J.O'Connell[1].
[1]Centre for Transplant and Renal Research, The Westmead Institute for Medical Research , University of Sydney, Sydney , Australia; [2]Immunology Research Centre, , St. Vincent's Hospital, Melbourne, Australia; [3]Division of Nephrology, Mount Sinai School of Medicine, New York, NY, United States; [4]Centre for Kidney Research, The Children's Hospital at Westmead, Sydney, Australia.

Introduction: A large renal allograft cohort from patients recruited to the GOCAR study showed that an intronic SNP (rs17319721) in SHROOM3 potentiates renal allograft fibrosis[1]. Renal ischemia-reperfusion injury (IRI) is a common clinical event and, in animal models, is a surrogate for transplant injury. This study aims to investigate the role of an intronic SNP of SHROOM3 (predicted to be pathogenic) in kidney infiltration, injury and fibrosis in mouse model of IRI.
Materials and Methods: IRI was performed on female and male C57BL/6 mice (11 -14 weeks) with homozygous SNP (C/C), heterozygous SNP (T/C) and wild-type (T/T) at an intronic SNP on position 92,951,065 of SHROOM3. Both renal pedicles were occluded for 30 mins at 37°C. At day 30, serum and urine creatinine, and proteinuria was assessed. Renal histology was examined for the degree of cellular infiltration and kidney injury. Fibrosis was quantified computationally using Sirius Red staining. Kidney injury was graded from 1-5 histologically.
Results and Discussion: Mice were followed out for > 9 months. There was no difference in body weight, growth or mobility between WT mice and mice homozygous or heterozygous for the SHROOM3 SNP. 30 days after the IRI procedure there was no difference in serum creatinine, urine creatinine or proteinuria between all groups. In male mice similar grades of kidney injury with matching cellular infiltration were found in all 3 mouse genotypes [2.8±1.7 in WT mice (n = 4)][(3.0 ± 1 in het-SNP mice (n = 4)] [(3.2 ± 1.5 in homo-SNP mice (n = 4)]. In female mice, SHROOM3 homo-SNP mice showed higher grades of kidney injury (1.6 ± 0.8)(n = 5) and more cellular infiltration than SHROOM3 het-SNP (0.6 ± 0.2)(n = 5) and WT mice (0.5 ± 0.5)(n = 4). Further the percentage of fibrosis in female mice was higher in homo-SNP mice (5.30 ± 1.59%) compared to het-SNP (3.72 ± 1.70%) and WT mice (1.92 ± 0.79%). Males were considered to be more susceptible to IRI in other animal studies whereas this mutation appears to primarily affect female mice[2].
Conclusion: This study suggests an intronic SNP in SHROOM3 may be a risk genotype for renal fibrosis in female mice, consistent with clinical observations and provides us with a model to study donor related factors following renal transplantation.
Australian National University provide SHROOM3 heter-SNP (T/C) breeding pairs
References:
[1] Menon, M. C., P. Y. Chuang, Z. Li, C. Wei, W. Zhang, Y. Luan, Z. Yi, H. Xiong, C. Woytovich, I. Greene, J. Overbey, I. Rosales, E. Bagiella, R. Chen, M. Ma, L. Li, W. Ding, A. Djamali, M. Saminego, P. J. O'Connell, L. Gallon, R. Colvin, B. Schroppel, J. C. He, and B. Murphy. 2015. Intronic locus determines SHROOM3 expression and potentiates renal allograft fibrosis. J Clin Invest 125: 208-221.
[2] Kang, K. P., J. E. Lee, A. S. Lee, Y. J. Jung, D. Kim, S. Lee, H. P. Hwang, W. Kim, and S. K. Park. 2014. Effect of gender differences on the regulation of renal ischemia-reperfusion-induced inflammation in mice. Mol Med Rep 9: 2061-2068

627.3

Role of IL-7 and IL-7/anti-IL-7Antibody complexes in Treg expansion and a murine skin allograft tolerance model

Min Hu[1], Heather Burns[1], David Liuwantara[1], Yi Wen QIAN[1], Yuan Min Wang[2], Geoff Zhang[2], Wayne Hawthorne[1], Shounan YI[1], Stephen I.Alexander[2], Philip J.O'Connell[1].
[1]Centre for Transplant and Renal Research, The Westmead Institute for Medical Research, , University of Sydney, Sydney, Australia; [2]Centre for Kidney Research, The Children's Hospital at Westmead, Sydney, Australia.

Introduction: IL-7 plays an important role on memory regulatory T cells (Tregs) for their maintenance in peripheral tissues[1]. The study aims to determine whether 1) IL-7 supports CD4+Foxp3+ Treg survival in the alloresponse and 2) IL-7/anti-IL-7mAb complexes induce Treg expansion in a murine skin allograft transplant model.
Methods: CD4+Foxp3+ Tregs were sorted from Foxp3-GFP (C57BL/6) mice. Two MLRs were performed. Responders from 1) splenocytes of Foxp3GFP mice, or 2) sorted CD4+Foxp3+ Tregs were both stained with CellTrace™Violet, and stimulated with irradiated splenocytes of NOD/LtSz-scidL2Rgnull mice as APCs. IL-7, anti-IL-7mAb, IL-7/anti-IL-7mAb, or IL-2 were added to the MLR. At day 5, cells were analysed using flow cytometry. CD4+Foxp3+ Tregs were adoptively transferred into Rag-/- mice. Skin transplantation with C57BL/6, DBA, B10.Br or CBA grafts were performed at day 2 after adoptive transfer. At >100 days post-transplant, Rag-/- mice were treated with IL-7/anti-IL-7mAb complexes once a week for 3 weeks. These mice then were challenged with CD4+ effector T cells at week 2 after IL-7/anti-IL-7mAb complexes treatment.
Results: At day 5 of MLR (Tregs), the proportion of Foxp3+ Tregs were similar under IL-7 (63%), IL-2 (68%), IL-7/anti-IL-7mAb (67%) conditions but not anti-IL-7mAb (21%) or control (19%). Expression of CD127 was present on Foxp3+ Tregs with IL-2 (22%) and anti-IL-7mAb (17%), but not IL-7 (2%) and IL7/anti-IL-7mAbs (3%) conditions. There were no differences in T cell proliferation or the proportion of memory (CD4+CD44+high) T cells using these conditions at day 5 of MLR (splenocytes). The proportion of Foxp3+ Tregs was higher in spleens of Rag-/- mice reconstituted with Tregs and IL-7/anti-IL-7mAb treatment group (7.0 ± 2.6%) when compared Tregs from control mice (2.6 ± 1.9%) at week 3 of treatment. However, CD4 effectors did not reject skingrafts in either group.
Conclusions: IL-7 supported Foxp3 Treg survival and IL-7/anti-IL-7mAb complexes can expand Tregs in a model of skin allograft tolerance.
References:
[1] Gratz, I. K., H. A. Truong, S. H. Yang, M. M. Maurano, K. Lee, A. K. Abbas, and M. D. Rosenblum. 2013. Cutting Edge: memory regulatory t cells require IL-7 and not IL-2 for their maintenance in peripheral tissues. J Immunol 190: 4483-4487.

627.4

Phospo-specific flowcytometry unveils the incomplete blockade of monocyte activation by tacrolimus in kidney transplant recipients

Nynke Kannegieter, Dennis A.Hesselink, Marjolein Dieterich, Rens Kraaijeveld, Gretchen N.de Graav, Michiel G.H.Betjes, Willem Weimar, Carla C.Baan.
Internal Medicine, Erasmus MC, Rotterdam, Netherlands.

Introduction: Monocytes have been identified as important contributors in triggering rejection processes. Surprisingly, little is known about the effects of immunosuppressive drugs on activation cascades of monocytes. Here single-cell phospho-specific flow cytometry was used to explore the effects of immunosuppression on signaling pathways in monocytes of kidney transplant patients.
Materials and Methods: We measured the phosphorylation of diverse signaling pathways including NFκB, MAPK (p38, ERK) and AKT in peripheral blood monocytes of kidney transplant patients (n=20) the first 6 months after transplantation. Patients received maintenance therapy consisting of tacrolimus, mycophenolate mofetil and prednisolone after receiving basiliximab induction therapy. Both *ex vivo* phosphorylation of these signaling proteins and phosphorylation capacity after PMA/Ionomycin stimulation were determined in CD14+ monocytes by phospho-specific flowcytometry. The individual effects of the prescribed immunosuppressive drugs on p38MAPK, NFκB, AKT and ERK phosphorylation were tested in blood samples from healthy volunteers (n=5).
Results and Discussion: Before transplantation *ex vivo* phosphorylation of p38MAPK, AKT and ERK, but not of NFκB, was highly expressed by monocytes (MFI: 1684, 1073, 492 and 271, respectively) compared to isotype controls (MFI: 605, 602, 191 and 287, respectively; $p<0.001$ for p38MAPK, ERK and AKT). After transplantation these phosphorylated signaling molecules significantly decreased by 23%, 20% and 35% for p38MAPK (figure 1), AKT and ERK, all $p<0.05$, respectively. After *in vitro* stimulation the maximum phosphorylation capacity (MFI: 4902, 2769, 1942, respectively) was 15%, 26% and 5% lower compared to pre-transplant values, all $p<0.05$, respectively.
Therapeutic concentrations of immunosuppressive drugs only partially block monocyte activation pathways. This observation suggests that these cells might be involved in anti-donor responses in kidney transplant patients. The incomplete blockade of monocyte signaling might be explained by autophosphorylation of p38MAPK, a non-canonical and alternative pathway of the MAPK signaling pathway.
To better define the impact of tacrolimus, MPA and prednisolone on p38MAPK phosphorylation, monocytes from healthy controls were studied in the presence of tacrolimus (50 ng/mL), prednisone (100ng/ml) and mycophenolic acid (MPA, 10 µg/ml). Only tacrolimus significantly inhibited the p38MAPK phosphorylation ($p<0.05$). Evidence for autophosphorylation was provided by the use of the MAPK inhibitor SB203580, showing a complete blocking of p38MAPK phosphorylation ($p<0.01$).
Conclusion: Currently prescribed immunosuppressive drugs can inhibit the activation of monocyte signaling pathways after transplantation, but the blockade is far from complete allowing for rejection processes to occur under immunosuppression.

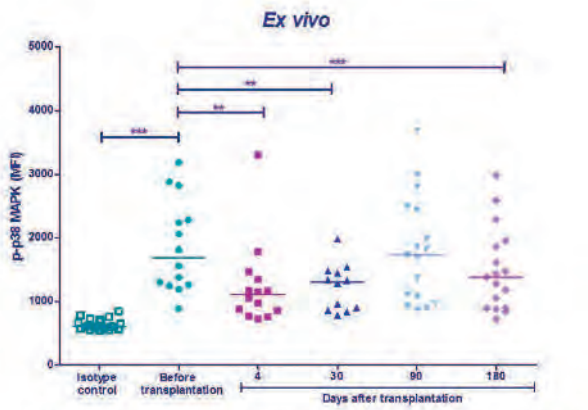


Fig. 1 **Example of decreased signaling pathway phosphorylation after transplantation.** *Ex vivo* phosphorylation of the intracellular signaling protein p38MAPK is significantly inhibited after transplantation compared to pre transplant values in kidney transplant recipients. **)p<0.01; ***) p<0.001

627.5

Placenta-derived decidual stromal cells for treatment of severe acute graft-versus-host disease

Olle Ringden[1], Martin Solders[1], Arjang Baygan[1], Mats Remberger[2], Tom Erkers[1], Silvia Nava[1], Britt Gustafsson[3], Jacek Winiarski[3], Magnus Westgren[4], Behnam Sadeghi[1], Helen Kaipe[1].
[1]Division of Therapeutic Immunology, Karolinska Institutet, Stockholm, Sweden; [2]Center for Allogeneic Stem Cell Transplantation, Karolinska University Hospital Huddinge, Stockholm, Sweden; [3]Dept. of Pediatrics, Karolinska University Hospital Huddinge, Stockholm, Sweden; [4]Dept. of Obstetrics and Gynecology, Karolinska Institutet, Stockholm, Sweden.

Introduction: We introduced mesenchymal stromal cells (MSCs) as therapy for acute GVHD with a dramatic response in some patients, but no response in others[1],[2]. The placenta protects the fetus from the mother's immune system. The placenta has also been used for hundred years to treat burn injuries in Africa. Decidual stromal cells (DSCs) differ from bone marrow-derived (BM) MSCs in greater expansion potential, being half the size, less differentiation capacity to bone or cartilage, and need direct contact with T-cells to inhibit alloreactivity in vitro. In a pilot study, we found that DSCs are immunosuppressive and may cure life-threatening acute GVHD[3].
Materials and Methods: Thirty-six patients were treated with DSCs for severe or steroid-refractory gastrointestinal acute GVHD. The first 17 patients received DSCs dissolved in saline with 5% AB-plasma and all had grade III acute GVHD (DSC group 1). Twenty-one patients were treated with DSCs dissolved in saline with 5% albumin and repeated doses, six had grade II and 15 had grade III acute GVHD (DSC group 2). We included 32 retrospective controls, nine with grade II and 23 with grade III acute GVHD who were not treated with stromal cells. All patients received prednisolone 2 mg/kg/day. Median age in the controls, DSC group 1 and DSC group 2, were 40 (range 3-67), 53 (0.8-65), and 48 (1.3-72.2), respectively.
Results and Discussion: Three patients had transient reactions during DSC infusions. Three patients had zygomycetes infection, three had graft failure and two squamous cell carcinoma. Such events will be elucidated with more patients. Causes of deaths, excluding GVHD, were the same in the DSC patients and the controls. The response rates, no response/partial response/complete response was 24/6/2 in the controls, 7/5/5 in the DSC group 1 and 0/10/11 in the DSC group 2. One-year survival was 3% in the controls, 47% in DSC group 1 and 75% in the DSC group 2 ($p<0.01$ vs. controls). The DSC group 2 also had a better one-year survival, as opposed to 23% among 22 patients treated with BM-MSC ($p<0.01$). Median steroid dose at four weeks after initiation of steroids was 1.67 mg/kg in the controls, 0.7 mg/kg in the DSC group 1, and 0.57 mg/kg in the DSC group 2. Serum albumin, which was decreased at initiation of therapy increased among patients in DSC group 2 ($p<0.01$).
Conclusion: DSCs is a promising treatment for severe and/or steroid-refractory acute GVHD.
References:
[1] Le Blanc K, et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet 2004; 363: 1439.
[2] Ringden O, et al. Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. Transplantation 2006; 81: 1390.
[3] Ringden O, et al. Fetal membrane cells for treatment of steroid-refractory acute graft-versus-host disease. Stem cells 2013; 31: 592.

627.6

Age-related changes in rat donor kidneys and other organs relevant for organ quality and function

Miriam Banas[1], Kathrin Eidenschink[1], Andreas Fuchs[1], Simone Wurm[1], Cornelius Bollheimer[2], Bernhard Banas[1].
[1]Nephrology, University Hospital Regensburg, Regensburg, Germany; [2]Institute of Biomedicine and Aging, University of Erlangen, Erlangen, Germany.

Background: Since the need of deceased donor kidneys for transplantation is much higher than organs available, the acceptance of allografts retrieved from older donors has been markedly increased. Higher donor age is known to be associated with reduced allograft survival. However, acceptance of elder organs is not standardized yet, since the underlying processes in physiological organ aging are not known so far.
Toll-like receptors (TLR), sensors in the innate immune system, have been best characterized as a system to recognize danger signals to the host arising from pathogen-associated molecular patterns. It has been shown that TLR4 activation by endogenous ligands may be one pathological link from unspecific primary allograft damage to subsequent chemokine release, infiltration and activation of immune cells leading to deterioration of renal function and induction of renal fibrosis (Bergler T et al, 2012).
Methods: Using a well-defined rat model we were interested to analyze not only functional and histomorphological changes in aging kidneys, but also age-dependent regulation of the TGF-beta system, of TLRs and of chemokines.
Results: 3 and 24 months old male Sprague Dawley rats were investigated. Comparing rats 3 months of age with 24 months old rats no changes in blood pressure and heart rate could be detected. Old rats showed significantly increased proteinuria. Computer-aided morphometry of glomeruli demonstrated significantly increased accumulation of matrix, collagen and desmin, a marker for podocyte damage, in old rat kidneys. KI 67 staining revealed a significantly reduced cell proliferation in older rat kidneys. TGFß1, TGFß2, Smad2, CCL5 and most TLR investigated (except TLR2,3 and 9) showed a significant higher mRNA expression in 24 months old rat kidneys. The most prominent increase could be noted for TLR 12 (5-fold, $p<0,001$), TLR 5 (4 fold, $p<0,05$) and TLR 10 (4-fold; $p<0,01$). Comparing these results with other organs a differential TLR expression could be noted. In the livers of old rats a significant up regulation of mRNA expression could be detected for TLR2, 3, 4, 6, 7 and 8. In the spleen only TLR7 and 8 showed an up regulation whereas lungs and heart did not reveal any differences. Investigating the TLR ligands and signaling molecules significant differences could be noted in the kidneys of old rats for Fibrinogen beta and gamma, HSP 22, 47, 60, 96, ICAM, Nfkb1 and Myd88. Livers and spleens showed again different expression patterns. As source of TLR ligands in the kidneys, a higher number of apoptotic cells, macrophages and T-cells could be detected.
Conclusion: Healthy 24 months old rat kidneys show significant structural changes compared to younger kidneys. Locally expressed markers of fibrosis and immune activation were associated with kidney aging and infiltration of immune cells was observed. Other organs suitable for transplantation showed a different TLR expression. Regarding these results increasing knowledge about aging will lead to novel methods and markers for classifying pre-transplant organ quality. An adequate selection, combination and dosing of immunosuppressive drugs should help to increase the long-term function of allografts accepted from donors with advanced biological age.

627.7

The function of CD8+/TCR- facilitating cells and regulatory T cells is impaired in Flt3-ligand knockout mice

Yiming Huang, Hong Xu, Thomas Miller, Yujie Wen, Suzanne T.Ildstad.
Institute for Cellular Therapeutics, University of Louisville, Louisville, KY, United States.

Introduction: $CD8^+TCR^-$ facilitating cells (FC) enhance engraftment of hematopoietic stem cells (HSC) in allogeneic mouse recipients without causing graft-versus-host disease. The major subpopulation in murine FC resembles plasmacytoid precursor dendritic cells and induces antigen-specific $CD4^+CD25^+FoxP3^+$ regulatory T cells (Treg) in vivo. The number of $CD8^+TCR^-$ FC and p-pre DC FC are significantly decreased in Flt3 ligand-knockout (FL-KO) mice. In the present study, we evaluated whether FL-KO FC facilitate engraftment of HSC in allogeneic mouse recipients (C57BL/6 to BALB/c).
Materials and Methods: HSC were sorted from the bone marrow (BM) of donor C57BL/6 (B6) mice, and $CD8^+TCR^-$ FC were sorted from donor FL-KO (B6 background) BM. 10,000 HSC and 30,000 FL-KO FC were transplanted into BALB/c recipients conditioned with 750 cGy of total body irradiation (TBI). Control mice received 10,000 HSC with or without 30,000 B6 FC.
Results: Twenty-nine percent (2 of 7) of BALB/c recipients of B6 HSC alone engrafted, with 33% donor chimerism (range 0.8% to 65%). Twenty percent (1 of 5) BALB/c recipients of B6 HSC + FL-KO B6 FC engrafted. In contrast, 53% (8 of 15) of recipients transplanted with B6 HSC + B6 FC engrafted, with an average of 39% donor chimerism (range 1% to 80%). We also tested the number and function of $CD4^+CD25^+Foxp3^+$ Treg in FL-KO mice. The percentage of Treg in peripheral blood (PB) and BM of FL-KO mice were significantly decreased compared with B6 mice ($p < 0.05$). The level of chemokine receptor CXCR3 expressed on Treg from FL-KO PB and spleen were significantly decreased compared with Treg from B6 mice ($p < 0.05$). To test the function of FL-KO Treg in facilitation of engraftment of HSC, Treg were sorted from the spleen of FL-KO mice. 10,000 B6 HSC + 100,000 FL-KO Treg were transplanted into BALB/c recipients conditioned with 750 cGy of TBI. Only 38% of mice transplanted with HSC + FL-KO Treg engrafted (n=8), with 3% donor chimerism. In contrast, 100% of recipients of HSC + B6 Treg engrafted (n = 10), with 62% donor chimerism.
Conclusion: Our data suggest that: 1) the number of FC and p-preDC was significantly decreased in FL-KO mice; 2) the number of Treg and $CXCR3^+$ Treg in PB and spleen was significantly decreased in FL-KO mice; 3) the function of FC from FL-KO BM or Treg from FL-KO spleen was impaired in facilitation of engraftment of allogeneic HSC.

627.8

Murine and human CD4 T cell migration across lymphatic endothelium is differentially regulated by sphingosine 1-phosphate (S1P) receptors

Yanbao Xiong[1], C. Colin Brinkman[1], Keli L.Hippen[2], Bruce R.Blazar[2], Jonathan Bromberg[1].
[1]Surgery, Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD, United States; [2]Division of Blood and Marrow Transplantation, Department of Pediatrics, University of Minnesota Cancer Center, Minneapolis, MN, United States.

T cells utilize S1P and S1PR1 to migrate from thymus across microvascular endothelium to the blood circulation, and across medullary lymphatic endothelium of the lymph node (LN) for egress to efferent lymphatics and the blood circulation. However, whether S1PRs regulate T cell migration from tissues across afferent lymphatic endothelium and into draining LN are unknown. We hypothesized that different S1PRs were used by CD4 T cells and lymphatic endothelial cells (LECs) to promote CD4 T cell afferent lymphatic migration.
Methods: MS-1 blood endothelial and SVEC4-10 lymphatic endothelial cell lines and human and murine primary LECs were used in transwell assays. Human and murine CD4 T cells placed in the upper chamber were migrated across endothelium to chemokines, cytokines or S1P.
Results: The interactions between human and murine CD4 T cells and LEC resulted in enhanced migration toward S1P. Enhance migration was not observed for CD4 T cell interactions with BEC, and was also not observed for several other chemokines and cytokines, including CCL19, CCL21, CXCL12, CCL2, CCL22, and IL6. CD4 T cell migration across LEC was S1P dose dependent, and was both chemokinetic and chemotactic. Pretreatment of CD4 T cells but not LEC with the non-specific S1PR inhibitors pertussis toxin, FTY720, or S1P inhibited CD4 T cell migration across LEC, suggesting that S1PRs played a critical role on CD4 T cell trans-lymphatic endothelial migration. The specific S1PR1, S1PR3, and S1PR4 inhibitors each blocked CD4 T cell function and migration, but did not affect the LEC functional ability to promote CD4 T cell migration. In contrast the S1PR2 inhibitor specifically blocked LEC and their ability to enhance CD4 T cell migration, but did not act on T cells to block migration. S1P upregulated VCAM-1 and VE-cadherin, but not ICAM-1 expression on LECs, and blocking anti-VCAM-1 or anti-VLA-4 mAbs inhibited CD4 T cell migration. Under inflammatory conditions, LEC treated with TNFa further enhanced CD4 T cell migration toward S1P.
Conclusion: CD4 T cells and LEC utilized distinct S1PRs to regulate CD4 T cell migration across afferent lymphatics in a VCAM-1 and VLA-4 dependent fashion. S1P engages active processes in both T cells and LEC to promote migration. These results demonstrate for the first time unique roles for S1PRs in regulating T cell and LEC functions in migration. These findings suggest new and specific drug targets for regulating lymphatic migration in immunity and tolerance.

627.9

Lymph node stromal laminins affect CD4+ T functions and polarization

Thomas Simon, Jonathan Bromberg.
Surgery, Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD, United States.

Two laminin isoforms are particularly associated with lymph node (LN) structure and function, laminin a4b1g1 and a5b1g1. Our recent work demonstrated that tolerance is associated with laminin a4 upregulation that favored regulatory T cell transmigration in the lymph node. In contrast, immunity is associated with laminin a5 upregulation that inhibited Treg migration. Here, we tested the hypothesis that laminins directly impact CD4 T cell activation and Treg polarization.

To investigate whether laminin a4b1g1 and a5b1g1 provide co-inhibitory or co-stimulatory signals, recombinant laminins and mAb anti-CD3 were co-immobilized and CFSE+ CD4 T cells were incubated for 3 days. Addition of laminin a4b1g1 to anti-CD3 stimulated cultures sharply inhibited CD4 T cell proliferation and activation in a dose-dependent manner. In contrast, addition of laminin a5b1g1 increased proliferation and activation marker expression. Addition of laminin a4b1g1 was able to inhibit the immunogenic effect of laminin a5b1g1. Addition of blocking mAb to integrin a6 completely abrogated the stimulatory effect induced by laminin a5b1g1, demonstrating that a6 integrin is the main receptor for laminin a5. We hypothesized that blocking the integrin binding site of laminin a5b1g1 would interfere with immunity. Mice were injected intravenously with blocking anti-a6 mAb along with allogenic cell immunization and transfer of allospecific CD4+ T cells. The systemic blockade of a6 integrin resulted in decreased proliferation and activation of alloantigen specific CD4 T cells in the LNs.

To assess whether laminin a4b1g1 and a5b1g1 affect Treg induction, recombinant laminins and anti-CD3 mAb were co-immobilized and purified CD4 T cells were incubated with IL-2 and anti-CD28 mAb with or without TGF-b for 5 days. The proportion of Treg cells induced by TGF-b decreased by 2 fold in presence of laminin a5b1g1. This effect was also abrogated by anti-a6 integrin blocking mAb. The proportion of Treg cells induced by TGF-b was unaltered by the presence of laminin a4b1g1. However, addition of laminin a4b1g1 was able to partially reverse the inhibitory effect of laminin a5b1g1 on Treg induction.

These results demonstrate that the laminin a4b1g1 and a5b1g1 are co-inhibitory and co-stimulatory ligands, respectively, for CD4 T cells. Laminin a5b1g1 promotes CD4 T cell activation and proliferation and a6 integrin is its primary receptor. Laminin a5b1g1 substantially reduced Treg generation, confirming the immunogenic role of this laminin. In contrast, laminin a4b1g1 strongly inhibited T cell activation and opposed the immunostimulatory effect of laminin a5b1g1. These findings demonstrate that laminins are not only LN structural molecules, but act as molecular switches for tolerance and immunity by directly influencing T cell proliferation and polarization.

628.1

Monitoring leukocyte trans-lymphatic endothelial migration with an in vitro model

Yanbao Xiong[1], C. Colin Brinkman[1], Keli L.Hippen[2], Bruce R.Blazar[2], Jonathan Bromberg[1].
[1]Surgery, Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD, United States; [2]Division of Blood and Marrow Transplantation, Department of Pediatrics, University of Minnesota Cancer Center, Minneapolis, MN, United States.

Trans-endothelial migration (TEM) is an essential process for leukocyte circulation. While much is known about blood to tissue migration, far less is known about tissue to lymphatic migration. There are several in vivo models for examining lymphatic TEM, such as ear pinna and footpad assays, and some in vitro models with complex equipment requirements. Our aim was to create a robust in vitro system to evaluate lymphatic TEM for various cell types, migration to several different chemo-attractants, and have the capacity to integrate the complexities of stromal variability and fluid flow.

Methods: MS-1 blood endothelial (BEC) and SVEC4-10 lymphatic endothelial cell (LEC) lines and human and murine primary LEC were used in transwell assays. Human and murine CD4 T cells placed in the upper chamber were migrated across endothelium to chemokines, cytokines, or sphingosine 1-phosphate (S1P); while stromal fibers and fluid flow variables were assessed.

Results: Human and murine CD4 T cells migrated through LEC, but not BEC, in a vectorial fashion, migrating only from the abluminal to the luminal surface but not in the reverse direction. The migration was chemotactic and dose-dependent to chemoattractants. Under interstitial-type flow conditions, CD4 T cells migrated more toward S1P and other chemokines across LEC than without flow. Mouse LEC lines and primary LEC cells were characterized by high expression of Lyve-1, Prox1, Vegfr3, CD31 and gp38, but not PNAd, confirming their LEC origin and phenotype. Stromal support of the LEC with collagens (gelatin), laminin a4 or a5, were equally effective in enhancing LEC growth and layer integrity and assuring leukocyte vectorial migration. S1P was also required for LEC growth and layer integrity. Expression of VCAM-1 and ICAM-1 by LEC was increased by the inflammatory cytokines TNFa or IFNg; and CD4 T cell migration toward S1P and CCL19 were increased across TNFa pretreated LEC, which was inhibited by anti-VCAM1/anti-VLA4 treatment. Activated and memory CD4 T cells and dendritic cell also showed vectorial migration, but CD8 T cells did not migrate in a vectorial fashion.

Conclusion: We devised a simple system that modeled lymphatic TEM through LEC monolayers, validated the vectorial migration, established the ability to assess different cell types migration to various chemo-attractants, showed that human and murine cells behaved identically, and that lymphatic flow and stromal structural element variables can be easily incorporated into the model. This system paves the way for high throughput screening of drugs for regulating lymphatic migration in immunity or tolerance.

628.2

Variable effects of different serotonin receptor subtype antagonists on the development of transplant vasculopathy in murine aortic allografts

Christian Heim, Annika Gocht, Jörg Distler, Bernd Spriewald, Martina Ramsperger-Gleixner, Michael Weyand, Stephan M.Ensminger.
University of Erlangen, Erlangen, Germany.

Previous studies suggest an important role of platelets on developing transplant vasculopathy, the hallmark feature of chronic rejection. The signaling molecule serotonin (5-HT) is stored within platelets until cell activation and is involved in proliferation of vascular smooth muscle cells. Therefore, the aim of this study was to investigate the effect of 5-HT2A and 5-HT2B receptor antagonists on transplant vasculopathy (TV).
Fully allogeneic C57BL/6 (H2b) donor aortas were transplanted into CBA (H2k) recipients. Recipient mice were treated with the specific 5-HT2A antagonist sarpogrelate (10 mg/kg/d), the specific 5-HT2B antagonist SB204741 (10 mg/kg/d), and the non-selective 5-HT2 receptor antagonist terguride (0.6 mg/kg/BD). Grafts were analysed by morphometry and immunohistology on day 30 after transplantation. Intragraft gene expression was measured using quantitative RT-PCR on day 14 after transplantation.
In mice treated with sarpogrelate, transplant vasculopathy was reduced as compared to untreated controls (intima proliferation 43%+/-8% vs. 54%+/-13% [control]/p<.05; n=7). Daily application of the 5-HT2B antagonist SB204741 did not reduce the development of TV (intima proliferation of 49% +/- 9% vs. 41% +/- 13% [control]/p=n.s.; n=8). Interestingly, the non-selective 5-HT2 receptor antagonist terguride did not reduce the formation of TV either (intima proliferation: 56% +/- 13% vs. 54% +/- 13% [control]/p=n.s.; n=10). In addition experimental groups revealed a down regulation of ICAM-1, PDGF, VEGF, IFNγ, MCP1, CD40L, P-Selectin, and TGF-β, and upregulation of eNOS after sarpogrelate treatment. Terguride treatment significantly reduced PDGF, IFNγ, and TGF-β expression within the graft. Immunohistochemical analysis of 5-HT2A receptor distribution revealed a significantly decreased expression within the neointima of sarpogrelate treated animals in contrast to SB204741 or terguride treated mice. Furthermore, sarpogrelate treatment resulted in a lower amount of infiltrating macrophages within the media.
These results demonstrate that antagonists of the serotonin subtype receptor 5-HT2A can dramatically reduce the development of TV in a mouse aortic allograft model. Antagonisation of 5-HT2B receptors alone or in combination did not result in reduced intima proliferation, suggesting that specific 5-HT2A antagonism appears to be a very attractive strategy for prevention of TV that deserves further experimental and clinical evaluation.

628.3

Various pathogenic microbes induce CNI-resistance in B-cells responding to blood group antigens through TLR–MyD88 pathway

Hiroshi Sakai, Yuka Tanaka, Hideki Ohdan.
Gastroenterological and Transplant Surgery, Hiroshima University, Hiroshima, Japan.

Introduction: Various microbial infections following ABO-incompatible transplantation (ABOi-Tx) are associated with refractory antibody (Ab)-mediated rejection (AMR) in humans. Here, we demonstrated that various pathogens can induce B cells, which respond to blood group A antigens (A-Ag), to be resistant to calcineurin (CN) inhibitors via TLR–MyD88 signaling (-sig). These pathogens possess agonistic activities towards Toll-like receptors (TLR).
Materials and Methods: We immunized cyclosporin (CsA)-treated Balb/c mice with A-Ag. The phenotype of B cells responding to A-Ag was analyzed by flow cytometry, while anti-A Ab levels in the mice sera were analyzed by ELISA. B cells from Balb/c splenocytes were stained with CFSE, and stimulated in vitro with anti-IgM F(ab')2 (a-IgM), with or without the respective TLR-agonist subtype (Pam3CSK4 for TLR1/2, poly(I:C) for TLR3, LPS for TLR4, FSL-1 for TLR6/2, and CpG-ODN for TLR9). B cells from MyD88- or TRIF-deficient mice were also stimulated with a-IgM and LPS in vitro. The phenotype and frequency of proliferating B cells were analyzed by flow cytometry, while NFATc1 expression in these cells was analyzed by western blot.
Results: The number of CD5+B-1a cells (B1a) with B-cell receptors (BCR) for A-Ag increased in Balb/c mice sensitized with A-Ag. Anti-A Ab production was inhibited by CsA. Consistently, B cells stimulated with a-IgM in vitro differentiated into B1a, which was abrogated by CsA. B cells stimulated with a-IgM and poly(I:C), which induces TLR3–TRIF-sig independent of MyD88-sig, differentiated into B1a. However, treatment with a-IgM and other TLR-agonists that activate MyD88-sig, induced differentiation of the B cells into CD5dimB-1b cells (B1b). We also analyzed the susceptibility of proliferating B cells to CsA, focusing on TLR4-sig, the only TLR subtype that activates both MyD88-dependent and –independent (TRIF-dependent) signals. B1b with BCR for A-Ag significantly increased in the mice after exposure to A-Ag together with LPS in vivo. Anti-A Ab production was not inhibited by CsA. Concurrently, when B-cells in Balb/c or TRIF-deficient mice were treated with a-IgM and LPS in vitro, they differentiated into CsA-resistant B1b. On the other hand, MyD88-deficient B cells differentiated into B1a, which was abrogated by CsA regardless of LPS administration. This indicated that B1b differentiation as well as resistance to CsA depends on MyD88-sig. Western blot results revealed that treating proliferating B1b with LPS along with a-IgM enhanced NFATc1 expression in these cells, but not in MyD88-deficient B cells.
Discussion: BCR-crosslinking activates CN/NFATc1, leading to differentiation to B1a, which can be inhibited by CsA. However, various pathogens induce TLR–MyD88-sig in B1b, independent of CN activity, and enhance BCR-sig by activating NFATc1, a downstream factor of CN. Therefore, the inhibitory effect of CsA on BCR-sig is disabled by the activation of MyD88-sig in B1b.
Conclusion: Various infections after ABOi-Tx may induce CsA-resistant AMR by activating B1b via the TLR–MyD88 pathway.

628.4

Alloreactivity drives the repopulation and the maintenance of human intestinal graft tissue-resident memory cells

Julien Zuber , Brittany Shonts, Sai Ping Lau, Aleksandar Obradovic, Jianing Fu, Suxiao Yang, Shana Coley, Joshua Weiner, Joseph Thome, Susan DeWolf, Donna Farber, Yufeng Shen, Govind Bhagat, Adam Griesemer, Mercedes Martinez, Tomoaki Kato[1], Megan Sykes. Columbia University Medical Center, Columbia University, New York, United States.

Large numbers of donor tissue-resident memory T cells are transferred within intestinal grafts. However, little is known about how Host-vs-Graft (HvG) and Graft-vs-Host (GvH) responses influence the turnover, phenotype and repertoire of graft-resident T-cell populations following transplantation and how the balance between HvG to GvH responses correlates with clinical outcomes. We hypothesized that HvG-reactive recipient cells would hasten the replacement of donor leukocytes in the presence of rejection and that normal, physiologic leukocyte turnover could be measured in the absence of rejection.

Phenotype and donor /recipient origin of graft-resident lymphocytes were investigated prospectively with multicolor flow cytometry from 148 fresh ileum graft biopsies from 13 intestinal transplant recipients. Low levels of recipient chimerism among CD4+ and CD8+ αβ TCR+ graft-resident T cells during the first 45 days were significantly associated with the absence of biopsy-proven rejection. Over time, cell replacement rates in the graft were significantly faster over the first 3 and 6 months post-transplant in patients with mixed (humoral plus cellular) rejection compared to those without (**Figure 1**).

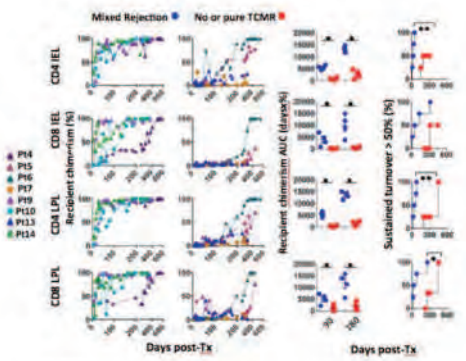


Figure 1: Relationship between rejection and recipient chimerism in intestinal allografts.
Depict 136 biopsies obtained from 8 patients with a longitudinal follow-up from POD0 to up to POD600. Recipient chimerism over time in CD4+ and CD8+ abTCR+ intra-epithelial lymphocytes (IELs) and lamina propria lymphocytes (LPL) isolated from serial biopsies, according to the occurrence of mixed rejection or not. Dots and triangles represent multivisceral transplant and isolated intestinal transplant recipients, respectively. AUC, Area Under the Curve; Pt, Patient; TCMR, T-cell-mediated rejection. The right panels show that patients with mixed rejection experienced faster replacement of donor cells by recipient cells than those without mixed rejection (* p<0.05, ** p<0.01).

The phenotype of recipient-derived T cells isolated from early biopsies differed significantly from that of donor cells. Moreover, at the times of rejection, we detected a large population of CD28+ NKG2Dhi CD8+ cells among recipient intra-epithelial lymphocytes ($p<0.0001$). However, recipient T cells eventually acquired tissue-resident phenotypic markers (CD103+ CD69+ CD28-).

Next, we identified (pre-transplant) and tracked the donor-reactive TCR repertoire in post-transplant biopsies using a recently developed high-throughput sequencing approach[1]. We investigated 5 biopsies with early rejection, from days 12 to 24. HvG-reactive clones (up to 80% of recipient clones) were highly enriched relative to non-HvG-reactive clones in the biopsies with rejection compared to pre-transplant peripheral lymphoid samples and declined after the resolution of rejection (**Figure 2**). However, the observed long-term persistence in the grafts of donor-reactive T cells, albeit at a lower frequency compared to rejection biopsies, may pose a constant threat to the allograft.

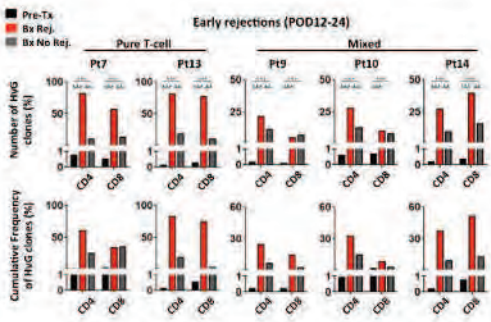


Figure 2: Evolution of HvG recipient T cell clones in intestinal allografts.
Frequency of HvG clones in rejecting biopsies (red) compared to pre-transplant lymph node (Pts 9, 13 and 14) or spleen (Pts 7 and 10) samples (black) and post-rejection biopsies (gray). The denominator includes all the clones that were identified in pre-transplant recipient samples (HvG plus all other clones). HvG clone frequencies were compared using Fischer exact test; *** $p< 10^{-25}$; ** $p< 10^{-10}$; * $p< 10^{-3}$.

Similarly, we identified GvH-reactive clones from donor pre-transplant samples. Remarkably, in the absence of overwhelming rejection, preexisting donor graft-resident T cells with GvH reactivity expanded in the graft in association with delayed replacement of donor cells by recipient T cells. Donor T cells far predominated over recipient cells at 1 year post-transplant in the two recipients with the highest GvH/HvG clones ratio within the graft, in contrast to the others.

To conclude, we found that the replacement rate and the phenotype of intestinal graft-resident T cells correlates strongly with both clinical outcome and the dynamics of the two-way alloimmune response.

The study was funded by the Schaefer research scholar program and NIAID grant P01 AI106697

References:

[1] (1): Morris H. et al. Sci Transl Med 2015 Jan 28; 7(272):272ra10

628.5

Vascular sequestration of donor-specific antibodies protects allogeneic islets from humoral rejection

Chien-Chia Chen[1], Eric Pouliquen[1], Alexis Broisat[2], Dimitri Chartoire[1], Alice Koenig[1], Mitra Ahmadi[2], Sandrine Bacot[2], Catherine Ghezzi[2], Emmanuel Morelon[1,3,4], Thierry Defrance[1], Olivier Thaunat[1,3,4].
[1]Unité 1111, INSERM, Lyon, France; [2]Unité 1039, INSERM, Grenoble, France; [3]Service de Transplantation, Néphrologie et Immunologie Clinique, Hospices Civils de Lyon, Hôpital Edouard Herriot, Lyon, France; [4]Unité de Formation et de Recherche Lyon Est, Université de Lyon, Lyon, France.

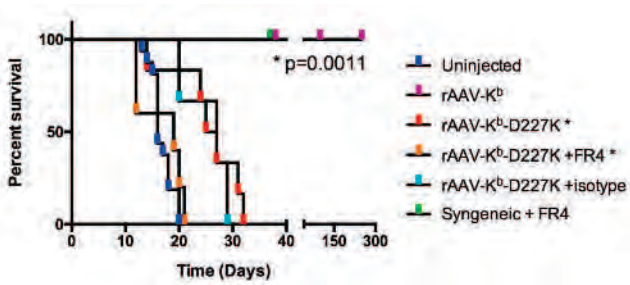
Introduction: Islets grafting restores endogenous insulin production in Brittle type 1 diabetic patients but long-term outcomes remain disappointing due to destruction of allogeneic islets by recipients' adaptive immune system.
In solid organ transplantation, antibody-mediated rejection (AMR) is now recognized as the first cause of transplant failure. This experimental murine study aimed at determining whether donor-specific antibodies (DSA) also contribute to islet grafts destruction.
Methods and Results: Diabetes was induced by streptozotocin injection in RAG2 KO C57BL/6 (H-2^b) mice, which lack T and B cells. Allogeneic (CBA, H-2^k) islets were not rejected by these immunocompromised recipients, which remained euglycemic until the end of the follow-up (120 days). DSA (either polyclonal immune sera or murine IgG2a anti H-2^k mAb) were able to bind to CBA islets and induce complement-dependent destruction on both β cell line and dissociated islet cells in vitro. This cytotoxic effect could be boosted by pre-incubation of β cells in interferon-γ, which increased the expression of MHC molecules. In contrast, repeated IV injections of DSA did not impact CBA islet grafts function in vivo, even if poly(I:C) IP injections were performed to upregulate MHC expression on islet cells. Flow cytometry analysis of dissociated islet after grafting showed endothelial cells from the recipient take place of those from the donor to re-establish vascularisation. Live imaging studies, using radiolabelled DSA, showed that alloantibodies were sequestrated in recipients' vascular bed. As a consequence radiolabelled DSA were able to bind to allogeneic endothelium of CBA heart transplant, but failed to reach CBA islets.
Conclusion: Our experimental study demonstrates that, in contrast with solid organ transplants, islet grafts are protected from humoral rejection. This is due to vascular sequestration of DSA and the fact that graft vascularization arise from the recipient. Our results are in line with the clinical observation we recently made that appearance of DSA did not accelerate the rate of islet graft attrition in the GRAGIL cohort of islet recipients.

628.6

Disruption of CD8-coreceptor binding abrogates tolerance induction via liver-directed expression of donor MHC class I

Moumita Paul-Heng[1], Eithne Cunningham[1], Daniel Bunker[1], Mario Leong[1], Zane Wang[1], Chuanmin Wang[1], Szun Tay[2,3], Claire McGuffog[2], Grant Logan[3], Ian Alexander[3], Patrick Bertolino[2], G. Alex Bishop[1], David Bowen[1,2], Alexandra Sharland[1].
[1]Transplantation Immunobiology Research Group, The University of Sydney, Sydney, Australia; [2]Liver Immunology Group, Centenary Institute, Sydney, Australia; [3]Gene Therapy Research Unit, Children's Medical Research Institute and The Children's Hospital at Westmead, Sydney, Australia.

Background: We use an AAV vector to express donor MHC (H-2K^b or H-2K^d) in recipient livers and induce donor-specific tolerance in a mouse skin transplant model. K^b expression in B10.BR mice led to impaired production of IFN-γ in response to K^b, despite persistence of K^b-specific CD8+ T cells, suggesting that tolerance was associated with functional silencing[1].
Aims and Methods: To determine the role of direct recognition of class I by CD8-dependent T cells in tolerance induction, we generated rAAV-K^b-D227K and K^d-D227K where a point mutation in the class I alpha 3 domain abrogates CD8 binding and direct allorecognition but does not interfere with indirect allorecognition. Expression of WT or mutant K^b or K^d and PD-L1 on hepatocytes was assessed by FACS and IHC. K^b-bearing 178.3 skin was grafted onto uninjected recipients and mice injected with rAAV-K^b-D227K or rAAV-K^b ± PD-L1 blo ckade.
Results: Uninjected B10.BR mice rejected 178.3 skin (MST=16 d, n=6), while grafts onto rAAV-K^b-injected mice survived long term (MST>250, n=5). rAAV-K^b-D227K only slightly prolonged graft survival (MST=27, n=6). These results were recapitulated in the B6.Kd to C57BL/6 model. The modest survival prolongation of K^b-bearing skin grafts in mice inoculated with K^b-D227K was abrogated when the recipients were treated with anti-FR4 (clone TH6) to deplete Tregs. K^b but not K^b-D227K expression in B10.BR livers or K^b expression in C57BL/6 livers increased hepatocyte surface PD-L1, suggesting that alloreactive CD8+ T cell activation results in PD-L1 upregulation on liver parenchyma. Blockade of PD-L1 plus rAAV-K^b caused hepatitis, with elevated ALT levels (721±73) compared to rAAV-K^b (33±4) or anti-PD-L1 (35±2) alone.



Conclusions: Disrupting CD8 coreceptor engagement abolishes tolerance induction via liver-directed expression of donor MHC class I, indicating that direct recognition by CD8-dependent T cells is essential for the process. Indirect recognition of Kb allopeptides and generation of Tregs can produce a modest prolongation of K^b-bearing skin graft survival but does not induce tolerance. PD-L1 upregulation might play a role in the functional silencing of CD8+ T cells in this model[1].
References:
[1] Cunningham EC et al., Transplantation. 2013 Jan 15;95(1):70-7.

628.7

Long-lasting expansion of memory-like natural killer cells in the liver after intraportal syngeneic islet transplantation in mice

Yoshihiro Saeki, Kohei Ishiyama, Nobuki Ishida, Yuka Tanaka, Ohdan Hideki.
Gastroenterological and Transplant Surgery, Hiroshima University, Hiroshima, Japan.

Introduction: It has been recently reported that liver-resident DX5$^-$ NK cells expressing TRAIL have potent memory functions, which include differentiation into a long-lived memory subset and robust antigen-specific expansion to a secondary challenge. We have previously demonstrated that liver TRAIL$^+$ NK cells play a crucial role in islet graft loss after intraportal islet transplantation (IT). Taken together with those facts, we investigated whether the acquisition of memory functions in liver DX5$^-$ NK cells after primary IT may cause the vigorous islet destruction at subsequent IT sites.
Methods: Liver NK cells from C57BL/6 mice were cultured for 24 h in the presence of TNFα, IFNγ, and IL-1β, which are predominantly and transiently produced under IBMIR conditions. To evaluate the phenotypic and functional alteration of liver NK cells from the early to the late phase after syngeneic IT, surface molecules on liver NK cells, collected from a recipient at 24 h and 35 d after IT, were analyzed. For evaluating the memory functions of NK cells, we established a second IT model; graft survival was assessed by determining plasma glucose levels of recipients after receiving 400 islets, which is normally sufficient to reverse diabetes, following a primary IT with 200 islets.
Results: The expression of CD69, TRAIL, and CXCR3 on liver DX5$^-$ NK cells was significantly higher than that on DX5$^+$ NK cells. The expression of both CD69 and CXCR3 was significantly higher on liver NK cells in vitro culture system mimicking IBMIR conditions (24 h/fresh ratio; 2.0, $p < 0.01$; 1.5, $p < 0.01$, $n = 7$, respectively). Furthermore, the population of DX5$^-$ NK cells expanded 2.4 times (73.1 ± 11.0% vs. 32.5 ± 5.6%, $p < 0.01$, $n = 7$) under the cytokine stimulation mainly associated with TNFα. The population of DX5$^-$ NK cells promptly expanded and the expression of CD69, TRAIL, and CXCR3 on liver NK cells were significantly increased at 24 h after IT, meanwhile the administration of anti- TNFα antibody suppressed DX5$^-$ NK cell expansion and activation. Consequently, a significant and persistent increase of DX5$^-$ NK cells, expression of CD69, TRAIL, and CXCR3 on liver NK cells was observed after IT (35 d/0 d ratio; 2.2, $p < 0.01$, 2.3, $p < 0.01$, 3.7, $p < 0.01$, 2.0, $p < 0.05$, $n = 8$, respectively). In addition, the expansion of DX5$^-$ NK cells and CXCR3 expression on liver NK cells was significantly correlated with disturbed graft engraftment at day 35 ($r = 0.89$, $p < 0.01$; $r = 0.77$, $p < 0.05$, $n = 8$, respectively). Furthermore, hyperglycemia could not be reversed in recipients that received a second IT following first priming.
Conclusions: Our data indicate that exposure of liver-resident DX5$^-$ memory-like NK cells to IBMIR early after intraportal syngeneic IT leads to persistent and long-lasting NK cell cytotoxicity even against subsequent islet grafts. These results suggest that regulating of liver DX5$^-$ memory-like NK cells may improve the engraftment of sequentially transplanted islets.

628.8

Blocking m-TOR pathway specifically in myeloid system abate chronic allograft rejection by inhibiting immune cell activation and infiltration

Chenglin Wu[1,2], Changxi Wang[1], Ronghai Deng[1], Longshan Liu[1], Linwei Wu[1], Xiaoshun He[1], Xianchang Li[2].
[1]Organ Transplant Center, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, People's Republic of China; [2]Immunobiology &Transplant Science Center, Houston Methodist Hospital Texas Medical Center, Houston, United States.

Despite remarkable improvements in short term transplant survival, the long-term outcome of allografts remains poor in the clinic, and most transplants are eventually lost due to chronic rejection. m-TOR pathway plays an important role in acquired immune cell activation and acute rejection, but its role in innate immune cell activation and chronic rejection remains poorly defined. This experiment is intended to explore the exact mechanism of m-TOR pathway in regulating the activation of innate immune cells and chronic allograft loss. We used a mouse heart transplant model to examine cell types that infiltrate chronically rejected allografts and their roles in chronic allograft loss. We found that treatment of B6 recipients with a single dose of CTLA-4Ig prolonged the survival of Balb/c heart allografts (MST=31d) as compared to untreated controls (MST=8 days). Histologically, the CTLA-4Ig treated grafts showed signs of extensive chronic rejection characterized by heavy leukocyte infiltration and prominent neointima formation. Phenotypically, macrophages were a key infiltrating cell type in the vascular lesions in the grafts. Then we established a conditional knockout mice in which the m-TOR gene was missing only in myeloid system (using Lyz - Cre and mTOR – Flox mice). Compared to the wild-type recipients, the graft survival time of conditional knockout recipients was significantly prolonged (60 day vs.30 day), which was associated with markedly reduced infiltrating immune cells and neointima generation in the grafts. Flow cytometry analysis revealed that the number of infiltrating macrophages and CD3+ lymphocytes decreased significantly. Interestingly, the ratio of mature macrophages who express CD86 exhibited the most remarkable reduction. More importantly, among the infiltrating lymphoid cells, both the number and ratio of CD4 +cells were significantly decreased (17.7% vs 0.96%), but this change was not obvious in spleen. In vitro, we found that during the process of bone marrow-derived macrophage differentiation, the expression of co-stimulating molecules CD80 and CD86 was lower in m-TOR myeloid knockout mice as compared to wild type mice. As antigen presenting cells, when cultured with naïve T cells, bone marrow-derived macrophage from knockout mice could significantly inhibit the proliferation and activation of naïve T cell. Our studies identified a previously unknown mechanism of chronic allograft loss and pinpointed m-TOR as a molecular target in the inhibition of immune cells infiltrating and chronic rejection.

628.9

Soluble BAFF is associated with increased risk of graft loss but not with pre-transplant IgM to post-transplant IgG DSA conversion

Anh Nguyen[1], Lorita M.Rebellato[2], Junchao Cai[1], Wei Huang[1], Carl E. Haisch[2], Kimberly P. Briley[2], Paul Bolin[2], Scott A. Kendrick[3], Claire Morgan[2], Angela Q. Maldonado[4], Matthew Everly[1].
[1]Terasaki Foundation Laboratory, Los Angeles, CA, United States; [2]East Carolina University, Greenville, NC, United States; [3]Eastern Nephrology Associates, Greenville, NC, United States; [4]Vidant Medical Center, Greenville, NC, United States.

Introduction: B cell activating factor (BAFF), a B cell survival factor and member of the TNF ligand superfamily, is involved in B cell survival, maturation, and activation. Recently, it has been linked to increased risk of DSA development and graft dysfunction. Our aim was to investigate if soluble BAFF (sBAFF) detection could predict post-transplant IgG DSA conversion from pre-transplant IgM specific to the donor and detriment to the graft.
Materials and Methods: We retrospectively analyzed 218 consecutive kidney transplant patients at a single center, transplanted between Jan 2006 and Dec 2010, with a minimum 3 year follow-up. We excluded 20 patients from the analysis due to HLA-matched transplant, insufficient patient information, or lack of patient sera. Sera sampling occurred pre-transplant, 1, 3, 6, 9, 12 months post-transplant and annually thereafter. Antibody identification was determined by LABScreen single antigen beads. In a cohort of patients with pre-formed IgM, but without IgG HLA antibodies specific for the donor (n=51), we checked sBAFF levels on the date of IgG DSA appearance post-transplant and 2 samples prior to that, when possible. We measured sBAFF using beads (made in-house) and used a positive cut-off of 3000MFI.
Results and Discussion: Of the 51 patients, 22 patients (43%) converted IgM to IgG DSA of the same specificity post-transplant, with >50% (12/22) converting within the 1st year. Five patients were not tested for sBAFF since their DSA appeared in the 1st sample post-transplant (n=4) and 1 patient had insufficient sera. A patient was considered positive if any of their 3 testing points tested positive. sBAFF positivity was not associated with pre-transplant IgM to post-transplant IgG DSA conversion (Fig. 1A), although there was a trend for sBAFF positive patients to convert (10/19 or 53% sBAFF positive patients convert vs. 8/27 or 30% sBAFF negative patients convert). However, sBAFF positivity was significantly associated with graft loss (Fig. 1B, p=0.0005). 58% (11/19) of sBAFF positive patients went on to have graft loss whereas only 7% (2/27) of sBAFF negative patients did so.

1A

	No Conversion	Conversion
sBAFF<3000	19	8
sBAFF>3000	9	10
		p=0.1371

1B

	No Graft Loss	Graft Loss
sBAFF<3000	25	2
sBAFF>3000	8	11
		p=0.0005

Conclusion: Elevated sBAFF levels are associated with graft loss in a cohort of patients with pre-transplant donor-specific IgM. We found no association with sBAFF and post-transplant IgG DSA conversion from IgM. These findings are within a small cohort and needs to be expanded to include patients without IgM pre-transplant.

629.1

Genetically modified porcine neonatal islet xenografts provide long-term survival in baboons

Wayne J. Hawthorne[1,2], Joanne Hawkes[1], Evelyn Salvaris[3], YiVee Chew[1], Heather Burns[1], Sussan Davies[1], David Liuwantara[1], Helen Barlow[3], Jamie Brady[4], Andrew Lew[4], Simon Robson[5], Mark Nottle[6], Philip J. O'Connell[1,2], Peter Cowan[3].
[1]The Centre for Transplant & Renal Research, The Westmead Institute for Medical Research, Westmead, Australia; [2]Westmead Clinical School, Sydney Medical School, University of Sydney, Sydney, Australia; [3]Immunology Research Centre, St Vincents Hospital, Melbourne, Australia; [4]Walter and Eliza Hall Institute, Melbourne, Australia; [5]Beth Israel Deaconess Medical Centre, Harvard Medical School, Boston, MA, United States; [6]Department of Obstetrics and Gynaecology, University of Adelaide, Adelaide, Australia.

Background: Xenotransplantation of porcine neonatal islet cell clusters (NICC) is a promising approach to expand the application of clinical islet transplantation. We have previously reported that deletion of the αGal xenoantigen (GTKO) and transgenic expression of the human complement regulators CD55 and CD59 protects NICC xenografts from IBMIR in baboons immunosuppressed with a clinical protocol (ATG, MMF, Tac). However, despite this early protection, analysis of biopsies revealed rejection of NICC within one month with a heavy lymphocytic infiltration. We therefore moved to a co-stimulation blockade-based immunosuppressive regimen, incorporating our chimeric anti-CD2 mAb to deplete T cells.
Aim: To achieve prolonged survival and function of NICC xenografts in baboons.
Methods: Long-term (>1 year) diabetic baboons (n=2) were transplanted intraportally with GTKO/CD55/CD59/H-transferase NICC at a dose of 50,000 or 10,000 IEQ/kg body weight. Immunosuppression consisted of anti-CD2, anti-CD154, belatacept and Tacrolimus. Graft function was assessed by measuring blood glucose levels multiple times daily and performing IVGTT and OGTT at regular intervals, complemented by immunohistochemical analysis of biopsies.
Results: The recipient transplanted with the higher dose of NICC showed a steady reduction in daily insulin requirement, and exogenous insulin was stopped altogether by 9 weeks. Insulin independence was maintained for more than 7 months, by which time immunosuppression had been reduced to monthly belatacept. HbA1c levels stabilised at around 5%. Biopsies showed well-formed islets containing cells that stained positive for insulin, glucagon or somatostatin, with no cellular infiltrate. Normal IVGTT responses were observed, with significant porcine insulin and c-peptide detected in response to glucose stimulation. The graft was rejected more than 12 weeks after the cessation of belatacept. Remarkably, insulin independence was also achieved in the animal receiving one-fifth the islet dose, albeit over a longer timeframe (4 months). This recipient is currently exhibiting normal glucose handling at 9 months post-transplant, under tapering immunosuppression.
Conclusions: This is the first demonstration of insulin independence in the stringent pig-to-baboon islet xenograft model. Furthermore, to our knowledge, the dose of islets used in the second recipient (10,000 IEQ/kg) is the lowest to achieve normoglycaemia in any nonhuman primate model. This supports the concept that neonatal pig islet xenografts will mature and expand to the extent required to efficiently regulate glucose metabolism in primate recipients.

629.2

Multi-transgenic donor pigs combined with targeted drug treatments extend life-supporting organ function in a xenogeneic lung transplantation model

Lars Burdorf[1], Christopher Laird[1], Dawn Parsell[1], Natalie O'Neil[1], Ivan Tatarov[1], Tianshu Zhang[1], Selin Sendil[1], Joseph Rabin[1], Siamak Dahi[1], Donald Harris[1], Natalia Kubicki[1], Elana Rybak[1], Carol J.Phelps[2], David L.Ayares[2], Agnes M.Azimzadeh[1], Richard NPierson [1].
[1]Surgery, University of Maryland, Baltimore, MD, United States; [2]Revivicor, Inc., Blacksburg, VA, United States.

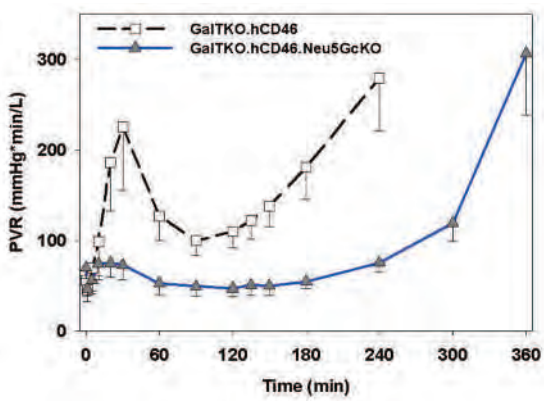
Purpose: Life-supporting (LS) lung xenograft (xLTX) function was tested in a rigorous pig-to-baboon model, using various multi-transgenic donor pigs, **coupled with mechanism-targeted drug treatments**.
Methods: Left single lung transplantations were performed using porcine donor organs with 6 genetic modifications (6GE) into baboons (n=22). All lungs were GalTKO.hCD46.hEPCR, also expressing 3 (of 7) additional transgenes (hCD55, hTBM, hCD47, hCD39, hTFPI, hHO-1 or A20). Recipients consistently received steroids, C1 esterase inhibitor, heparin, thromboxane synthase inhibitor, histamine receptor blockers and anti-GPIb Fab, and donor pigs were treated with DDAVP prior to lung procurement, to deplete pig endothelial VWF, all based on our prior work in an ex vivo xenolung perfusion model. In some recipients, immunosuppression (ATG, MMF, and Tacrolimus or aCD40 AB), anti-IL6 receptor blocker (Tocilizumab) and/or Alpha-1 Antitrypsin were given. LS xenograft function was assessed intermittently by occluding the right (contralateral to the transplant) pulmonary artery, flow measurements to the transplanted graft and CT scans.
Results: Unlike various other 6–gene pigs lungs (maximum survival ~12 hours), GalTKO. hCD46.hCD55.hEPCR.hCD47.hTFPI lung recipients (n=13) usually exhibited prolonged LS lung function (up to >30hrs; range 20->30). Two recipients with persistent life-supporting lung function exhibited unexpected ventricular fibrillation at 30 hrs under sustained general anesthesia: in these 2 cases the lung appeared macro- and microscopically grossly normal. Chest closure and extubation was successfully performed in 7 baboons (of 10 attempted). Post-transplant survival of 6GE lung recipients ranged from 1 to 8 days. Failure of the transplanted organ was predominantly associated with vascular barrier function injury and lung consolidation.
Conclusion: The combination of 6GE pig lungs with drugs blocking other known pathogenic pathways (VWF/GPIb interaction, thromboxane and histamine release, cytokine elaboration), is associated with prolonged LS xeno lung function; some recipients exhibit life-supporting lung xenografts beyond 24 hours. We hypothesize that this model will be useful to unveil residual mechanistic obstacles to clinical xenograft application.

629.3

Knock-out of N-Glycolylneuraminic acid (Neu5Gc) in GalTKO.hCD46 pig lungs improves pulmonary function in a xenogeneic pig-to-human lung perfusion model

Lars Burdorf[1], Siamak Dahi[1], Franchesca Ali[1], Emily Redding[1], Dawn Parsell[1], Beth French[1], Evan Schwartz[1], Elizabeth Kang[1], Xiangfei Cheng[1], Gheorghe Braileanu[1], Evelyn Sievert[1], Tianshu Zhang[1], Elana Rybak[1], Carol J.Phelps[2], David L.Ayares[2], Agnes M.Azimzadeh[1], Richard NPierson [1].
[1]Surgery, University of Maryland, Baltimore, MD, United States; [2]Revivicor, Inc., Blacksburg, VA, United States.

Background: Due to a gene mutation, humans cannot synthesize the Neu5Gc form of sialic acid. Anti-Neu5Gc antibodies are often found in human sera, and have been associated with xeno-inflammation. Here we describe effects associated with a Neu5Gc knock-out in GalTKO.hCD46 lungs in an ex vivo perfusion model.
Methods: 5 GalTKO.hCD46.Neu5GcKO pig lungs were perfused for 6h with fresh heparinized human blood. 7 GalTKO.hCD46 lungs without additional KO served as a reference. Functional parameters as well as blood and tissue samples were collected at pre-defined time points. Plasma cytokines and inflammatory markers were measured by Luminex.
Results: All Neu5GcKO organs kept adequate blood oxygenation and "survived" until elective termination whereas one of the "reference" lungs failed prior to the 4h time point. Thromboxane elaboration (e.g. ΔTBX 14.1±4.7 vs. 51.9±8.1 ng/ml at 4h; p=0.004), thrombin generation (e.g. ΔF1+2 7.0±1 vs. 26.0±5 nM at 4h; p=0.008), and histamine elaboration (e.g. 100.6±17.8 vs. 200.0±37.4 ng/ml at 4h; p=0.04) were significantly lower with additional Neu5GcKO. Activation of platelets, measured by both, CD62P and βTG, was lower in Neu5GcKO experiments when compared to the reference group. Pulmonary vascular resistance (PVR) rise was significantly attenuated and delayed in GalTKO.hCD46.Neu5GcKO lungs (e.g. 75±9 vs. 279±58 mmHg*min/L at 4h). Complement activation (C3a) was not altered. MCP-1, ELA2, and LTF levels were blunted in Neu5GcKO perfusions (p<0.05 vs. reference at 1 and 4h).



Conclusion: Neu5GcKO in GalTKO.hCD46 lungs significantly reduces TBX, F1+2 and histamine levels in the perfusate and attenuates PVR elevation in the xenolung. The findings suggest that additional knock-out of the Neu5Gc sialic acid reduces the inflammation process, perhaps by blunting activation of pulmonary macrophages and platelets that elaborate TBX.

629.4

Carbon monoxide reduces acute pulmonary dysfunction in GalT-KO pig-to-cynomolgus monkey orthotopic lung transplantation

Hisashi Sahara[1], Hiroshi Nagashima[2], Mitsuhiro Sekijima[1], Takahiro Murokawa[1], Takehiro Iwanaga[1], Yuichi Ariyoshi[1], Yurika Ichinari[1], Kazuaki Nakano[2], Hitomi Matsunari[2], Akira Shimizu[3], Kazuhiko Yamada[1].
[1]Division of Organ Replacement and Xenotransplantation Surgery, Center for Advanced Biomedical Science and Swine Research, Kagoshima University, Kagoshima, Japan; [2]Department of Life Sciences, School of Agriculture, Meiji University, Kawasaki, Japan; [3]Department of Analytic Human Pathology, Nippon Medical School, Tokyo, Japan.

Background: After the production of α1,3 galactosyltransferase knock-out (GalT-KO) swine, the results of both heart and kidney xenotransplantation have been encouraging. In contrast to these organs, the results of lung xenotransplantation have been worse, and the longest survival of pulmonary xenograft is only 5 days. There thought to be several factors to overcome acute pulmonary xenograft dysfunction, especially, severe endothelial cell injury after lung xenotransplantation activates several factors such as severe inflammation or severe coagulation. We have recently reported that the early activation of endothelial cells and further inflammatory cascades within organ allografts resulted in the early graft loss using porcine lung allotransplant model from brain-dead donor, and also donor cytoprotective preconditioning with carbon monoxide (CO) during organ retrieval was sufficient to improve lung graft survival. In this study, we evaluated the effects of CO on reducing hyperacute dysfunction and the development of microangiopathy using GalT-KO pig-to-cynomolgus monkey orthotopic lung transplant model.
Methods: Nine cynomolgus monkeys received left lung from GalT-KO swine. In Group 1 (n=5), only GalT-KO donors were treated with inhaled CO for 180 min. In Group 2 (n=2), both donors and recipients were treated with CO (donor: 180 min; recipient: 360 min). Concentration of inhaled CO was adjusted by the level of COHb in the blood (COHb: 15-20 %). In the non-CO-treated group, two cynomolgus monkeys received left lung from GalT-KO swine without CO therapy (Group 3). Following perfusion, complete blood counts and other key variables were followed serially and graft function was assessed with blood gases, chest X-rays and lung biopsies.
Results: The graft was grossly normal at 2 hours reperfusion with or without CO inhalation. However, the physiological assessments by blood gas analysis exhibited better PaO2 in the recipients of CO-treated lungs compared with non-CO-treated group, and moreover, histological analysis showed decreased inflammatory cell infiltrations, intravascular thrombus or hemorrhage in the CO-treated group. These findings were more pronounced in Group 2 compared with Group 1. Although, animals did not survive over 4 days after transplantation due to hypoxemia or hypotension in both CO-treated and non-CO-treated graft, the longest survivors treated with CO (Group 2) revealed fewer inflammatory cell infiltartes or microthrombus compared with the recipient who received non-CO-treated GalT-KO lung (Group 3). Also, immunohistochemistry 2 days after transplantation showed less IgM/IgG and C4d deposition was observed in the CO-treated group compared to non-CO-treated group.
Conclusion: Perioperative treatment with low-dose inhalation of CO successfully maintained pulmonary graft function associated with reduction of the degree of inflammation, intravascular thrombus and hemorrhage using orthotopic pulmonary xenotransplant model at early time point. Although further studies are required to prevent thrombotic microangiopathy completely, protect ion of the graft damage from innate immune response by CO treatment would be one of the therapeutic strategies for prolonging pulmonary xenograft survival.

629.5

Empirical study of GTKO Pig - Tibetan macaque splenic fossa heterotopic auxiliary liver xenotransplantation

Kefeng Dou, Kaishan Tao, Zhaoxu Yang, Xiao Li, Zhuochao Zhang, Dengke Pan, Hui Chen, Liang Zhou, Hongchen Ji, Ben Ma.
Department of Hepatobiliary Surgery, Xijing Hospital, the Fourth Military Medical University, Xi'an, People's Republic of China.

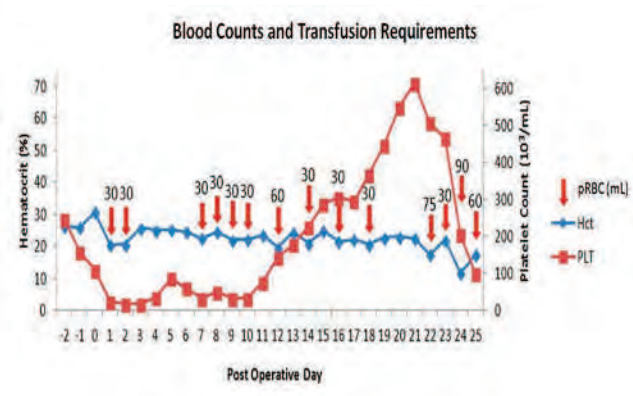
Objective: To address xenogeneic immune rejection, blood clotting disorders after liver xenotransplantation, we developed a novel surgical approach to liver xenotransplantation and explored perioperative anticoagulation therapy.
Methods: Three GTKO pig and one wide type miniature pig were used as donors. The recipients were 4 Tibetan macaques. Donor surgery: Under general anesthesia, the donor liver was lavaged quickly with "Celsior + Salviae miltiorrhizae" composite graft perfusion; the lobus sinister lateralis of the donor liver was then cut and trimmed. Recipient surgery: Under general anesthesia, we removed the spleen of the Tibetan macaque and transplanted the lobus sinister lateralis of the donor liver into the spleen fossa. We divided the left renal vein and then anastomosed the recipients' distal left renal vein with the hepatic portal vein of the graft, the recipients' proximal renal vein with the hepatic vein of the graft, and the recipients' splenic artery with the hepatic artery of the graft; we placed the biliary drainage tube in sequence. Before the abdomen was closed, B-mode ultrasonic equipment was used to observe blood flow in the liver graft. We then utilized the "TAC + MMF + Solae + hormone" immunosuppressive regimen and used Salvia alone for anticoagulation therapy.
Results: The WT pig liver underwent hyperacute rejection. Cellular infiltration and antibody and complement deposition were confirmed. No bile flow out from the drainage tube. Recipient animal was euthanized on 12 hour post transplantation. In three recipients with GalT-KO grafts, few minutes after intraoperatively opening the blood flow of the liver graft, inaurate bile was observed within the drainage tube. The physiological and biochemical indices and liver function gradually returned to normal physiological ranges. The coagulation ability and platelet count remained stable, and no pathological changes were observed in the coagulation system. An ELISA test was used to detect the porcine albumin and platelet-activating factor in the recipients' blood. The recipient in the first case survived for 26 h; autopsy indicated death due to acute pulmonary edema and left ventricular failure, and a pathological examination showed acute immune rejection. The direct cause of death was considered to be too much fluid uptake. The recipient and liver graft in the second case survived for 2 weeks, eventually succumbing to hypostatic pneumonia, respiratory failure, and heart failure. Autopsy results indicated that the shape and color of the liver graft were good, the quality of the liver graft was approximately 300g. A pathological examination found no hyperacute or acute immune rejection. The direct cause of death was a serious lung infection. The recipient and liver graft in the third case survived for 10 days, due to thrombosis of hepatic artery, we did not observe the flow out of bile from the biliary drainage tube and recipient animal was euthanized.
Conclusion: Overall, our study lengthens the survival time of GTKO porcine liver transplants in nonhuman primates and provides new avenues for the clinical application of liver xenotransplantation.

629.6

Marked improvement in pig-to-baboon liver xenograft survival: a major step towards clinical application

Jigesh Shah, Nalu Navarro-Alvarez, Matthew W.DeFazio, Nahel Elias, Heidi Yeh, Ivy Rosales, Robert B.Colvin, A. Benedict Cosimi, Martin Hertl, James F.Markmann, David H.Sachs, Parsia A.Vagefi.
Center for Transplantation Sciences, Massachusetts General Hospital/Harvard Medical School, Boston, United States.

Background: The use of porcine livers is currently impeded not by xenogeneic rejection, but by incompatibilities in the coagulation and complement systems resulting in severe coagulopathy, thrombotic microangiopathy (TMA) and graft thrombosis. We recently reported a novel approach of human coagulation factors administration that controlled coagulopathy, maintained circulating platelets and prevented TMA in pig-to-baboon liver xenotransplantation (LXT). However, survival beyond 9 days has never been reported.
Methods: LXT was performed using a pCMV-free GalT-KO donor. Post-LXT the baboon received an infusion of human prothrombin concentrate complex (Octaplex). Immunosuppression consisted of Thymoglobulin induction (10 mg/kg; Day -3), and maintenance therapy with Belatacept (20 mg/kg; Days -1, 0, 4, 7, 14 and 21), continuous FK-506 (0.20 mg/kg/day; target levels 10-20 ng/ml), and a methylprednisone taper.
Results: The baboon recovered post-LXT without incident, never requiring re-exploration. LFT's were noted to increase on POD 7, prompting pulse steroids and with improvement of transaminases. Notably, baboon anti-pig IgM appeared on POD 7 by FACS, disappearing by POD 14. Thrombocytopenia began recovering spontaneously by POD 11 (peak platelet count 614,000 on POD 21). PRBC requirements were minimal, with 44% (255 mL) of the volume given in the final 72 hours of life (Figure 1). Euthanasia occurred POD 25 for progressively worsening cholestasis. Terminal liver biopsy demonstrated common bile duct necrosis, focal hepatic necrosis (30%), and intact portal tracts without evidence of rejection or TMA.
Conclusion: This result represents the longest reported survival of a non-human primate following LXT. Current studies are determining the relative importance of exogenous human coagulation factors, use of a pCMV-free donor, and belatacept, in achieving this prolonged survival. Consistent achievement of 4-week survival could justify LXT clinical application as a bridge to allotransplantation for patients with acute hepatic failure.



629.7

Use of multi-transgene expression for prevention of cardiac xenotransplantation associated consumptive coagulopathy

Joshua Chan[1], Avneesh K.Singh[1], Philip C.Corcoran[1], Marvin L.Thomas[2], Billeta G.Lewis[2], David L.Ayares[3], Keith A.Horvath[1], Muhammad M.Mohiuddin[1].
[1]Cardiothoracic Surgery Research Program/National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD, United States; [2]Division of Veterinary Resources/Office of Research Services, National Institutes of Health, Bethesda, MD, United States; [3]Revivicor, Inc., Blacksburg, VA, United States.

Objective: The unique potential for cardiac xenotransplantation to address the current issues of scarce donor resources has immense implications for the future of heart failure management. Essential in the success of xenotransplantation is overcoming incompatibilities between xenograft and recipient. The development of post-xenotransplant critical consumptive coagulopathy has often precluded long-term survival. Expanded use of genetic modification and the inclusion of additional humanized factors within xenografts may prevent disruption of the recipient coagulation system. We now detail the use of six-gene modified cardiac xenografts and its effects on reducing xenotransplant-associated consumptive coagulopathy.
Methods: Specific pathogen-free baboons (8-12 kg) underwent heterotopic cardiac transplant using weight-matched genetically engineered swine donor hearts. Transgenic modification included GTKO.CD46.EPCR.DAF in addition to either TFPI.CD47 (n=2) or CD39.TBM (n=1). Post-transplant immunosuppressive regimen was comprised of conventional anti-rejection therapies as well as anti-CD40 targeted immunosuppression. Recipient and xenograft status were closely monitored with continuous cardiac telemetry and video monitoring as well as weekly or biweekly complete blood count panel, coagulation analysis, and echocardiography.
Results: Heterotopic heart transplants were completed without technical difficulty. Compared to historical controls of three-gene cardiac xenotransplants with GTKO.CD46.TBM (n=5), subjects with six-gene xenografts were found to have significantly higher fibrinogen (170 mg/dL vs. 126 mg/dL, p=0.02) and lower antithrombin (282% vs. 516%, p=0.38) during the first 14 days post-operatively. These findings were maintained at 90 days (fibrinogen: 211 mg/dL vs. 164 mg/dL, p=0.04; antithrombin: 80% vs. 100%, p<0.001) and were consistent with superior protection against dysregulated coagulopathy in the six-gene cohort. Levels of D-dimer evaluated at two weeks following transplantation were noted to have a non-significant elevation in the three-gene subjects compared to those with six-gene grafts (921 ng/mL vs. 790 ng/mL, p=0.19). Three-gene xenotransplant recipients were also observed to have a lower hematocrit in the first three months (33% vs. 35%, p=0.04) and required a greater number of transfusions. Subjects with six-gene xenografts have not experienced any clinically significant hemorrhage or mortality.
Conclusion: The inclusion of additional humanized genes in cardiac xenografts is associated with improved levels of multiple factors in the coagulation-fibrinolysis cascade. It is postulated that the added expression of TFPI and DAF (to regulate the generation and propagation of tissue factor) as well as EPCR (for modulation of activated protein C production) contribute in preventing critical consumptive coagulopathy. Implementation of further transgenic modifications may be central to achieving improved outcomes in xenotransplantation.

629.8

Human anti thrombotic genes do not obviate the need for anticoagulation in aiding long term xenograft survival

Muhammad Mohiuddin[1], Avneesh Singh[1], Joshua Chan[1], Philip Corcoran[1], Marvin Thomas[2], Billeta Lewis[2], David Ayares[3], Keith Horvath[1].
[1]Cardiothoracic Surgery Research Program, NHLBI /NIH, Bethesda, MD, United States; [2]Division of Veterinary Resources, NIH, Bethesda, MD, United States; [3]Revivicor, Inc., Blacksburg, VA, United States.

Introduction: Survival of heterotopic cardiac xenografts is dependent on suppression of the antibody response and of complement activation while addressing the anti coagulatory pathway. Recent advances in the genetic engineering of pigs have addressed these issues and as a result significant graft survival has been achieved. Here we study the role of continuous heparin infusion in long term graft survivors.
Methods: Two pig hearts expressing (GTKO.CD46.EPCR.DAF.TFPI.CD47) and one heart expressing (GTKO.CD46.EPCR.DAF. CD39.TBM) were heterotopically transplanted in young specific pathogen free baboons. Recipient baboons were treated with a short course of anti CD20 antibody, cobra venom factor, anti thymocyte globulin, and were maintained on anti CD40 antibody (clone 2C10R4), mycophenolate mofetil and tapering doses of steroids. All baboons received continuous intravenous heparin. The dose of heparin was adjusted based on ACT levels designed to maintain the ACT at twice the baseline level. Graft survival was monitored with continuous telemetry, periodic echocardiography and manual palpation. Blood work (CBC, chemistry, troponin and ACT) was performed at 1-2 week intervals.
Results: One heart from each group is still contracting after 207 and 142 days of transplantation. One heart rejected around day 46. In both the surviving baboons heparin infusion was stopped for more than once due to infection, traumatic bleeding or cardiac fibrillation episodes. In all instances, discontinuation of heparin resulted in slowing down of cardiac contractility, and the ventricular cavity rapidly filled with thrombus, obliterating the LV cavity as observed on ultrasound. However with resuming the heparin infusion, contractility slowly improved and the ventricular thrombus slowly resolved.
Conclusion: The available genetic modifications and novel immunosuppression described provided benefit in prolonging cardiac xenograft survival. However, episodes with discontinuation of heparin resulted in a decrease in graft function and rapid thrombus formation. This finding clearly demonstrates the necessity of continuous heparin or anticoagulation therapy in maintaining heterotopic cardiac xenograft survival.

629.9

The effect of IL-6 blockade on revascularization of islet after transplantation in pig to nonhuman primate (NHP) islet xenotransplantation

Byoung-Hoon Min[1,4], Jun-Seop Shin[1,2,3,4], Jong-Min Kim[1,3], Chung-Gyu Park[1,2,4,5,6].
[1]Xenotransplantation Research Center, Seoul National University, College of Medicine, Seoul, Korea; [2]Department of Microbiology and Immunology, Seoul National University, College of Medicine, Seoul, Korea; [3]Institute of Endemic Diseases, Seoul National University, College of Medicine, Seoul, Korea; [4]Cancer Research Institute, Seoul National University, College of Medicine, Seoul, Korea; [5]Department of Biomedical Sciences, Seoul National University, College of Medicine, Seoul, Korea; [6]Department of Experimental Animal Research, Biomedical Research Institute, Seoul National University, College of Medicine, Seoul, Korea.

Background: Pig to NHP islet xenotransplantation is currently being explored as a pre-clinical model for treatment of type 1 diabetes patients with hypoglycaemia unawareness. However, immune responses to the xenogeneic antigens in xenotransplantation are stronger than in allotransplantation in some aspects, which should be overcome by appropriate immunosuppressants. Moreover, isolation of the islets from the pancreas can damage vasculature around the islets and revascularization after transplantation is essential for proper functioning of the transplanted islets. Therefore, we want to examine the effects of various combination of immunosuppressants on revascularization process after islet transplantation.
Method: Pig islets were isolated from DPF SNU miniature pigs and transplanted via portal vein into streptozotocin-induced diabetic Rhesus macaque monkeys. The control group (n=2) were treated with basal immunosuppression comprised of ATG, anti-CD40 antibody (2C10R4), sirolimus and tacrolimus. Tocil group (n=3) were treated with basal immunosuppression + Tocilizumab (IL-6 receptor antagonist). To assess the revascularization status of transplanted islets, a biopsy of the distal portion of the liver was performed on 20 or 21 days after transplantation. For immunohistochemical staining, anti-insulin and anti-CD31 antibodies were used. Also, semi-quantitative histological analysis of CD31+ cells on the peri- and intra-islet area was conducted.
Result: We consistently observed many pig islets positively stained with insulin in the liver of recipient monkeys with basal immunosuppression irrespective of the addition of Tocilizumab on 20 days post transplantation (Fig. 1). In control group treated with basal immunosuppression, well-developed endothelial cells were observed on the peri- and intra islet area. By contrast, in Tocil group, the extent of CD31+ cells' distribution in the intra-islet space was significantly reduced (Fig.2) suggesting that tocilizumab treatment might prevent revascularization.

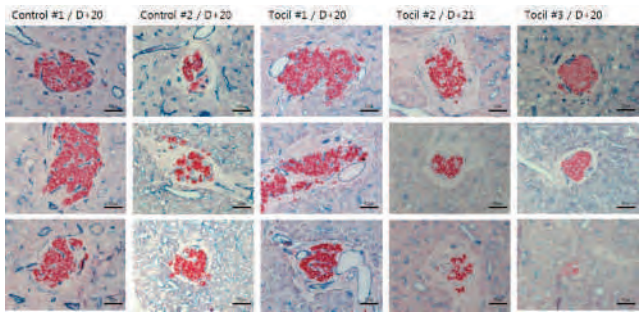


Fig. 1. Insulin and CD31 distribution in the islets transplanted to the liver of recipient monkeys. Liver samples taken on 20 or 21 days after pig islet transplantation. Figure shows well-engrafted islet beta cells (red) and lining of CD31+ endothelial cells (Blue).

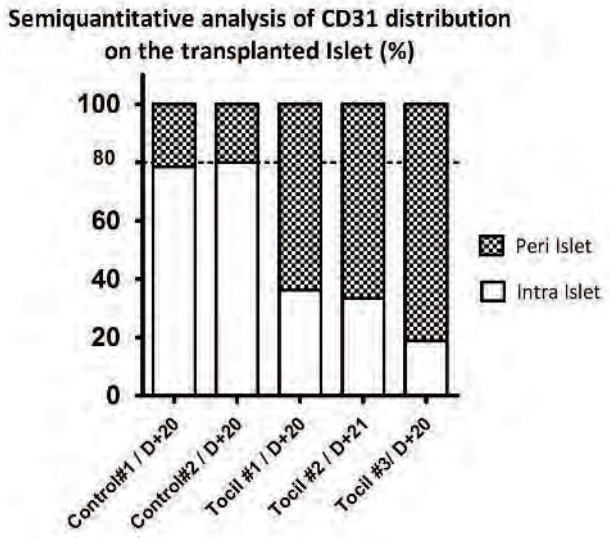


Fig. 2. Semi-quantitative histological analysis of CD31+ cells on the peri- and intra-islet area. Control group (basal immunosuppression) showed well-developed endothelial cells on the peri- and intra islet area. In tocil group (basal immunosuppression+tocilizumab), the extent of distribution of CD31+ cells in the intra-islet space was reduced.

Conclusion: Revascularization of the transplanted islets is essential for graft outcome in pig-to-NHP islet xenotransplantation. Our results demonstrated that IL-6 blocking during islet engraftment period reduced vascularization, however, this effect had no significant correlation with the overall islet graft survival.

This study was supported by a grant from the Korea Healthcare Technology R&D Project, Ministry for Health & Welfare, Republic of Korea (Project No. HI13C0954); Anti-CD40 antibodies used in these studies were provided by the NIH Nonhuman Primate Reagent Resource (R24 RR016001, and NIAID contract HHSN 2722000900037C)

P.1000

Solving the structural basis for alloantibody recognition of Asian HLA in solid organ transplantation

Gu Yue[1,2], Too Chien Tei[1], Paul A. MacAry[1,2].
[1]Department of Microbiology and Immunology, National University of Singapore, Singapore, Singapore; [2]NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, Singapore, Singapore.

Kidney transplantation is currently the optimal treatment for end-stage kidney failure patients. However, a significant number of transplant recipients suffer from chronic allograft rejection. It has been proposed that this is largely attributed to donor-specific alloantibodies (DSAs) present in the recipient, mainly targeting mismatched human leukocyte antigen (HLA) molecules on the donor graft. However, there have not been detailed mechanistic studies on the human anti-HLA alloantibody repertoire and whether these target specific, pathogenic determinants on foreign HLA. In this study, we aim to characterize the form and function of alloantibodies in patients. A detailed map of the pathogenic determinants on foreign HLA will provide a framework for the development of better prognostic tests and future therapeutic candidates.
We have produced human monoclonal alloantibodies by screening a naive library representative of the Singaporean population against recombinant HLA-A*11:01 monomer using phage-display method. ELISA was used to assess the binding specificity towards various common Asian HLAs. The in vitro relevance of these monoclonal antibodies were determined by flow cytometry binding to a collection of EBV-transformed B-lymphoblastoid cell line (BLCLs). The antigen-binding fragment (Fab) of human alloantibodies were then engineered onto the crystallizable fragment (Fc) of a murine antibody, in preparation for competitive assays with patient serum.
We have expressed twelve distinct fully-human monoclonal antibodies exhibiting variable binding specificities, suggesting that they target a spectrum of epitopes on HLA-A*11:01. Particularly, one of the antibodies was detected to be binding to HLA-A*11:01, A*01:01 and A*26:01 but not other common Asian HLA alleles such as HLA-A*24:02, A*30:01 nor HLA-B*40:01. Sequence alignment and comparison of these HLA alleles allowed a prediction of the epitope-determining amino acids targeted by this alloantibody.
To the extend of our knowledge, this is the first time that fully human monoclonal alloantibodies against HLA have been developed. The same methodology will be applied to other HLA alleles frequently occurring in the Asian population, including HLA-A*24:02 and HLA-B*40:01. Further characterization of the alloantibodies will be conducted in future to map clinically relevant deleterious epitopes and to assess in vitro ability of these antibodies to block or neutralize HLA-induced immune responses.

P.1001

Phenotypic patterns of B cells subsets in the peripheral blood of patients with long-term kidney allograft function

Katarzyna Kościelska-Kasprzak, Marta Myszka, Alina Szewczyk, Tomasz Baran, Maria Boratyńska, Marian Klinger.
Department of Nephrology and Transplantation Medicine, Wrocław Medical University, Wrocław, Poland.

B lymphocytes are involved both in allograft injury and tolerance. Increased numbers of naive and transitional B cells with suppressive function as well as transcriptional B cell signature have been shown in kidney transplant (KTx) recipients with operational tolerance.
The goal of this study was to assess the patterns of B cell subsets in long term KTx recipients in relation to allograft function, time after transplantation, and immunosuppressive protocol.
Materials and Methods: The study group included 42 KTx recipients with the stable allograft function 14.6±5.0 y. after KTx and 14 with chronic allograft dysfunction 14.4±4.4 y. after KTx. The patients were treated with calcineurin inhibitor (47) or mTOR inhibitor (9), and mycophenolate mofetil or azathioprine. All but 3 patients received steroids. The control group consisted of 24 healthy volunteers. The B cell phenotype in peripheral blood was assessed using flow cytometry and included: naive $CD19^{+}CD24^{int}CD38^{int}$, memory $CD19^{+}CD24^{int}CD38^{-}$, B10 $CD19^{+}CD24^{high}CD27^{+}$, and transitional B cells (regulatory B cells) $CD19^{+}CD24^{high}CD38^{high}$. The absolute counts were calculated with Trucount tubes.
Results: KTx recipients had much lower B cell counts as well as presented altered phenotype distribution of B lymphocyte subsets compared to healthy controls. Only memory B cells were at a level close to the control group. Decreased numbers of naive, transitional and B10 B cells were found in KTx recipients (IQR, P vs control):

Subsets	Control	Stable		CAD	
naive B cells/µl	131 – 178	11 – 76	P < .001	13 – 90	P < .001
memory B cells/µl	3.8 – 17.5	1.4 – 7.8	P = .003	2.3 – 10.6	ns
transitional B cells/µl	4.9 – 14.2	0.1 – 1.2	P < .001	0.2 – 0.6	P < .001
B10/µl	50 – 95	17 – 39	P < .001	18 – 94	P = .04

There was no difference in B cell subsets between Stable and CAD groups. However, the percentage of transitional B-cells in the CAD group was lower, with poor statistical significance (P=.08).
Time after KTx did not affect the levels of transitional (regulatory) and B10 B cells. Nevertheless, it was related (P<.001) to decrease in naive and memory B cells.
The treatment with tacrolimus was associated (P=.009) with a higher count of naive B cells (24 – 161/µl) than treatment with cyclosporine A (8 – 74/µl). No other B cell subsets were influenced by the type of calcineurin inhibitor or mTOR inhibitor.
The stable KTx patients treated with CsA and mycophenolate mofetil presented increased counts of transitional (0.3 – 1.4/µl, P=.007) and naive B cells (17 – 87/µl, P=.01) compared to patients treated with CsA and azathioprine (0.02 – 0.2/µl and 6 – 14/µl, respectively).
Conclusion: We found low levels of transitional, B10, and naïve B cell subsets in long-term KTx recipients. These abnormalities in phenotype distribution were prevalent both in the stable patients and in CAD. They were affected by immunosuppressive treatment and time after KTx. It seems that assessment of B cell phenotypes may not be useful to identifying renal transplant patients predisposed to developing tolerance.

The research was supported by the scientific grant from the Polish Transplantation ociety

P.1002

Human ABH antibody repertoire elucidated by coupled high throughput sequencing and proteomics

Liu Logen[1], Wang Yi[1,2], Shi Bingyi [3], Lin Tao[4], Chen Gang[5], Liu Hongtao[6], Wen Jiqiu[7], Changxi Wang[8], Luo Zhigang[2], Gui Peigeng[2], Jiang Hongtao[2], Zhou Hao[2], Qin Guoqing[2], Zhang Yunsheng[1], Zou Yizhou[9].
[1]Clinical Research Center, The Second Affiliated Hospital, University of South China, Hengyang, People's Republic of China; [2]Organ Transplantation Unit/Department of Urology, The Second Affiliated Hospital, University of South China, Hengyang, People's Republic of China; [3]Institute of Organ Transplantation, The 309th Hospital of Chinese People's Liberation Army, Beijing, People's Republic of China; [4]Department of Urology /Organ Transplantation Center, West China Hospital, Sichuan University, Chengdu, People's Republic of China; [5]Institute of Organ Transplantation, Tongji Hospital, Huazhong University of Science and Technology, Wuhan, People's Republic of China; [6]The Organ Transplantation Center, Anhui Provincial Hospital, Hefei, People's Republic of China; [7]National Clinical Research Center of Kidney Disease, Jinling Hospital, Nanjing University School of Medicine, Nanjing, People's Republic of China; [8]Organ Transplantation Center, The First Affiliated Hospital of Sun Yat-sen University, guangzhou, People's Republic of China; [9]Department of Immunology, Xiangya Medical School, Central South University, Changsha, People's Republic of China.

Background: Blood group ABH antigen is important for organ transplantation. Failed ABO blood type match need pre-transplantation plasma treatment of the receptor. Then, the transplanted organ would not be rejected by recurrent incompatible ABH antibodies, which is called immune accommodation. The underlying mechanism, despite heavily researched, is not fairly clear. To uncover this, further knowledge of the nature of ABH antibody and its reactivity is emergently required. Here, we present the first human Anti-A blood group antibodies repertoire.
Materials and Methods: CD19+CD5+ B1 cells, CD19-CD5- B2 cells, CD19-CD38hiCD27hiCD138+ plasma cells, CD19+CD38-CD27+ memory B cells from healthy blood type B human donors were sorted by flow cytometry. High through-put sequencing were performed with these cells to construct an immunoglobulin repertoire. Plasma from the same individual was affinity purified with Blood group type A antigen coupled to agarose beads, or with agarose beads only as control. The bound polyclonal anti-A antibody was subjected to multi-enzymes digestion respectively, and identified by nano flow high performance liquid chromatography coupled to tandem mass spectrometry, with previous constructed immunoglobulin repertoire as reference database. To pair heavy chain and light chain, antibodies were also cloned from single B cells (plasma cells) sorted to 96 well plates. Anti-A antibodies were expressed in cultured 293T cell line.
Results and Discussion: We isolated about 105 B1 cells, 2x 106 B2 cells, 5 x 105 memory B cells and plasma cells from 100ml peripheral blood of healthy human donor. And we got about 10Gb sequence data totally. This a relatively large database, since previously studies often isolate B cells from 10ml blood, while it is not comparable to studies performed in mouse models because most B cells are located in spleen. Sequences from plasma cell is of significantly important, because present plasma treatment methods in ABO incompatible (ABOi) organ transplantation do not eliminate plasma cells. MS analysis identified antibodies of high confidence and high abundance. Among them, several antibodies were also successfully cloned in single B cell (plasma cell) sorted into 96 well plates. This single cell cloning process reflects the truly heavy chain and light chain pair, and is prior to bioinformatics prediction or experimental combination of all possible pairing. Western blot further verified that our cloned antibodies response to A antigen but not B.
Conclusion: These results described the first human blood type A antibody repertoire in both genomic and proteomics levels. By comparing the antibody repertoire change before and after ABOi organ transplantation, it may help us in elucidating mechanisms of immune accommodation.

P.1003

mHLA-G expression on CD4+CD25(high) T cell and CD16+ natural killer cell associated with clinical status in renal transplantation patients

Bilkay Baştürk[1,2], Bircan Kantaroğlu[2], Aytül Noyan[3], İlker Arel[4], Nihan Tekkarışmaz[5].
[1]Immunology, Baskent University Faculty of Medicine, Ankara, Turkey; [2]Immunology Tissue Typing Laboratory, Baskent University Research and Medical Center, Adana, Turkey; [3]Pediatrics, Baskent University Research and Medical Center, Adana, Turkey; [4]General Surgery, Baskent University Research and Medical Center, Adana, Turkey; [5]Nephrology, Baskent University Research and Medical Center, Adana, Turkey.

Introduction: Human leukocyte Antigen-G (HLA-G) molecules are HLA class Ib antigens characterized by tolerogenic and immune-inhibitory functions and have been associated with allograft acceptance. HLA-G expression correlates with low frequency of liver, kidney and heart acute rejection. HLA-G modulates antural killer (NK) cells, T cells, and DC maturation through its interactions with various inhibitory receptors. HLA-G protects HLA class-I deficient targets from NK cells-mediated lysis and its expression was related to allograft acceptance. The aim of this study was to investigate HLA-G expression on CD16+ NK cells and CD4+CD25high T cells and allograft rejection in renal transplant patients.
Material and Methods: HLA-G expression profiles are routinely determined in patients which are enrolled in regular follow-ups. 40 renal transplant patients results (2010-2015) were investigated before and post-transplantation. Results compared with previous study control grup' results. Patients were followed similarly pre-and post-treatment during ATG usage before treatment and diagnosis of rejection post-transplantation. To investigate the expression of HLA-G on the surface of CD4+CD25(high) Treg cells and CD16+ natural killer cells, we used anti-CD45, anti-CD3, anti-CD4, anti-CD25- anti-CD16 and anti-HLA-G monoclonal antibodies in flow cytometer analysis. Statistical analysis was performed using SPSS software
Results and Discussion: CD4+CD25high Treg CD4+CD25high HLA-G+ cellular subgroups were found to be statistically different in patients and healthy controls($p<0.05$). Although there was a difference between pre-and post-transplantation patients in single cases, the mean values within the patients as a group were not statistically significant. Our findings indicated that the increases in CD4+CD25high cellular subgroup and CD4+CD25highHLA-G+ cellular subgroup (r: 0,325) as well as CD4+CD25highHLA-G+ cellular subgroup and CD3-CD16+ HLA-G+ cellular subgroup (r:0,580) correlated with each other. Furthermore, plasmapheresis and ATG treatment were shown to upregulate the percentage of CD4+CD25high HLA-G+ cell subgroup. Increased HLA-G surface expression in these cells post-transplantation could contribute to tolerogenic effect and increased HLA-G expression on the surface of NK cells could contribute to inhibitory effect by using inhibitory receptors.
Conclusion: HLA-G expression is affected by a myriad of variables including polymorphisms, primary disease and post-transplantation treatment protocols. Thus, follow-ups which are using the means as reference values do not indicate significant differences and it's difficult to determine a threshold value. Of note, evaluating the patients as unique cases within themselves has a strong treatment follow-up predictive capacity.
References:
[1] XIAO Li, et al., HLA-G expression in the peripheral blood of live kidney transplant recipientsChin Med J 2013;126 (14)
[2] H.-L. Jin, et al., Clinical Relevance of sHLA-G-Mediated With Better Graft Acceptance in Early Posttransplantation. Transplantation Proceedings. 2015; 44: 1259–1261
[3] K. Poláková, et al., Analysis of HLA-G expression in serum and biopsy samples of kidneytransplant recipients. Immunobiology. 2015; 533–537
[4] J. Lazarte et al., New Developments in HLA-G in Cardiac Transplantation, Hum. Immunol.2016
[5] Edgardo D. Carosella et al., The tolerogenic interplay(s) among HLA-G, myeloid APCs, and regulatory cells. Blood. 2011;6499-6505
[6] Mindi R.Walker et al., Induction of FoxP3 and acquisition of T regulatory activity by stimulated human CD4+CD25– T cells. J. Clin. Invest.2003; 112:14371443
[7] Nathalie Rouas-Freiss et al., HLA-G in Transplantation: A Relevant Molecule for Inhibition of Graft Rejection? American Journal of Transplantation 2003; 3: 11–16
[8] Bruqiere O et al., Role of HLA-G as a predictive marker of low risk of chronic rejection in lung transplant recipients: a clinical prospective study.Am.J. Transplant.2015;461-471
[9] WEI-YU HU et al., Expression of human leukocyte antigen-G and acute rejection in patients following liver transplantation. EXPERIMENTAL AND THERAPEUTIC MEDICINE 2014;1291-1295
[10] Pankratz S et al., Human CD4+ HLA-G+ regulatory T cells are potent suppressors of graft-versus-host disease in vivo. FASEB L. 2014;3435-3445

P.1004

Ectopic lymphoid follicles are present in type I T-cell mediated kidney transplant rejection

Kitty Leur[1,2], Gretchen N.de Graav[1], Karin Boer[1], Dennis A.Hesselink[1], Janneke N.Samsom[3], Marian C.Clahsen-van Groningen[4], Carla C. Baan[1].
[1]Internal Medicine, Transplantation, Erasmus Medical Center, Rotterdam, Netherlands; [2]Department of Surgery, Division of HPB and Transplant Surgery, Erasmus Medical Center, Rotterdam, Netherlands; [3]Department of Pediatrics, Erasmus Medical Center, Rotterdam, Netherlands; [4]Department of Pathology, Erasmus Medical Center, Rotterdam, Netherlands.

Background: In renal transplantation, T follicular helper (Tfh)-cell driven B-cell activation and immunoglobulin production may occur in Ectopic Lymphoid Follicles (ELFs) formed in the allograft. In this study, we investigated the presence of ELFs in different types of acute renal allograft rejection.
Materials and Methods: A total of 11 renal transplant biopsies were included in this study. Primary diagnosis of these samples were 1) C4d+ antibody-mediated rejection (ABMR, n=2), 2) T-cell mediated rejection type I (TCMR I, n=4), and 3) T-cell mediated rejection type II (TCMR II, n=5). Four µm sections of the formalin fixed, paraffin embedded tissue were cut and sequentially stained for Tfh cells (CD3, CD4, Bcl-6), B-cells (CD20), and immunoglobulins (IgM and IgG). Slides were analyzed for both the presence and composition of infiltrate.
Results: In all biopsies, infiltrates of CD3+ and CD4+ cells were detected. Remarkably, in TCMR I biopsies, CD20+ B-cells formed aggregates in the tubulo-interstitium and co-localized with CD4+ T-helper cells which tightly surrounded the aggregates of B-cells, i.e. forming ELFs. CD4+ T-cells co-localized with Bcl-6, which is a transcriptional factor for Tfh cells. Two biopsies showing TCMR I and 3 biopsies showing TCMR II showed high and low expression, respectively, for IgG and IgM, while no immunoglobulin expression was found in ABMR biopsies.
Conclusions: ELFs are formed in the renal allograft during type 1 acute T-cell–mediated rejection. This presence argues for a pivotal role for Tfh cell-driven activation of B-cells in type I acute T-cell mediated rejection.

P.1005

The role of DNA methylation of interferon gamma and programmed death 1 in allograft rejection after kidney transplantation

Karin Boer[1], Elly E.A.de Wit[1], Dennis A.Hesselink[1], Leo J.Hofland[2], Michiel G.H.Betjes[1], Carla C. Baan[1].
[1]Internal Medicine - Nephrology and Transplantation, ErasmusMC, University Medical Center Rotterdam, Rotterdam, Netherlands; [2]Internal Medicine - Endocrinology, ErasmusMC, University Medical Center Rotterdam, Rotterdam, Netherlands.

Introduction: DNA methylation is a well-known epigenetic mechanism which regulates gene expression. Variations in DNA methylation profiles are increasingly associated with immune-mediated diseases. However, the role of DNA methylation in the regulation of the alloimmune response after organ transplantation is unknown. Here, we studied the methylation of regulatory cytosine phosphate guanine sites (CpGs) in genes involved in alloreactivity; the pro-inflammatory cytokine *interferon γ* (*IFNγ*) and the co-inhibitor molecule *programmed death 1* (*PD1*) in kidney transplant recipients.
Materials and Methods: DNA methylation of regulatory CpGs (2 for *IFNγ* and 8 for *PD1*) was determined by pyrosequencing of FACS-sorted pure naïve, central memory (CM), effector memory (EM) and terminally differentiated EMRA CD8+ T-cells of kidney transplant recipients, before, and 3 and 12 months after transplantation. Both recipients who developed a biopsy-proven acute rejection within the first three months after transplantation (rejectors) and recipients who remained free from rejection (non-rejectors) were included. As infection with cytomegalovirus (CMV) may significantly alter DNA methylation, only CMV seronegative kidney transplant recipients and CMV seronegative healthy donors were studied.
Results: Both *IFNγ* and *PD1* were significantly ($p<0.001$) higher methylated in the naïve CD8+ T-cells compared to the memory subsets (*IFNγ*: 55% in naïve versus 16% in CM, 12% in EM and 6% in EMRA; *PD1*: 43% versus 17%, 11% and 12%, respectively). Remarkably, the methylation status of both *IFNγ* and *PD1* did not significantly change in the rejectors during the immunologically challenging first year after transplantation in any of the studied CD8+ T-cell subsets. Comparing the methylation status of *IFNγ* and *PD1* of rejectors with non-rejectors at the different time points did not demonstrate significant differences. The methylation status of both *IFNγ* and *PD1* in the kidney transplant recipients was comparable to healthy donors in the different studied CD8+ T cell subsets.
Conclusion: Kidney transplant recipients do not demonstrate significant alterations in DNA methylation of either *IFNγ*, or *PD1* in CD8+ T-cells throughout the first post-transplant year as compared to healthy individuals. Based on these findings we conclude that variations in DNA methylation of either *IFNγ* or *PD1* is not associated with allograft rejection after kidney transplantation.

P.1006

Mechanism of FK506 induced TNF related apoptosis In Jurkat T cells

Sang Young Chung, Soo Jin Na Choi.
[1]Department of Surgery, Chonnam National University Hospital, Gwangju, Korea.

Purpose: To elucidate the mechanism of FK506 induced TRAIL(TNF related apoptosis inducing ligand) –R1(DR4) and TRAIL-R2(DR5) in FK506-treated Jurkat T cells. And signal transduction pathway of TNF-related events was studied.
Methods: The catalytic activation of caspase-3 and caspase-9 proteases was determined by digestion of fluorogenic biosubstrates and western blot with anti-caspase-3 and anti-caspase-9 antibodies. The levels of mRNA and proteins for p53, Bax, PUMA, proline oxidase, TRAIL(TNF related apoptosis inducing ligand), TRAIL-R1(DR4), TRAIL-R2(DR5), Fas, FasL, TNF-α, IL-6, and NKκB were measured by RT-PCR and western blot with specific antibodies. Also we further examined the localization of TRAIL family proteins using by fluorescent microscope with specific TRAIL family antibodies.
Results: FK506 decreased the viability of Jurkat T cells dose- and time-dependently along with catalytic activation of caspase-3 and caspase-9, p53 phosphorylation, and changes in expression levels of Bax, PUMA, and proline oxidase protein. It caused an increase in expression of TRAIL, TRAIL-R1(DR4), TRAIL-R2(DR5), Fas, and FasL in the levels of mRNA and proteins of Jurkat T cells. Furthermore, FK506 increased extracellular release of TNF-α and IL-6 cytokines in Jurkat T cells. It also induced the transactivation of NKκB through the dephosphrylation of Ser486 residues in Jurkat t cells.
Conclusion: These results suggest that FK506 induces apoptotic death of Jurkat cells through activation of caspase family protease, Bcl-2 family protein-related mitochondrial dysfunction, and activation of death-receptor mediated signaling pathways.

P.1007

Isatis tinctoria L. combined with co-stimulatory molecules blockade inhibits accelerated rejection mediated by allo-primed CD4+ memory T cells in mice

Shifeng Zhang[1,2], Ruwen Yang[1], Junjie Xia[1], Zhongquan Qi[1], Feifei Du[1].
[1]Organ Transplantation Institute, Xiamen University, Xiamen, People's Republic of China; [2]Department of Gastrointestinal Surgery, Zhongshan Hospital, Xiamen University, Xiamen, People's Republic of China.

Background: Accelerate rejection mediated by CD4+ memory T cells is a key barrior in organt ransplantation, and can hardly be inhibited by current routine immunosupressive agents, such as CsA, FK-506 and rapamycine. In our previously study, we have learned that cpd K, a compond from a famous chinese herbs named Isatis tinctoria L., can pronglong heart survival of re-transplantation recipient and change the proprtion of memory T cells.
Purpose: Here, we want to know whether cpd K could directly inhibit accelarat rejection mediated by CD4+ memory T cells and induce allograft tolerance in a CD4+ memory T cells transfered model, and study the mechnism.
Results: We transferred B6 mice reactive CD4+ memory T cells to naïve B6 mice mice and build a transferred model for this study. And the results demonstrated that cpd K combined with co-stimulatory blockade anti-CD40L and anti-LFA-1 mAbs could effectively inhibit the accelerate rejection mediated by CD4+ memory T cells, and induce allograft tolerance. The treatment could significantly reduce the secretion of IL-2 and IFN-γ, and increase the concentration of TGF-β in the periphery. Of note, a high proportion of regulatory T cells appeared after treatment.
Conclusions: This sudy confirmed that cpd K could inhibit accelerate rejection mediated by CD4+ memory T cells in combination with co-stimulatory blockade, and induce alograft tolerance. This result represent a important step forward in Tm inhibition, whereas detailed mechnism need to be study in the future.

P.1008

A delay of bone marrow transplantation after low dose irradiation impairs engraftment

Hong Xu, Yiming Huang, Suzanne T. Ildstad.
Institute for Cellular Therapeutics, University of Louisville, Louisville, KY, United States.

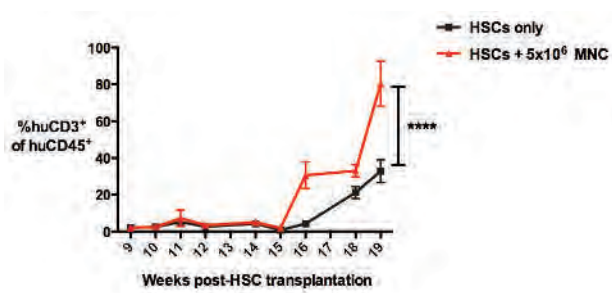
Introduction: We previously reported that using an allogeneic mouse bone marrow transplantation (BMT) model superior engraftment was achieved in mice conditioned with 500 or 600cGy total body irradiation (TBI) if bone marrow cells (BMC) were infused with a 1 to 8-day delay. The optimum engraftment occurred with a 4-day delay. Since a 1 to 3-day delay of BMC infusion after 100-300cGy low dose TBI has been practiced clinically and experimentally, we examined the optimal timing of BMC infusion with respect to low dose TBI on engraftment.
Materials and Methods: B6 ($H2^b$) mice were conditioned with 100-300cGy TBI on day 0 in combination with anti-αβTCR+anti-CD154+rapamycin (TCR/MR1/Rapa). $15x10^6$ BMC from MHC-disparate BALB/c ($H2^d$) mice were transplanted on days 0 (4-6 hours post TBI), +1, or +3.
Results and Discussion: With 300cGy TBI, engraftment occurred in all recipients when BMT was carried out on day 0, +1, and +3. The donor chimerism was 27.9±7.7% at 1 month when BMT was on day 0 and it was significantly higher than donor chimerism in mice that received BMT on day +1 (19.4±2.4%, P=0.011) or day +3 (5.7±4.1%, P=0.00001). With 200cGy TBI, 100% of recipients engrafted if BMC were infused at day 0 or day +1. However, only 16.7% mice engrafted if BMC were infused on day +3. With 100cGy TBI, 57.1% of recipients engrafted when the marrow was infused on day 0, but only 33.3% engrafted with 1 day delay of BMT. Engraftment did not occur for 3-day delay. These observations were confirmed using 200 or 100cGy alone without TCR/MR1/Rapa treatment in CD45.1 B6 to CD45.2 B6 congeneic BMT. Lower doses of TBI (100 to 300 cGy) partially decreased the immune cells, but significant populations of T and NK cells persisted. The impaired engraftment after low dose TBI might be because the evidence that significantly more $CD44^{high}/CD62L^{low/-}$ CD8+ T effector cells were detected on day +1 to +3 compared with day 0 after 100 or 200cGy TBI. Our data indicate that a delay between low dose TBI and BMT impairs engraftment, which is opposite with higher TBI of 500-600cGy with that the delay of BMT improves engraftment. The optimal timing of BMC infusion with low dose TBI should be the same day with TBI.
Conclusion: This study suggests that the immune recovery in BMT after low dose TBI conditioning and its effects on BMC engraftment differs from 500-600cGy higher dose TBI. Moreover, our findings may have significant clinical impact in determining optimal timing of conditioning with low dose TBI in BMT.

P.1009

Investigation of a novel multi-lineage human-mouse chimeric model for in vivo transplantation research

Fadi Issa, Joanna Hester, Kathryn Wood.
Nuffield Department of Surgical Sciences, University of Oxford, Oxford, United Kingdom.

Background: Humanized mouse models have proven a valuable tool in transplantation research. The vast majority of models employ peripheral blood mononuclear cells (PBMCs) for immune system reconstitution, which results in the production of a T cell-biased model. While useful, this prevents the investigation of human B and innate immune cells. Moreover, these models are prone to a human versus mouse xenograft-versus-host disease (XvHD). Models using haematopoeitic stem cells (HSCs) as donor cells may provide a method for the development of a wider range of human leukocytes. T cells developing in such a model would also be unlikely to cause XvHD. In this study we hypothesized that HSC-humanized mice produce multilineage cells with the capability of rejecting a human allograft.
Materials and Methods: Neonatal $Rag2^{-/-}c\gamma^{-/-}$ mice received myeloablative total body irradiation followed by $5x10^6$ umbilical cord blood (UCB)-derived HSCs (n=10 mice, two separate HSC donors). Peripheral blood human $CD45^+$ chimerism was detectable from week 6 onwards. However, overall chimerism levels were low and we therefore hypothesised that the presence of a human skin allograft or the addition of UCB-derived mononuclear cells (MNCs) would improve chimerism levels. Neonatal mice received HSCs as above and 6 weeks later a human skin allograft. One group received no further cells and another group received $5x10^6$ MNCs (n=5 mice per group). Mice receiving a skin graft with or without MNCs had significantly higher HLC levels at week 10 compared with mice without a skin graft ($p<0.05$). In mice receiving a skin allograft, the addition of MNCs was not associated with an additional improvement in the numbers of human leukocytes (p=0.56). Over a 19-week period none of the mice developed XvHD. At week 19 the percentage of leukocytes that were T cells was significantly higher in mice receiving MNCs (see figure, $p<0.0001$). In mice receiving skin grafts with or without MNCs, approximately 80% of developing cells were B cells, although there was a decline after week 15 which coincided with an increase in T cell numbers. Significantly more B cells developed in mice receiving HSCs alone compared with those receiving HSCs and MNCs ($p<0.05$). NK and dendritic cells were not detectable, although a small number of monocytes were observed (mean 0.4%). Human IgG and IgM were detectable although the former was found to be non-specific to the skin donor HLA type by Luminex. Although there was engraftment of multiple human cell types, skin graft rejection was not observed despite grafts being infiltrated with T cells, B cells and macrophages.
Conclusion: While the HSC mouse model recreates a more complete picture of the human immune system, the functionality of these cells is poor. It is likely that developing T cells are mouse MHC restricted and therefore ineffective in a human alloresponse. Methods to tackle this include the use of HLA-transgenic mice which allow for appropriate thymic selection.



P.1010

Effects of metformin on hyperglycemia in an experimental model of tacrolimus- and sirolimus-induced diabetic rats

Jian Jin, Sun Woo Lim, Long Jin, Ji Hyun Yu, Hyun Seon Kim, Kang Luo, Byung Ha Chung, Chul Woo Yang.
Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, South Korea.

Background and Aims: Metformin (MET) is a first-line drug for type II diabetes mellitus (DM); its effect on new-onset diabetes after transplantation caused by immunosuppressant therapy is unclear. We compared the effects of MET on DM caused by tacrolimus (TAC) or sirolimus (SRL).
Methods: DM was induced by injection of TAC (1.5 mg/kg) or SRL (0.3 mg/kg) for 2 weeks in rats, and MET (200 mg/kg) was injected for 2 more weeks. The effects of MET on DM caused by TAC or SRL were evaluated using an intraperitoneal glucose tolerance test (IPGTT) and by measuring plasma insulin concentration, islet size, and glucose-stimulated insulin secretion (GSIS). The effects of MET on the expression of AMP-activated protein kinase (AMPK), a pharmacological target of MET, were compared between TAC- and SRL-treated islets.
Results: IPGTT showed that both TAC and SRL induced hyperglycemia and reduced plasma insulin concentration compared with vehicle. These changes were reversed by addition of MET to SRL but not to TAC. Pancreatic islet cell size was decreased by TAC but not by SRL, but addition of MET did not affect pancreatic islet cell size in either group. MET significantly increased GSIS in SRL- but not in TAC-treated rats. AMPK expression was not affected by TAC but was significantly decreased in SRL-treated islets. Addition of MET restored AMPK expression in SRL-treated islets but not in TAC-treated islets.
Conclusions: MET has different effects on hyperglycemia caused by TAC and SRL. The discrepancy between these drugs is related to their different mechanisms causing DM.
Keywords: New onset diabetes after transplantation; Tacrolimus; Sirolimus; Metformin.

P.1011

Clinical features indicating irreversible rejection after uterus allotransplantation in cynomolgus monkey

Lori Kisu[1], Yojiro Kato[2], Yohei Yamada[3], Kentaro Matsubara[4], Hideaki Obara[4], Katsura Emoto[5], Masataka Adachi[1], Kiyoko Umene[1], Yuya Nogami[1], Kouji Banno[1], Yuko Kitagawa[4], Daisuke Aoki[1].
[1]Department of Obstetrics and Gynecology, Keio University School of Medicine, Tokyo, Japan; [2]Department of Transplant Surgery, International University of Health and Welfare, Mita Hospital, Tokyo, Japan; [3]Department of Pediatric Surgery, Keio University School of Medicine, Tokyo, Japan; [4]Department of Surgery, Keio University School of Medicine, Tokyo, Japan; [5]Department of Pathology, Keio University School of Medicine, Tokyo, Japan.

Objective: Uterus transplantation has been performed overseas with subsequent live birth and no serious rejection. However, rejection remains as a concern in this procedure. Organ elimination due to rejection after transplantation of a life-supporting organ generally results in a fatal outcome, but clinical features indicating irreversible rejection of the uterus are unclear. The objective of this study was to analyze symptoms associated with irreversible rejection after uterus allotransplantation in cynomolgus monkey.
Methods: Uterus allotransplantation was conducted in three cynomolgus monkeys (cases 1 to 3) that had subsequent rejection that was confirmed histologically in biopsy of the uterine cervix and was unresponsive to treatment. Clinical features present at the time of rejection and thereafter were evaluated retrospectively. Endpoints included performance status, hematology, transabdominal ultrasonography and histopathological findings in the uterus after extraction by laparotomy.
Results: In cases 1 to 3, rejection of the uterine cervix was diagnosed histopathologically on postoperative days 11, 35 and 35, respectively. Hematology in all animals showed increased WBC, LDH and CRP before and after rejection was diagnosed. In transabdominal ultrasonography, the uterus was swollen at the time rejection was diagnosed. The uterus then showed time-dependent shrinkage, which made it difficult to identify blood flow in the uterine artery. Performance status were good at the time of diagnosis of rejection and thereafter. Laparotomy conducted on postoperative days 85, 196 and 126, respectively, showed a white contracted uterus that was highly adhesive with surrounding tissues. Histopathological findings in each extracted uterus showed hyalinization in all uterine layers, endometrial deficit, interstitial lymphocytic infiltration and vascular intimal swelling and occlusion.
Conclusion: Irreversible rejection after uterus allotransplantation in cynomolgus monkeys was associated with increases in WBC, LDH and CRP and uterine shrinkage after transient swelling. These clinical features are important for monitoring of uterine rejection. Performance status was good even after the uterus failed due to rejection, which suggests that uterine transplantation rejection is not fatal, in contrast to rejection of life-supporting organs.

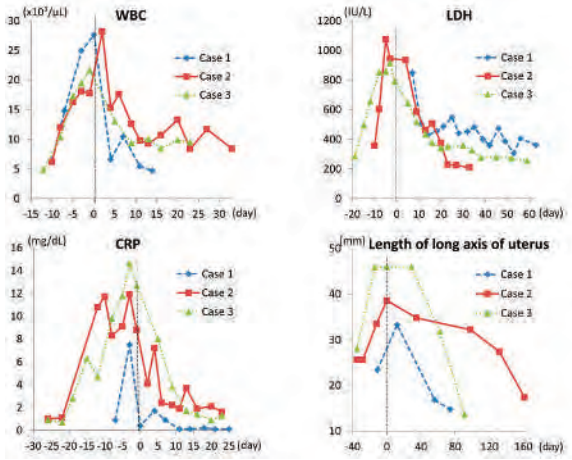


Figure 1. Changes in WBC, LDH, CRP and lengths of long axis diameter of uterus before and after rejection (day 0 = the day when rejection was histologically diagnosed)

This study was supported by the Adaptable and Seamless Technology Transfer Program through target-driven R&D, JST (AS2525033N); We are grateful to Hideaki Tsuchiya, Iori Itagaki, Ikuo Kawamoto, Takahiro Nakagawa, Hayato Narita and Atsushi Yoshida of Research Center for Animal Life Science, Shiga University of Medical Science for experimental support.

P.1012

Hepatic miR-301a: its mode of action on T-helper 17 cell-mediated rejection via targeting interleukin-6/STAT3 axis in rats

Chia-Yun Lai[1], Toshiaki Nakano[1,2], I-Hsuan Chen[1,2], Hui-Peng Tseng[1,2], Shih-Ming Yang[1,2], Li-Wen Hsu[1], Yu-Fan Cheng[1], Shigeru Goto[1,3,4], Chao-Long Chen[1].
[1]Liver Transplantation Program, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan; [2]Graduate Institute of Clinical Medical Sciences, Chang Gung University College of Medicine, Kaohsiung, Taiwan; [3]Fukuoka Institution of Occupational Health, Fukuoka, Japan; [4]Department of Nursing, Josai International University, Togane, Japan.

Introduction: The precise diagnosis of acute rejection (AR) is an important issue for optimal regulation of immune responses. This study aimed to characterize AR-related microRNAs (miRNAs) and the underlying AR mechanisms in liver transplantation.
Materials and Methods: Using a rat orthotopic liver transplantation (OLT) model and microarray assay, we compared miRNA expression profiles between naïve and AR livers at day 7 after OLT with short- (<14 days, DA-LEW) or long-term (>60 days, DA-PVG) survival fates. The AR-related miRNAs and pro-inflammatory cytokines were validated with quantitative real-time PCR. The AR-related miRNA-mediated inflammatory responses were confirmed by overexpression of the target miRNA in rat primary hepatocytes.
Results: The microarray revealed that miR-301a in the DA-LEW lethal AR livers was significantly higher than its expression in tolerogeneic DA-PVG AR livers. Overexpression of hepatic miR-301a induced interleukin (IL)-6 production in rat primary hepatocytes. The splenocytes co-cultured with miR-301a-overexpressing hepatocytes differentiated into retinoic acid-related orphan receptor γt (RoRγt)-positive and IL-17-secreting T-helper (Th)17 cells with the elevation of miR-301a and the reduced protein inhibitor of activated STAT3 (PIAS3) expression.
Discussion: Our present data suggest that excessive expression of hepatic miR-301a is linked to the formation of Th17 population via targeting the IL-6/STAT3 axis in the AR liver, resulting in the systemic elevation of Th17 cells in lethal AR.
Conclusions: Hepatic miR-301a may be one of potential targets for diagnosis and therapeutics in liver transplant rejection and tolerance induction.

P.1013

Combining monoclonal antibodies with 1,25(OH)2D3 treatment to prolong the survival time of cardiac allograft in accelerated rejection models of mice

Yan Guoliang[1,2], Xu Shuangyue[1,2], Li Yaguang[1,2], Qi Zhongquan[1,2].
[1]Organ Transplantation Institute, Xiamen University, Xiamen, People's Republic of China; [2]Fujian Provincial Key Laboratory, Organ and Tissue Regeneration, Xiamen, People's Republic of China.

In this study, we investigated the combination of 1,25(OH)2D3 and monoclonal antibodies anti-CD154/anti-LFA-1 to induce long-lived heart allograft acceptance in memory T cells-based adoptive mice model. The protective effects on grafts by different treatments were evaluated and the effective combination treatment and its action mechanism was explored.
In the transplantation model of adoptive transferred alloreactive T cells, the grafts would have the longest survival time by combining costimulation blockade which could be 80 days maximum. It was declared in pathology that the lymphocytic infiltration and the damage of tissue structure was lightest. The levels of IL-2, IFN-γ and IL-10 were obviously down-regulated in Group 1,25(OH)2D3 and Ab+1,25(OH)2D3, while the gene expression levels of TGF-β and Foxp3 were up-regulated. It's suggested that both of the treatments could increase the expression of Tregs in grafts. Detected by Flow Cytometry Method, we found that the alloreactivity and the proportion of memory CD4+ and CD8+ T cells were obviously decreased with treatment of Group 1,25(OH)2D3 and Ab+1,25(OH)2D3. The grafts could survival for a long time off the drugs, which was related with inducing numerous Tregs.
The combined recipe treatment could induce Th1 transdifferentiated to Th2. This treatment not only could significantly decrease the immune reaction of grafts and receptors, but also induce numerous Th3 phenotype which secreted TGF-β, and increased the proportion of Tregs.

P.1014

Effect of adipose-derived mesenchymal stem cells in a rat model of chronic renal allograft rejection

Rafael Pepineli, Priscila Q.Gouveia, Filipe M.O.Silva, Samirah A.Gomes, Giselle L.Tavora, IreneL. Noronha.
Renal Division, University of Sao Paulo Medical School, Sao Paulo, Brazil.

The use of stem cells is considered of great promise for tissue repair and regenerative medicine in organ transplantation. Mesenchymal stem cells (mSC) may represent a new strategy to prevent allograft rejection due to its anti-inflammatory and immunomodulatory properties. Besides being considered immune privileged dells, mSC have been shown to exert immune regulatory functions by secreting soluble factors that promote an immunosuppressive milieu. Among different sources of mSC, adipose tissue has the advantage to provide a large number of adipose-derived mSC (ADmSC) through a less invasive procedure. In the present study, the effect of ADmSC on the chronic renal allograft rejection model was analyzed.
The chronic rejection model was carried out by performing orthotopic kidney transplantation using male Fisher rats (F344) as donors and male Lewis rats (LEW) as recipients, without immunosuppression. Rats underwent orthotopic kidney transplantation, by placing the donor kidney on the left abdominal side, after a left nephrectomy. One day later, a right nephrectomy was performed. ADmSC were isolated from Lewis rats and expanded until the 4th passage, characterized by flow cytometry and by their potential ability to differentiate into mesenchymal lineages.
Transplant rats were divided into 3 groups (n=6 per group): Syngeneic (SYNG), untreated LEW rats receiving kidney from LEW rats (LEW-to-LEW); Allogeneic (ALLO), LEW rats receiving allogeneic kidney from F344 (F344-to-LEW); and ALLO+ADmSC, ALLO rats (F344-to-LEW) treated with ADmSC (3 doses of 1x106 cells at 0, 1 and 3 months after transplantation). The first administration of ADmSC or saline was performed on day 1, at the time of the right nephrectomy. The second and the third administration of ADmSC or saline (at 1 and 3 moths) were performed intravenously. All animals were followed up for 6 months. Blood pressure, urinary protein excretion, creatinine clearance, renal histology, immunohistochemistry for macrophages and T-cells, and qPCR for inflammatory cytokines were analyzed. Results are presented as mean±SEM; *p<0.05 vs SYNG, #p<0.05 vs ALLO.
At 6 months, the ALLO group presented significantly increased levels of blood pressure and urinary protein excretion and decreased creatinine clearance. Treatment with ADmSC ameliorated all these parameters. ALLO animals developed significantly interstitial fibrosis compared with the SYNG group, partially reversed by ADmSC treatment. In addition, ADmSC provided amelioration of allograft inflammation, characterized by decreased inflammatory cells and cytokine expression.

	SYNG	ALLO	ALLO+ADmSC
Blood Pressure (mmHg)	145±2	166±1*	147±2#
Urinary Protein Excretion (mg/24h)	79±17	167±29*	119±8#
Creatinine clearance (ml/min)	1.2±0.1	0.6±0.4*	1.5±0.3#
Interstitial Fibrosis (%)	7.2±0.6	24.4±2.3*	17.2±0.6*#
MØ (cells/mm²)	8±2	31±4*	4±0#
T-cells (cells/mm²)	8±3	26±3*	10±2#
IFN-γ (cells/mm²)	1.0±0.1	2.1±0.2*	0.4±0.3#
TNF-α (cells/mm²)	1.0±0.1	2.1±0.4*	1.0±0.4#
IL-1β (cells/mm²)	1.0±0.2	2.1±0.3*	1.0±0.3#
IL-6 (cells/mm²)	1.0±0.2	5.4±0.3*	3.0±0.4*#

In conclusion, in the chronic allograft rejection model Fisher to Lewis, administration of ADmSC was effective in protecting kidney allograft function, interstitial fibrosis and tissue inflammation. These findings may have important implications in the clinical settings and deserve further investigation.

P.1015

Activation of Human CD4+CD25+CD127loFOXP3+Treg by IL-4 and alloantigen induces activated Treg that do not express CD45RA and express the IL-5 receptor alpha (CD125)

NirupamaD. Verma[1,2], Catherine M.Robinson[1,2], Giang T.Tran[1,2], Rachelle M.Hall[1,2], Paul Wilcox[1,2], Suzanne J.Hodgkinson[1,2], Bruce M.Hall[1,2].
[1]Immune Tolerance Laboratory, Department of Medicine, University of New South Wales, Liverpool, Australia; [2]Ingham Institute, Liverpool Hospital, Liverpool, Australia.

Transplant tolerance induction requires $CD4^+CD25^+FoxP3^+$Treg known as tTreg. There is interest in expanding tTreg for therapy, but they are not antigen specific and are required in large numbers to induce tolerance. In our rat model, we found IL-4 in the absence of IL-2 can induce changes in naïve tTreg that have TCR for stimulating donor antigen, inducing the receptor for IL-5(IL-5Ra). These activated Treg are further expanded by IL-5 to increase their capacity to suppress allograft rejection.

In this study we examined if isolated human $CD4^+CD25^+CD127^{lo}FoxP3^+$Treg from a healthy donor could be activated by IL-4 and alloantigen to become activated Treg that expressed the IL-5 receptor. Treg were isolated from healthy human blood using Human $CD4^+CD127^{lo}CD25^+$Regulatory T cell isolation Kit. The unfractionated and isolated ($CD25^+CD127^{lo}$) cells were subjected to multicolour flow cytometry using mouse anti-human antibodies for Treg markers. CD25+ cell population was subjected to invitro culture for 3-4 days with allogeneic stimulator cells for assessment of proliferation and expression of cytokine and cytokine receptors.

First, we compared proliferation of tTreg cultured with rIL-2 or IL-4 in MLC with fully allogeneic donor cells. There was significant proliferation at days 3-7 with a peak at day 4, the level of proliferation was less than with IL-2 however. Further studies cultured for 4 days, and the yield of cells after 4 days was increased by 50%.

FACS analysis showed a reduction in $CD45RA^{hi}$ and an increase in $CD45RA^{intermediate}$ population, and a marked increase in $CD45RA^-$ cell population (43% vs 11%). CD45RO and $CD45RB^+$ population also increased. Nearly all cells continued to express CD62L. There was no induction of CCR8, the Th2 associated chemokine receptor.

RT-PCR of cultured Treg compared to the unstimulated cell population and Treg cultured with IL-2 and alloantigen, showed induction of IL-5Ra (CDw125) in only nTreg cultured with IL-4 and alloantigen and not in other cells.

These studies showed that human $CD4^+CD25^+CD127^{lo}FoxP3^+$Treg can be activated to express the IL-5 receptor, These are in line with our studies in rodents, suggesting IL-5 may promote the activation of antigen specific Treg. Culture with IL-4 appears to induce polyclonal activation of nTreg whilst the increased $CD45RA^-/CD45RO^+$ population was consistent with activation of antigen specific Treg. These studies suggest the alternate pathway for activation of tTreg may produce more potent antigen specific Treg for therapy circumventing the need for high numbers of nTreg requirements.

References:

[1] Verma ND. CD4+CD25+ T cells alloactivated ex-vivo by IL2 or IL4 become potent alloantigne-specific inhibitors of rejection with different phenotypes suggesting separate pathways of activation by Th1 and Th2 responses *Blood*. 2009: 113: 479-487.

P.1016

Regulatory T-cell mediated suppression of T-cell responses induced by primary human hepatocytes in vitro is cell-contact dependent

Daphne E.DeTemple[1], Moritz Kleine[1], Christine S.Falk[2,3], Wolf Ramackers[1], Mark D.Jäger[1], Jürgen Klempnauer[1], Michael Bock[2,4], Hüseyin Bektas[1], Florian Vondran[1,2].
[1]General, Visceral and Transplant Surgery, Hannover Medical School, Hannover, Germany; [2]German Centre for Infection Research (DZIF), Partner Site Hannover-Braunschweig, Hannover, Germany; [3]Institute of Transplant Immunology, Integrated Research & Treatment Centre Transplantation (IFB-Tx), Hannover Medical School, Hannover, Germany; [4]Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany.

Background: Hepatocyte transplantation (HCTx) is considered a promising therapeutic approach for the treatment of various chronic and acute liver diseases. The liver is considered an immunoprivileged organ, nevertheless, transplanted allogeneic hepatocytes so far only survived for a few months despite application of conventional immunosuppression. Encouraging results achieved in animal models thus could not successfully be transferred to clinical practice, yet. In recent years, the important role of regulatory T-cells (T_{reg}) for the development of tolerance after transplantation of solid organs was defined. T_{reg} are able to suppress T-cell mediated immunity and to modulate effectors of the innate immune system. Consequently, we anticipated that T_{reg} might also have the potential to reduce postoperative cell-loss and to enhance the long-term allograft-acceptance after HCTx. This study hence characterized T-cell responses induced by primary human hepatocytes (PHH) as well as the ability of T_{reg} to modulate this process *in vitro*.

Material and Methods: PHH were isolated using a 2-step perfusion technique from partially resected liver specimens and subsequently cultured as monolayers. $CD4^+CD25^{high}$ T_{reg} were sorted from human peripheral blood lymphocytes (HPBL) and expanded with CD3/CD28-expander beads and high doses of interleukin-2. Allogeneic T-cell responses in terms of cell proliferation were investigated in mixed lymphocyte cultures (MLC) and mixed lymphocyte hepatocyte cultures (MLHC) by labeling responder cells with PKH-26 and using flow cytometry for read-out. T_{reg} were co-cultured in a single-well and trans-well setup with PHH and HPBL. Multi-colour flow cytometry was applied for characterization of T-cell subpopulations. The Bio-Plex technology was used for analysis of cytokine profiles from culture supernatants.

Results: Proliferative T-cell responses to allogeneic stimulation with PHH (MLHC) was noticeably reduced and showed a delayed onset compared to conventional MLC. Especially CD4+ T-cells appeared to be the mediator of this immune reaction with $CD8^+$-subpopulations only showing a slight proliferation. Nevertheless, for the latter population a considerable up-regulation of the early activation marker CD69 was observed. Co-culture of T_{reg} within the same well resulted in efficient suppression of PHH-induced T-cell activation, whereas this effect was significantly reduced when T_{reg} were cultured in a trans-well setup. The regulatory potential of T_{reg} could not only be demonstrated for the proliferative T-cell response but also the associated cytokine profiles showing significantly reduced secretion of inflammatory mediators. In line with the cell-contact dependency of the suppressive effect, interleukin-10 was not found to play key role in this immune reaction.

Conclusion: T-cell responses to allogeneic PHH are mainly $CD4^+$ T-cell driven and efficiently can be suppressed when co-cultured with T_{reg}. The immunomodulatory effect of T_{reg} was shown for T-cell proliferation as well as cytokine expression and seems to be cell-contact dependent.

P.1017

A Traditional chinese herbal medicine "Huaier" induced prolongation of fully MHC-mismatched murine cardiac allograft

Enzhlyin[1], Masateru Uchiyama[1], Takako Yanagisawa[1], Satoko Mizuno[2], Etsuo Hoshino[3], Masanori Niimi[1].
[1]Surgery, Teikyo University, Tokyo, Japan; [2]Neurology, Saitama International Medical Center, Saitama Medical College, Saitama, Japan; [3]Cancer Institute Ariake Hospital, Tokyo, Japan.

Background: Huaier is one of mushrooms and consists of Trametes Robiniophila Murr. Huaier has been commonly used in China for cancer complementary therapy recently. However, concerning the mechanisms of its effects, there is little knowledge at present. We investigated the effects of Huaier in the survival of fully MHC-mismatched murine cardiac allograft transplantation.
Methods: CBA mice (H2k) underwent transplantation of C57BL/6 (B6, H2b) hearts and received oral administration of 6g/kg/day of Huaier on the day of transplantation until 7 days afterward. Immunohistochemical (IHC) study was performed to assess the degree of CD4/CD8/CD68+ cell-infiltration to cardiac allografts. Cell proliferation was also performed.
Result: Untreated CBA mice rejected B6 cardiac grafts acutely (median survival time [MST], 7 days). When CBA recipients were treated with Huaier for 7 days, the allograft survival was a little prolonged to MST, 16 days. Meanwhile, interestingly, approximately 20% of the Huaier-treated CBA recipients rejected B6 cardiac allograft on 6 days after transplantation. Cell proliferation of splenocytes from Huaier-treated recipients was suppressed compared with that from untreated recipients. IHC study on 6 days after grafting showed that CD4/CD8/CD68+ cell-infiltration to myocardium of allografts from Huaier-treated CBA recipients was suppressed. On the other hand, CD8/CD68+ cell-infiltration around coronary artery and intimal thickening of coronary artery in allografts from Huaier-treated CBA recipients were relatively enhanced.
Conclusion: Administration of Huaier might induce prolongation of fully MHC-mismatched cardiac allografts by less infiltration of inflammatory cells, however, show angiopathic effects. A traditional Chinese herbal medicine "Huaier" could have two different aspects, immunosuppressive and anti-cancer effects.

P.1018

Transplant tolerance induced by a single infusion of extracorporeal photopheresis treated donor splenocytes prior to transplantation

Zheng Zhang[1], Xin Kang[1], Junsheng Ye[1,2], Xin Yi Yeap[1], Mireia Caralt Caralt[1], Jiao-Jiang Wang[1], Jennifer Schneiderman[3].
[1]Surgery/Organ Transplantation, Northwestern University, Chicago, IL, United States; [2]Surgery/Organ Transplantation, Nanfang Hospital, Guangzhou, People's Republic of China; [3]Pediatric Hematology/Oncology/Stem Cell Transplant, Feinberg School of Medicine, Northwestern University, Chicago, IL, United States.

Extracorporeal Photopheresis (ECP) is an immune therapy in which blood leukocytes are treated with 8-methoxypsoralen followed by UV-A light, and then returned to patients. It has been approved by the US Food and Drug Administration (FDA) for the treatment of advanced cutaneous T-cell lymphoma (CTCL) since 1988. In addition to CTCL, ECP has been shown to have efficacy in the treatment of other disorders, including transplant rejection, graft versus host disease, and atopic dermatitisIts by treating the host immune cells. However, it is not known whether it plays a role in tolerance inducation. This study was aimed to determine whether treatment of donor splenocytes (DSP) with ECP (ECP-DSP) prior to transplant induces allotranspalnt tolerance. DSPs (5×10^7) from BALB/c donors were isolated and treated with ECP and then infused, i.v. into fully mismatched allogeneic C57BL/6 (B6) recipients 7 days prior to receiving heterotopic heart transplantation with BALB/c hearts. The graft survival was determined by the ceasation of heart beating. Immunophenotyping and histology were performed at pre-selected time points and at the endpoint of rejection. The results showed that recipients treated ECP-DSPs had significantly prolonged cardiac allograft survival (from 7 days to 21days, $p<0.01$) compared to untreated controls. Combining ECP-DSPs with transient RAPA further extended allograft survival to 59 days compared to RAPA alone (27days, $P<0.05$). Graft protection was donor specific as ECP-DSP treated recipients rejected 3rd party C3H cardiac allografts. ECP-DSPs markedly reduced intragraft infiltrates, decreased peripheral $CD44^{hi}CD62L^{low}$ effectors and IFN-γ producing T cells, and diminished donor specific antibodies ($p<0.05$, vs controls). Since the liver is considered the most tolerogenic organ transplanted, we tested whether the ECP-DSP approach promotes the tolerance in liver allografts, using an orthotopic liver transplantation. DSPs from ACI rats treated with ECP were infused into Lewis rat recipients 7 days prior to being transplanted with livers from ACI donors. The results revealed that all liver allografts treated with ECP-DSP survived beyond POD150 with normal liver function and histology, while the untreated rejected the liver grafts within 15 days. The long-surviving allografts accepted donor-type but not 3rd-party (BN) skin grafts, suggesting that a state of donor-specific tolerance. Furthermore, significant increases in the frequencies of Tregs ($CD4^+CD25^+Foxp3^+$) were observed in ECP-DSP recipients as examined at day 7 post-transplant and persisted at day 14 and day 28. In parallel, higher percentages of donor chimerism ($RT1^{av+}$), primarily myeloid derived cells, were also detected in the ECP-DSP treated liver transplants.
In conclusions, pre-transplant infusion of ECP-DSPs differentially regulates T effector vs Tregs and significantly prolonged liver and cardiac allograft survival and induces donor specific tolerance, therefore, has significant potential for clinical translation in solid organ transplantation.

P.1019

The effect of donor's chemokines (CXCL9, CXCL10 and CCL5) and growth factor (TGFβ1) on the renal allograft dysfunction

Sadaf Firasat[1], Ali Raza[1], Aiysha Abid[1], Abdul Rafay[1], Shafaq Mahmood[1], Tahir Aziz[2], Muhammad Mubarak[3], Anwar Naqvi[4], S Adibul Hasan Rizvi[4], Syed Qasim Mehdi[1], Shagufta Khaliq[1].
[1]CHGMM, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [2]Nephrology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [3]Pathology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [4]Urology and Transplantation, Sindh Institute of Urology and Transplantation, Karachi, Pakistan.

Introduction: Early non-invasive biomarkers are needed that indicate renal allograft dysfunction. Donors characteristics (such as donor age, HLAs match) have shown significant impact on graft functioning. It is interesting to know what biochemical or molecular changes are induced in donor after implantation of its kidney into the recipient.
Objective: We aimed to evaluate the patterns of expressions of selected biomarkers (CXCL9, CXCL10, CCL5 and TGFβ1) in renal transplant recipients and their respective donors during the course of rejection.
Methods: For the pilot study, a total of 339 urine samples from live-related renal transplant recipients (a; at transplant and b; at graft biopsy) and their respective donors (a; at nephrectomy for transplant, b; at rejection of their recipient) were collected. The samples were quantified for biomarkers using the ELISA kits and were analyzed using the SPSS and MedCalc software. Due to non-parametric distribution of the biomarkers, Mann-Whitney U test and Kruskal-Wallis H test were applied and P-value less than 0.05 were considered to be significant. The data were analyzed; first, biomarker levels of donors' sample (at (1) transplant time and (2) recipient's rejection time) were compared to know any change during time interval (time between transplant and rejection) and second, donor's samples at transplant and at rejection time were also compared with recipient rejection time to detect changes in the donor –recipients samples.
Results: We observed an overall trend of increase in the expression levels of markers in donors after transplantation. The data showed that expression levels of the biomarkers in the donors were raised significantly for the CXCL10 ($p=0.002$), CXCL9 ($p=0.034$) and was non-significant for the CCL5 ($p=0.519$) and TGFβ1 ($p=0.812$) at the time of recipient's rejection when compared to their levels at nephrectomy. Interestingly we found a significant difference between the recipient's and donor's levels at the time of allograft rejection (CXCL10 $p<0.0001$; CXCL9 $p=0.046$; CCL5 $p=0.006$; TGFβ1 $p=0.045$).
Conclusion: The observation of significant difference between the recipients and the donor's samples at the time of rejection may indicate that the donor's derived inflammatory markers have little or no role in recipient's rejection. However, the levels were increased significantly in the donors after transplantation. These results should be interpreted with caution. It is therefore required to recruit a large number of samples to further evaluate the role of donor's derived markers in kidney graft outcome.

P.1020

Negative influence of liver irradiation on liver resident NK cells

Ryosuke Nakano, Masahiro Ohira, Takuya Yano, Hideki Ohdan.
Department of Gastroenterological and Transplant Surgery, Hiroshima University, Hiroshima, Japan.

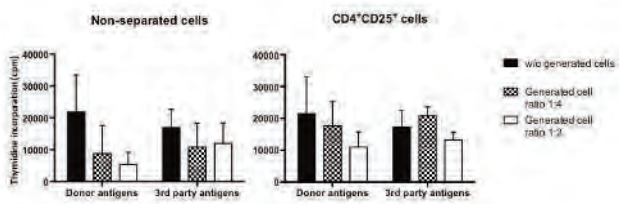
Introduction: In recent years, there has been increased focus on the development of radiation therapy post solid organ transplantation, to treat of liver tumor. However, there is limited information regarding the effect of liver irradiation on the immune systems in the liver.
Liver resident natural killer (lrNK) cells play important roles in the innate immune system of the liver. We previously reported that these lrNK cells demonstrate potent anti-tumor activity against hepatoma cells in hepatectomized mice model. The lrNK cells strongly expressed tumor necrosis factor-related apoptosis-inducing ligand (TRAIL); however, such expression was significantly reduced after partial hepatectomy or partial liver transplantation. Augmentation of TRAIL expressing lrNK cells inhibited the growth of hepatoma in the liver. In addition, the lrNK cells have recently been reported to develop from hematopoietic progenitor cells in the liver.
A central question is whether liver irradiation can influence the development and function of the lrNK cells. The aim of this study was to assess the accompanying effect of irradiation on the lrNK cells. The findings were expected to provide a rationale for the combination cellular therapy following irradiation in patients with hepatoma.
Method: Hepatic irradiation in mice was performed as follows: anesthetized C57BL/6 mice were first subjected to laparotomy, followed by intraoperative irradiation of all hepatic lobes using a tungsten sheet. In each animal, a sheet was placed behind the liver to shield the other abdominal organs and another sheet was placed to cover the whole body, except the laparotomy area. The animals were treated with single irradiation dose of 5, 10, or 20 Gy. For investigation, the mice were sacrificed 1, 2, 3, and 4 weeks post irradiation. Subsequently, we determined the population of intrahepatic lymphocytes and the phenotype of the lrNK cells.
Result: After whole-liver irradiation, there is no difference in the percentage of T cells and NKT cells in the liver. Whereas, there was a gradual decrease in the percentage of NK cells after whole-liver irradiation over the course of weeks. Particularly, the lrNK cells decreased significantly post whole-liver irradiation (before irradiation: 43.4%, 4 weeks post irradiation: 27.5%, 25.2%, and 19.6%, in the 5 Gy, 10Gy, and 20Gy groups, respectively). The expression of TRAIL on the liver NK cells was reduced because of hepatic irradiation (before irradiation: 40.4(MFI), 4 weeks post irradiation: 19.1, 12.6, and 11.5, in the 5 Gy, 10Gy and 20Gy groups, respectively). We also found a positive correlation between the dose of irradiation and lrNK cell function.
Discussion: We demonstrated that liver irradiation negatively influences the liver resident NK cells including the number and function of the cancer cells for at least a period of 1 month. It is suggested that whole-liver irradiation can abolish the hematopoietic progenitor cells in the liver. These findings thus provide a rationale for the combination cellular therapy following radiation in transplant recipients with hepatoma.

P.1021

Ex vivo generation of alloantigen-specific Immunomodulatory cells with co-stimulation blockade: do we need Treg purification for cell therapy?

Ming H.Yao, Makiko Kumagai-Braesch, Torbjörn Lundgren, Dennis Andersson, Bo-Göran Ericzon, Masaaki Watanabe.
Transplantation Surgery, Karolinska Institutet, Stockholm, Sweden.

Purpose: Regulatory T cells (Tregs) have been used to minimize the use of immunosuppressants and/or to induce tolerance. However, the complexity and extremely high cost of Treg purification process hamper the widespread application of Treg cell therapy. Adoptive transfer of ex vivo generated alloantigen-specific immunomoduratory cells by using CD28/B7 co-stimulatory blockade has been shown to achieve operational tolerance[1]. In this clinical trial, generated cells were transferred without any Treg purification processes. Herein, we generated alloantigen-specific immunomodulatory cells by using CTLA4-Ig (Belatacept®), and separated $CD4^+CD25^+$ cells to investigate the efficacy of Treg purification on immunomodulatory effects.
Methods: Human peripheral blood mononuclear cells (PBMCs) (50 x 10^6 cells) were co-cultured with irradiated HLA-mismatched donor PBMCs (20 x 10^6 cells) in the presence of CTLA4-Ig. At day 7, irradiated donor PBMCs, culture media, and CTLA4-Ig were replenished. At day 14, generated cells were collected and separated $CD4^+CD25^+$ cells. Phenotypes and immunomodulatory effects of these separated cells and non-separated cells were evaluated.
Results: After 14 days of co-culture $CD4^+CD25^+CD127loFoxp3^+$ Tregs increased from 4.1±1.0% to 7.3±2.6%. Non-separated generated cells effectively inhibited proliferative responses of freshly isolated recipient PBMC against donor-antigen in mixed lymphocyte reactions, in a generated cell-number dependent fashion. While, this effect was minor against third-party antigens (% response of the proliferation at the responder : generated cell ratio 1 : 0.5; against donor antigen 27±17 % and against the 3rd party antigens 78±53% (n=3)). $CD4^+CD25^+$ cells, which supposed to contain Tregs, suppressed the proliferation responses (% response against donor antigen; 56±15% and against 3rd party antigens; 79±13%). There was no significant differences of the inhibitory efficacy between non-separated and $CD4^+CD25^+$ cells. No significant differences were observed in the production of IFN- γ and IL-10.



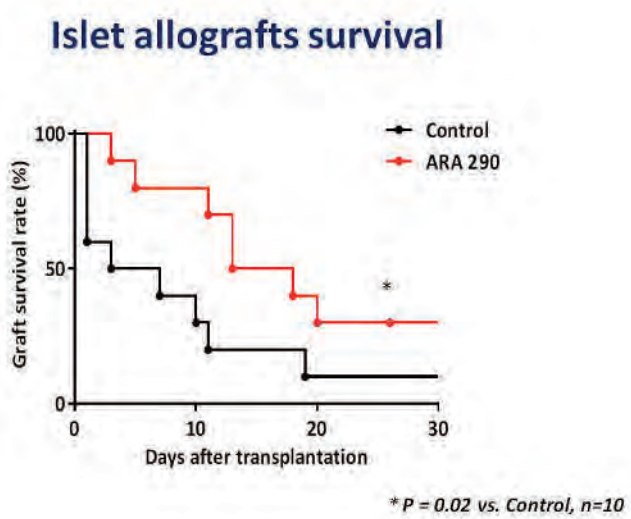
Conclusions: Non-separated and $CD4^+CD25^+$ separated alloantigen-specific generated cells with CTLA4-Ig showed comparable immunomodulatory effects.
References:
[1] Todo S et.al. A Pilot Study of Operational Tolerance with a Regulatory T Cell-Based Cell Therapy in Living Donor Liver Transplantation. Hepatology. 2016 Jan 16. doi: 10.1002/hep.28459. [Epub ahead of print]

P.1022

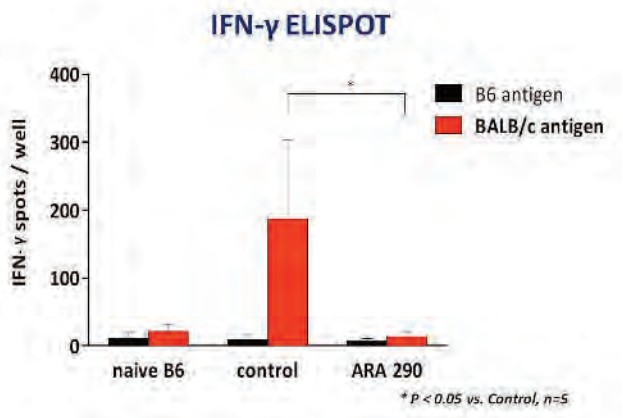
A non-hematopoietic erythropoietin analogue, ARA 290, prolonged allogeneic islet graft survival in a mouse model

Masaaki Watanabe[1], Ming H.Yao[1], Bo-Göran Ericzon[1], Anthony Cerami[2], Michael Brines[2], Torbjörn Lundgren[1], Makiko Kumagai-Braesch[1].
[1]Transplantation Surgery, Karolinska Institutet, Stockholm, Sweden; [2]Araim Pharmaceuticals, Ossining, NY, United States.

Purpose: Erythropoietin exerts anti-inflammatory, anti-apoptotic and cyto-protective effects in addition to its hematopoietic property. We have recently shown that ARA 290, a non-hematopoietic erythropoietin analogue, protected pancreatic islets from cytokine-induced damage and apoptosis and also ameliorated inflammatory responses after pancreatic islet transplantation (PITx) through the inhibitory effects on macrophages, and results in a successful engraftment even with a small amount of transplanted islets in the syngeneic mouse model[1]. In this study we further investigated the effect of ARA 290 on mouse allogeneicPITx.
Methods: Immunomodulatory effects of ARA 290 were assessed by allogeneic mouse mixed lymphocyte culture (MLC) and bone marrow derived dendritic cells (DCs). Balb/c mice (H-2d) islets (3-400 islets) were transplanted of streptozotocin-induced diabetic C57BL/6N mice (H-2b) via the portal vein. Recipients were given ARA 290 (120 μg/kg) intraperitoneally just before and at 0, 6, and once per day during 14 days after PITx. Blood glucose level was monitored and graft survival was assessed. Immunological status was assessed at 5 days after PITx.
Results: The treatment with ARA 290 significantly prolonged graft survival time compared to non-treated control group (MGT; 15.0 days vs. 5 days, *p=0.02, n=10 in each group).



The frequency of allo-reactive IFN-γ producing precursors in the spleen was significantly lower in the ARA 290 treatment group than in the control group (IFN-γ spot/well:13±7,6 vs 187±116, *p<0,05, n=5 in each group).



In vitro study, ARA 290 did not inhibit T cell proliferation nor IFN-γ production in allogeneic MLC. While, ARA 290 treated DCs showed less LPS driven CD86 up-regulations, and IL-6, TNF-α, and IFN-γ production.

Conclusion: ARA 290 significantly prolonged islet grafts survival following allogeneic PITx.With having anti-inflammatory and anti-apoptotic properties, ARA 290 may become a promising modality in clinical PITx.

References:

[1] Watanabe M. A Nonhematopoietic Erythropoietin Analogue, ARA 290, Inhibits Macrophage Activation and Prevents Damage to Transplanted Islets.Transplantation 2015, Dec. 17 (e-pub)

P.1023

Effects of addition of a dipeptidyl peptidase IV inhibitor to metformin on sirolimus-induced diabetes mellitus

Long Jin, Sun Woo Lim, Jian Jin, Byung Ha Chung, Chul Woo Yang. Transplant Research Center and Division of Nephrology, Department of Internal medicine, Seoul, St Mary's Hospital, The Catholic University of Korea, Seoul, Korea.

Background: The contribution of the immunosuppressant sirolimus (SRL) to the development of post-transplant diabetes mellitus (PTDM) are being increasingly recognized. This study was performed to evaluate whether the combined treatment of dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) with metformin results in lower glycaemia than DPP IV inhibitor or metformin administered alone in SRL-induced diabetes mellitus.

Method: Male Sprague-dawley rats were treated initially with SRL (0.3 mg/kg, s.c) for 3 weeks and then started to treated with DPP IV inhibitor (LC15-0444, 5 mg/kg/day, oral gavage) and/or metformin (200 mg/kg/day, oral gavage) for further 3 weeks. Body weight, urine volume, and water intake were measured before sacrifice. The effect of combined treatment of LC15-0444 and metformin was evaluated by intraperitoneal glucose tolerance test (IPGTT), serum insulin level, and islet size. Area under the curve for glucose (AUCg) from IPGTT graph also calculated by trapezoidal estimation. The influence of oxidative stress was evaluated by measuring 8-hydroxy-2`-deoxyguanosine (8-OHdG) level in pancreas tissue sections. Insulin secretion capacity was evaluated by glucose-stimulated insulin secretion (GSIS) test using normal isolated rat islets treated with SRL (90 ng/mL), exendin-4 (100 ng/mL) and/or metformin (165 ng/mL). Multiple comparisons among groups were performed by one-way analysis of variance with the post hoc Bonferroni test.

Results: After 6 weeks, SRL treatment decreased changes of body weight and increased water intake and urine volume for 24-h compared with vehicle (VH) group. SRL treatment significantly increased AUCg from the values obtained during the IPGTT, but LC15-0444 or metformin treatment group recovered these value compared with the SRL alone. Combined treatment with LC15-0444 and metformin has more significantly decreased blood glucose level than LC15-0444 or metformin treatment alone. Consistently, serum insulin level showed higher in combination group (LC15-0444+metformin) than LC15-0444 or metformin treatment alone. Islet size which was significantly decreased in SRL group was recovered with combined treatment of LC15-0444 and metformin group compared with single treated group. SRL treatment significantly increased intense nuclear expression and larger positive area for 8-OHDG, but LC15-0444 or metformin treatment group recovered these values. Combined treatment with LC15-0444 and metformin has more significantly decreased expression of 8-OHdG than LC15-0444 or metformin treatment alone. The result of GSIS also showed more significantly higher insulin level in combined group than LC15-0444 or metformin treatment alone.

Conclusion: Combined treatment with LC15-0444 and metformin showed better glucose tolerance by improving insulin secretion than single therapy. This finding provides the rationale for the use of DPP IV inhibitor in PTDM taking sirolimus and metformin.

P.1024

Immune modulatory effect of thalidomide and dexamethasone co-treatment on T cell subsets

Eun Jee Kim[1,2], Joon Ye Kim[1], Beom Seok Kim[1,3], Dong Jin Joo[1,4], Kyu Ha Huh[1,4], Myoung Soo Kim[1,4], Yu Seun Kim[1,4].
[1]The Research Institute for Transplantation, Yonsei University College of Medicine, Seoul, Korea; [2]Brain Korea 21 PLUS Project for Medical Science, Yonsei University, Seoul, Korea; [3]Nephrology Division, Department of Internal medicine, Yonsei University Health System, Seoul, Korea; [4]Department of Transplantation Surgery, Yonsei University Health System, Seoul, Korea.

Background: Thalidomide (TM) is known to have anti-cancer and anti-inflammatory properties; however, its mechanism on T cells is still unclear. Previously we showed immune modulatory effect of TM on T cells, and corticosteroid potentiates therapeutic effect of TM on lupus nephritis model. Here we examined whether TM/corticosteroid co-treatment has synergistic immune modulatory role on T cells.
Methods: Splenic naive T cells (Tnaives) from C57BL/6 mice were sort-purified and cultured for $CD4^+$ T cell proliferation and regulatory T cells (Tregs) conversion with TM and/or dexamethasone (DX) treatment. Also T cell suppression assay was performed to evaluate the function of converted Tregs. All samples were analyzed by flow cytometry after stained with anti-mouse CD4, Foxp3, OX40 (CD134), or glucocorticoid-induced TNFR-related protein (GITR; CD357).
Results: TM significantly decreased the proliferation of $CD4^+$ T cells in dose-dependent manner ($P <$. 01) and low dose DX co-treatment further decreased the proliferation synergistically ($P < .03$). In contrast, TM/DX co-treatment ameliorated the inhibitory effect and function of isolated DX on Treg conversion ($P < .04$). Furthermore DX treatment impaired the functions of converted Treg, which was recovered by TM/DX co-treatment. Also, Reduced GITR and OX40 expressions by DX treatment were ameliorated by TM/DX co-treatment (GITR; $P <$ .01, OX40; $P < .04$).
Conclusion: Considering the selective effect of TM on different T cell subsets, TM may have an immune modulatory role and DX co-treatment could further enhance the effect partially by the change of GITR and OX40 expression on Tregs. Further study is required to elucidate the underlying link between corticosteroid and thalidomide effect on T cells.

P.1025

Effect of isolated intraperitoneal sirolimus treatment on the kidneys of C57BL/6 mice

Yuri Cho[3], Beom Seok Kim[1,3], Hyojung Lee[3], Joon Ye Kim[3], Eun Jee Kim[3], Dong Jin Joo[2,3], Kyu Ha Huh[2,3], Myoung Soo Kim[2,3], Yu Seun Kim[2,3].
[1]Nephrology Division, Department of Internal Medicine, Severance Hospital, Yonsei University Health System, Seoul, Korea; [2]Department of Transplantation Surgery, Severance Hospital, Yonsei University Health System, Seoul, Korea; [3]The Research Institute for Transplantation, Yonsei University College of Medicine, Seoul, Korea.

Sirolimus, a mammalian target of rapamycin inhibitor, is widely used in transplantation. However, the mechanism by which sirolimus induces adverse effects such as proteinuria and edema remains unclear. To determine whether isolated sirolimus treatment induces proteinuria, we intraperitoneally injected C57BL/6 mice with different doses of sirolimus (0, 3, 10, or 30 mg/[kg·d]) for 24 d. Urinary albumin excretion was then quantified with a double-sandwich enzyme-linked immunosorbent assay, and serum creatinine levels were measured with a single dry-film chemistry auto-analyzer. mRNA expression levels of various genes were also measured by polymerase chain reaction. Urinary albumin was not detected in the sirolimus-treated mice; however, serum creatinine levels were found to increase in a dose-dependent manner and were significantly higher in the group treated with 30 mg/kg of sirolimus than for the control. Glomerular mRNA expression profiling showed decreased levels of podocyte-related genes (WT-1, synaptopodin, nephrin, CD2AP, and podocin), and the fibrosis marker TGF-beta in the sirolimus-treated mice. Furthermore, reduced expression of the antiapoptotic genes Bcl-2 and Bcl-xL was seen. Also, these proteins expression were decreased by sirolimus treatment. Although sirolimus treatment decreased the expression of slit diaphragm-associated genes and increased serum creatinine levels, it failed to induce proteinuria in this model. Further studies are required to evaluate the condition in which sirolimus induces proteinuria.
References:
[1] Reiser J, Sever S. Podocyte biology and pathogenesis of kidney disease. *Annu Rev Med* 2013;64:357-66.
[2] Tryggvason K, Patrakka J, Wartiovaara J. Hereditary proteinuria syndromes and mechanisms of proteinuria.*N Engl J Med* 2006;354:1387-401
[3] Flechner SM. Sirolimus in kidney transplantation indications and practical guidelines: de novo sirolimus-based therapy without calcineurin inhibitors. *Transplantation* 2009;87:S1-6.
[4] Jhaveri KD, Schatz JH, Young JW, Flombaum C. Sirolimus (rapamycin) induced proteinuria in a patient undergoing allogeneic hematopoietic stem cell transplant. *Transplantation* 2008;86:180-1.
[5] Sun YB, Qu X, Zhang X, Caruana G, Bertram JF, Li J. Glomerular endothelial cell injury and damage precedes that of podocytes in adriamycin-induced nephropathy. *PLoS One* 2013;8:e55027.

P.1026

Synergistic anticancer effect of metformin in combination with immunosuppressant on hepatocellular carcinoma cell lines

Sukwon Suh, Kwang-Woong Lee[2], Yoo-Shin Choi[1].
[1]Surgery, Chung-Ang University, Seoul, Korea; [2]Surgery, Seoul National University, Seoul, Korea.

Background: After liver transplantation for hepatocellular carcinoma (HCC), immunosuppression is required to avoid rejection and graft loss; however, it can stimulate the recurrence and progression of HCC[1]-[6]. Previous studies have shown metformin's anticancer effects on HCC[7]-[13]. This study was aimed at evaluating the interactions between metformin and immunosuppressive agents, including sirolimus, tacrolimus, and mycophenolate mofetil, for antitumor activity.
Methods: Three HCC cell lines (Huh7, HepG2, and Hep3b) were investigated. Cell viability was determined using the MTT assay while western blot analysis of mTOR pathway-related proteins was performed to elucidate underlying mechanisms.
Results: Metformin and sirolimus showed synergistic antiproliferative effects and sirolimus plus metformin supplemented with mycophenolate mofetil showed synergistic antiproliferative effects in specific HCC cells.

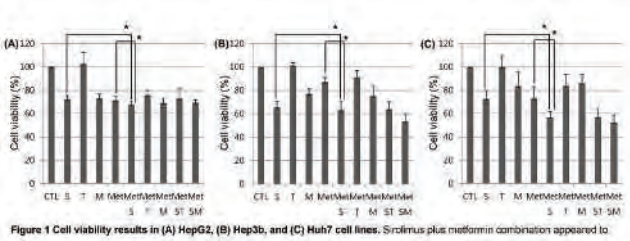


Figure 1 Cell viability results in (A) HepG2, (B) Hep3b, and (C) Huh7 cell lines. Sirolimus plus metformin combination appeared to synergistically induce significantly lower cell viability than sirolimus or metformin alone did. *P < 0.01, †sirolimus plus metformin supplemented with mycophenolate mofetil (MMF) showed additional significant synergistic inhibition (P < 0.01)

A synergistic effect of metformin and sirolimus via inhibition of mTOR and its downstream molecules p70S6K and p-4EBP1 was also observed. Metformin and sirolimus also synergistically downregulated livin and survivin expressions in HepG2 and Hep3b cells.
Conclusions: Metformin synergistically interacted with sirolimus, to produce enhanced anticancer effects against HCC cells and the underlying mechanism might involve the mTOR pathway. These results may provide a foundation for further studies on the use of this combination to enhance therapy for patients who have undergone liver transplantation for HCC.

References:

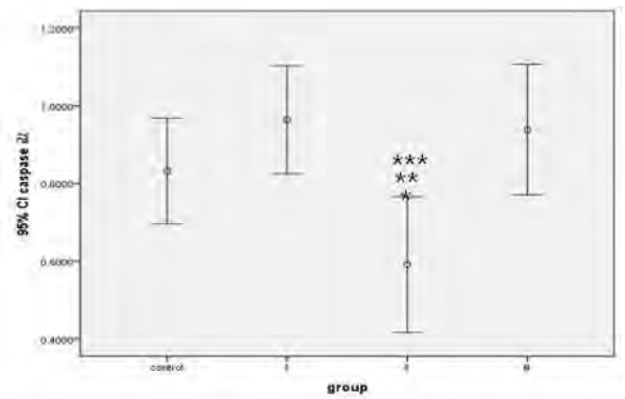
[1] Bruix J, Sherman M. Management of hepatocellular carcinoma. Hepatology 2005; 42: 1208-1236.
[2] Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. Lancet 2003; 362: 1907-1917.
[3] Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. N Engl J Med 1996; 334: 693-699.
[4] Vivarelli M, Cucchetti A, La Barba G, Ravaioli M, Del Gaudio M, Lauro A et al. Liver transplantation for hepatocellular carcinoma under calcineurin inhibitors: reassessment of risk factors for tumor recurrence. Ann Surg 2008; 248: 857-862.
[5] Vivarelli M, Cucchetti A, Piscaglia F, La Barba G, Bolondi L, Cavallari A et al. Analysis of risk factors for tumor recurrence after liver transplantation for hepatocellular carcinoma: key role of immunosuppression. Liver Transpl 2005; 11: 497-503.
[6] Maluccio M, Sharma V, Lagman M, Vyas S, Yang H, Li B et al. Tacrolimus enhances transforming growth factor-beta1 expression and promotes tumor progression. Transplantation 2003; 76: 597-602.
[7] Panwalkar A, Verstovsek S, Giles FJ. Mammalian target of rapamycin inhibition as therapy for hematologic malignancies. Cancer 2004; 100: 657-666.
[8] 8. Guba M, von Breitenbuch P, Steinbauer M, Koehl G, Flegel S, Hornung M et al. Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. Nat Med 2002; 8: 128-135.
[9] 9. Ben Sahra I, Le Marchand-Brustel Y, Tanti JF, Bost F. Metformin in cancer therapy: a new perspective for an old antidiabetic drug? Mol Cancer Ther 2010; 9: 1092-1099.
[10] 10. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J et al. Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest 2001; 108: 1167-1174.
[11] 11. Decensi A, Puntoni M, Goodwin P, Cazzaniga M, Gennari A, Bonanni B, et al. Metformin and cancer risk in diabetic patients: a systematic review and meta-analysis. Cancer Prev Res (Phila) 2010; 3: 1451-1461.
[12] 12. Li D, Yeung SC, Hassan MM, Konopleva M, Abbruzzese JL. Antidiabetic therapies affect risk of pancreatic cancer. Gastroenterology 2009; 137: 482-488.
[13] 13. Donadon V, Balbi M, Mas MD, Casarin P, Zanette G. Metformin and reduced risk of hepatocellular carcinoma in diabetic patients with chronic liver disease. Liver Int 2010; 30: 750-758.

P.1027

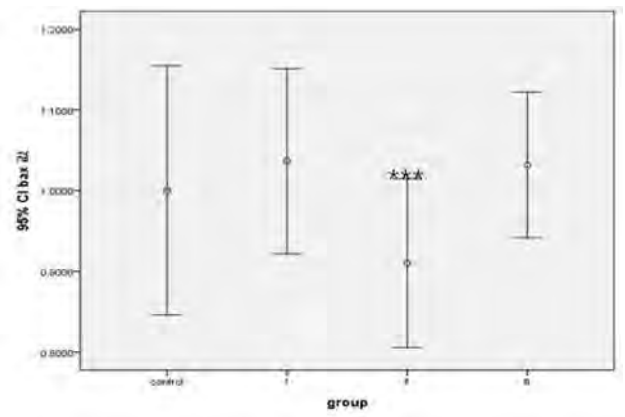
The liver-wrapping nanofiber releasing nitirc oxide downregulated the cleaved Caspase-3 expression on rat hepatic ischemia-reperfusion injury

HYUNG JOON AHN, Min Su Park, Sun Hyung Joo, Bum Soo Kim, Mi Kyung Hong.
Department of Surgery, Kyung Hee University, Seoul, Korea.

Purpose: Hepatic ischemia-reperfusion injury (IRI) is a major problem liver surgery. And the nitric oxide (NO) induced the protective effect on hepatic IRI via decreasing the apoptosis. The aim of this study was to investigate the effect of the nitiric oxide releasing nanofiber on hepatic IRI in a rat model. Methods: Twenty four rats were divided into four groups: (1) Control, the only IRI group (n=6); (2) group I, the hepatic IRI with wrapping the liver using polymer nanofiber matrix (n=6); (3) group II, the hepatic IRI with wrapping the liver using NO rapid releasing-polymer nanofiber matrix (n=6); (4) group III, the hepatic IRI with wrapping the liver using NO slow releasing-polymer nanofiber matrix (n=6). Results: The level of cleaved Caspase-3/β-actin in groups II were significantly lower than in control, group I and III ($p<0.05$, <0.01, and <0.01, respectively, fig 1).



The level of Bax/β-actin in group II was significantly lower than in group III ($p<0.01$, fig 2).



The cleaved Caspase-3/β-actin ratio for control, groups I, II, and III were 0.83 ± 0.21, 0.96 ± 0.22, 0.59 ± 0.28, and 0.94 ± 0.29 respectively. The Bax/β-actin ratio for control, groups I, II, and III were 1.00 ± 0.24, 1.03 ± 0.18, 0.91 ± 0.16, and 1.03 ± 0.17 respectively. However, there were no significant differences between groups in Bcl-2/β-actin ratio. Conclusion: NO rapid releasing nanofiber on rat hepatic IRI downregulated the cleaved Caspase-3 expression.

P.1028

Remote perconditioning on rat hepatic ischemia-reperfusion injury downregulated the Bax and cleaved Caspase-3 expression

HYUNG JOON AHN, Min Su Park, Sun Hyung Joo, Mi Kyung Hong, Ho Chul Park.
Department of Surgery, Kyung Hee University, Seoul, Korea.

Purpose: Hepatic ischemia-reperfusion injury (IRI) is considered a major cause of hepatic damage in liver surgery. The aim of this study was to investigate the effect of the remote ischemic perconditioning method on hepatic IRI in a rat model.
Methods: Seventeen rats underwent hepatic IRI for 30 minutes followed by reperfusion, and were divided into three groups: group I, the only hepatic IRI (n=5); group II, the hepatic IRI with remote perconditioning (n=7); and group III, the hepatic IRI with remote postconditioning (n=5).
Results: For Bax/β-actin, mean values of the three groups (± SD) were 1.29 ± 0.26 (I), 0.89 ± 0.15 (II), and 1.02 ± 0.23 (III). The level of Bax/β-actin in group II was significantly lower than in group I ($P<0.01$). The cleaved Caspase-3/β-actin ratio for groups I, II, and III were 0.93 ± 0.22, 0.46 ± 0.16, and 0.63 ± 0.22, respectively. The level of cleaved Caspase-3/β-actin in groups II and III were significantly lower than in group I ($P<0.01$ and $P<0.05$, respectively) The Bcl-2/β-actin ratio for groups I, II, and III were 1.01 ± 0.09, 1.19 ± 0.39, and 1.20 ± 0.12, respectively. However, there were no significant difference between groups II & III and group I.
Conclusion: The remote perconditioning on rat hepatic IRI downregulated the Bax and cleaved Caspase-3 expression.

P.1029

Supplemented celsior and peri-mortem heparin enhances recovery of rodent DCD hearts

Hong Chee Chew[1], Jeanette Villaneuva[1], Ling Gao[1], Mark Hicks[1], Andrew Jabbour[1,2], Kumud Dhital[1,2], Peter MacDonald[1,2].
[1]Transplant Laboratory, Victor Chang Cardiac Research Institute, Sydney, Australia; [2]Heart Lung Clinic, St Vincent's Hospital, Sydney, Australia.

Aim: Development of a Donation after Circulatory Death (DCD) rodent model and to investigate the role of adjunct therapies in enhancing the recovery of DCD heart.
Method: Male Wistar rats (n=24), between 350-450g, are subjected to DCD conditions. Carotid artery is cannulated with a pressure catheter and oxygen saturation monitored using handheld veterinary pulse oximetry. Asphyxiation is performed by ligation of the trachea. Five groups were studied in total, as below:
I.Electrical Asystole + Celsior Flush (CA)
II.Celsior without perimortem heparin (C w/o Hep)*
III.Celsior with perimortem heparin (C + Hep)*
IV.Supplemented Celsior[1] without perimortem heparin (Cs w/o Hep)*
V.Supplemented Celsior with perimortem heparin (Cs + Hep)*
(*Heart was subjected to 10 minutes of warm ischaemia and 2 minutes of stand-off time.)
The heart is excised and preserved in cold celsior solution immediately. Preservation flush solution (60mls) is delivered via an aortic cannula at a flow rate between 20-30ml/min. After delivery of preservation flush, the aorta and atrial appendage are cannulated as per Langendorff system and the heart is reperfused with oxygenated Krebs-Henseleit solution at 37oc for 30 minutes. After this period of 'resting', flow is redirected through the left atrial appendage to mimic 'working' model for a further 30minutes. Functional data including, heart rate (HR), aortic pressure (MAP), coronary flow (CF) and aortic flow (AF) are recorded.
Results: Warm ischaemic time in Group 1 is 27.2 ± 3.6 vs 17.6 ± 1.8min in all other groups. Mean HR, MAP, CF and AF recovery in each group at 30 mins is presented in table below:

	I	II	III	IV	V
HR (bpm)	157.23	185.19	243.87	246.1	249.82
MAP (mmHg)	49.22	27.24	54.95	43.18	82.45
AF (ml/min)	-1.175	-0.61	5.73	3.78	10.42[@]
CF (ml/min)	2.74	7.38	16.8	5.38	16.58[#]
CO (ml/min)	2.13	6.77	22.53	9.16	27*

• @ p<0.05 vs groups II and IV; # p < 0.05 vs group IV; * p < 0.05 vs groups I, II and IV

Superior CF recovery is seen with the addition of peri-mortem heparin whilst supplementation of baseline preservation solution leads to enhanced AF recovery. The combination of supplemented celsior and peri-mortem heparin resulted in recovery of all hearts within the group.
Conclusion: Current model exemplifies the importance of peri-mortem heparin delivery in enhancing coronary flow recovery and its additive effect to enhance overall cardiac functional recovery when used in conjunction with triple supplemented celsior.
References:
[1] Watson AJ. Enhanced preservation of the rat heart after prolonged hypothermic ischemia with erythropoietin-supplemented Celsior solution. J Heart Lung Transplant. 2013 Jun;32(6):633-40. doi: 10.1016/j.healun.2013.03.014

P.1030

Pretreatment of cilostazol attenuates hepatic ischemia/reperfusion injury in rats

Taku Fujii[1], Hideaki Obara[2], Kentaro Matsubara[2], Hiroshi Yagi[2], Taizo Hibi[2], Yuta Abe[2], Minoru Kitago[2], Masahiro Shinoda[2], Osamu Itano[2], Yuko Kitagawa[2].
[1]Department of Surgery, Hiratsuka City Hospital, Kanagawa, Japan; [2]Department of Surgery, Keio University School of Medicine, Tokyo, Japan.

Background: Cilostazol is a type III phosphodiesterase inhibitor, and has been used worldwide to treat intermittent claudication symptoms. Recent studies have demonstrated that cilostazol is able to decrease ischemia/reperfusion (I/R) injury in the brain, the small bowels, the spinal cord, and the kidney[1]-[4].
Objectives: We aimed to evaluate the effects of cilostazol in a model of liver I/R injury in rats.
Materials and Methods: Male wister rats were divided into two groups (n=15 each): I/R injury with and without cilostazol. I/R injury in liver was induced by 1 h of warm ischemia of median and left lateral lobes, followed by 3h of reperfusion. The rats were euthanized at the end of reperfusion. The serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), IL-1β and IL-6 levels were measured. Mann-Whitney U test was used to compare the significant differences between two groups. Histologic examination was evaluated in rat liver tissues. We also performed survival study to confirm the protective effect of cilostazol on the mortality. For this survival study we used I/R injury model with ischemia time of 1.5 h and observed survival for 1 week. We employed Kaplan-Meier analysis and log-rank testing to assess survival rate.
Results: Significant reductions in the serum AST, ALT, IL-1β and IL-6 levels were seen in the cilostazol group compared with the control group. Cytoplasmic vacuolization are seen in the hepatocytes at the periphery of necrotic area in control group. Mild to moderate vacuolization and mild sinusoidal congestion are observed in cilostazol-treated group, and no necrotic hepatocytes are seen. Administration of cilostazol also significantly improved the survival rate of rats with liver I/R injury.
Conclusions: In a rat model, administration of cilostazol attenuated the effects of liver I/R injury, and improved the survival rate.
References:
[1] Morikawa T. The effects of cilostazol on tissue oxygenation upon an ischemic-reperfusion injury in the mouse cerebrum. *Adv Exp Med Biol*. 2010; 662: 89-94.
[2] Iba T. Comparison of the protective effects of type III phosphodiesterase (PDE3) inhibitor (cilostazol) and acetylsalicylic acid on intestinal microcirculation after ischemia reperfusion injury in mice. *Shock*. 2006; 26(5): 522-526.
[3] Moreira Neto, A. A. Effects of cilostazol in kidney and skeletal striated muscle of Wistar rats submitted to acute ischemia and reperfusion of hind limbs*Acta Cir Bras*. 2012; 27(11): 783-788.
[4] Kurtoglu T. Effects of cilostazol on oxidative stress, systemic cytokine release, and spinal cord injury in a rat model of transient aortic occlusion. *Ann Vasc Surg* 2014; 28(2): 479-488.

P.1031

Donor pretreatment with carbon monoxide prevents ischemia/reperfusion injury following heart transplantation in rats

Noritomo Fujisaki[1], Keisuke Kohama[1,2], Takeshi Nishimura[1], Hayato Yamashita[3], Michiko Ishikawa[3], Akihiro Kanematsu[4], Taihei Yamada[1], Sungsoo Lee[5], Joji Kotani[1], Atsunori Nakao[1].
[1]Emergency, Disaster and Critical Care Medicine, Hyogo College of Medicine, Nishinomiya, Japan; [2]Senri Critical Care Medical Center, Saiseikai Senri Hospital, Suita, Japan; [3]Graduate School of Health Sciences, Kobe University, Kobe, Japan; [4]Urology, Hyogo College of Medicine, Nishinomiya, Japan; [5]Thoracic and Cardiovascular Surgery, Yonsei University College of Medicine, Seoul, Korea.

Introduction: Inhaled carbon monoxide (CO) provides potent anti-inflammatory and antioxidant effects against ischemia reperfusion (I/R) injury. We hypothesized that prolonged CO pretreatment of a potential organ donor may reduce I/R injury of the cardiac grafts. This study was designed to determine whether organs are suitable and secure for transplantation when donors in the ICU are treated with inhaled CO for a prolonged period, since brain death can occur only in the ICU.
Methods: Hearts were heterotopically transplanted into syngeneic Lewis rats after eight hours of cold preservation in University of Wisconsin solution. Donor rats were exposed to CO at a concentration of 250 ppm for 24 hours via a gas-exposure chamber. Severity of myocardial injury was determined by total serum creatine phosphokinase (CPK) and troponin I levels at three hours after reperfusion. The gross morphology of the grafts assigned a transplant score based on contractility, color and hardness at six hours post-reperfusion. The levels of messenger ribonucleic acid (mRNA) for interleukin (IL)-6, intercellular adhesion molecule-1 (ICAM-1), tumor necrosis factor-α (TNF-α), inducible nitric oxide synthase (iNOS), endothelin (ET)1, vascular endothelial growth factor (VEGF), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were quantified using real-time reverse transcription-polymerase chain reaction (RT-PCR). For detecting neutrophils in the cardiac muscle, hematoxylin and eosin stain, naphthol AS-D chloroacetate esterase stain and immunostaining were perfomed. In addition, Affymetrix gene array analysis of messenger ribonucleic acid (mRNA) transcripts was performed on the heart graft tissue prior to implantation.
Results: Recipients of grafts from CO-exposed donors had lower levels of serum troponin I and CPK. Cardiac grafts pretreated with CO demonstrated quicker reanimation time and improved gross structural appearance, indicating earlier functional recovery. Less upregulation of mRNA for IL-6, ICAM-1, iNOS and TNF-α showed significantly. In histopathological examination, grafted heart exposed to CO also attenuated neutrophil infiltration into cardiac myocytes. Although donor pretreatment with CO altered the expression of 49 genes expressly represented on the array, we could not obtain meaningful data to explain the mechanisms by which CO potentiated the protective effects.
Discussion and Conclusion: Pretreatment with low dose CO gas before organ procurement effectively protected cardiac grafts from I/R-induced injury and induced downregulation of proinflammatory mediators in a rat heterotopic cardiac transplant model. A clinical report review indicated that CO-poisoned organ donors may be comparable to non-poisoned donors. Although these studies do not support the therapeutic use of CO during transplantation per se, they do not provide any evidence contraindicating the practice.
This work was supported by JSPS KAKENHI Grant Number 15K20354

P.1032

Effects of Ulinastatin on liver cold ischemia/reperfusion injury and graft survival in mice

Lianyue Guan[1], Hongyu Liu[1], Peiyao Fu[2], Zhuonan Li[1], Lijuan Xie[3], Wei Li[1].
[1]Hepatobiliary-Pancreatic Surgery, China-Japan Union Hospital of Jilin University, Changchun, People's Republic of China; [2]Liver Surgery, Zhongshan Hospital of Fudan University, Shanghai, People's Republic of China; [3]Vascular Surgery, China-Japan Union Hospital of Jilin University, Changchun, People's Republic of China.

Introduction: Liver ischemia reperfusion injury (IRI) is a common cause of liver graft nonfunction or function failure post transplantation. It is also a main reason which restricts the use of marginal liver donors. The mechanisms of liver IRI is very complicated and have no effective method for prevention and treatment yet. In this study, we aim to explore the protective effects of trypsin inhibitor Ulinastatin (UTI) on liver graft IRI and the underlying mechanisms.
Materials and Methods: In bred C57BL/6 mouse and liver cold IRI and orthotopic transplantation models were established. The liver injury score and functions were evaluated by liver ALT, AST, LDH enzyme release and histological examination. The apoptosis of hepatocyte was detected by TUNEL staining and the cytokines were detected by western blot and QT-PCR methods.
Results: UTI supplementation to the perfusion and preservative LR solutions significantly protects the liver from cold ischemia injury. The apoptosis and Caspase-3 and Bax gene expression of liver grafts were reduced and Bcl-2 was increased significantly in UTI treated groups. The cytokines IL-6, TNF-α, and IFN-γ expression were reduced, but IL-10 expression was increased significantly in the liver grafts of UTI groups. Moreover, the liver grafts with extended cold preservation time of 1 hour in the UTI supplemented LR solution demonstrated improved graft survival time post transplantation.
Conclusions: UTI treatment prevents liver from cold IRI significantly and improves liver graft survival with the donors from extended cold preservation time. The underlying mechanisms of UTI on liver IRI may be mediated by inhibition of proinflammatory cytokines, increasing anti-apoptotic gene Bcl-2 and decreasing pro-apoptosis genes of Caspase-3 and Bax expression, and further protects hepatocytes from apoptotic death and improves the liver function.
Nature Science Foundation of China (NSFC) (81170416; 81273264); Techpool Research Fund (01201046)

P.1033

Effects of Ulinastatin on liver warm ischemia reperfusion injury in mice

Peidong Li[1,2], Peiyao Fu[1,3], Zhuonan Li[1], Yanshuo Ye[1], Zhanpeng Wang[1], Hongyu Liu[1], Wei Li[1], Lianyue Guan[1].
[1]Hepatobiliary-Pancreatic Surgery, China-Japan Union Hospital of Jilin University, Changchun, People's Republic of China; [2]Oncology Medicine, Tumor Hospital of Jilin Province, Changchun, People's Republic of China; [3]Liver Surgery, Zhongshan Hospital of Fudan University, Shanghai, People's Republic of China.

Introduction: Ulinastatin, a trpsin inhibitor, has been reported that can inhibits the liver inflammation and improves the liver function. The role of UTI on liver warm ischemia reperfusion injury (IRI) and the underlying mechanisms remain to be further investigated.
Materials and Methods: In bred C57BL/6 mice and liver warm IRI model were employed. The ligamentum of hepatoduodenale was clamped for 45 mins and followed with reperfusion of 1 hour, 6 and 24 hours, respectively. UTI was treated preoperatively. Serum enzyme release, liver cell histology and apoptosis, and Caspase-3 mRNA & Fas mRNA expression from the liver tissues were examined, respectively.
Results: ALT/AST levels were remarkably lower in UTItreated group than IRI control group at reperfusion time of 1hour, 6 and 24 hours.Liverhistological examine showed that hepatic cells edema?degeneration, vacuolization, some patchy necrosis, and sinusoidal congestion were obviously in the IRI control group at 6 hours reperfusion, While UTI treatment reduced the liver tissue damage significantly. The expression of Caspase-3 mRNA and Fas mRNA were detected significantly higher in IRI control group than that in UTI treated group $P<0.05$. The production of IL-6 and TNF-α were elevated in the IRI control group and suppressed by UTI treatment ($P<0.05$). Moreover, the apoptotic activate of liver cells in the IRI+UTI group was reduced significantly than that in IRI control group.
Conclusion: Ulinastatin treatment prevents liver IRI significantly. The underlying mechanisms of the protective role of UTI on liver IRI may through inhibition of proinflammatory cytokines IL-6 and TNF-α, down-regulation of pro-appoptotic genes Caspase-3 mRNA and Fas mRNA expression and prevention of liver cells from apoptotic death.
Nature Science Foundation of China (NSFC) (81170416; 81273264); Techpool Research Fund (01201046)

P.1034

Perfusion of Porcine Kidneys with Macromolecular Heparin Ameliorates Early Ischemia Reperfusion Injury

Amir Sedigh[1], Bo Norlin[3], Rolf Larsson[2], Fredrik Lennmyr[3], Gunnar Tufveson[1], Peetra Magnusson[2], Sofia Nordling[2], Tomas Lorant[1].
[1]Department of Surgical Sciences, Section of Transplantation Surgery, Uppsala University, Uppsala, Sweden; [2]Department of Immunology, Genetics and Pathology, Section of Clinical Immunology, Uppsala University, Uppsala, Sweden; [3]Department of Surgical Sciences, Section of Anaesthesiology and Intensive Care, Uppsala University, Uppsala, Sweden.

Background: Ischemia-reperfusion injury (IRI) is the most significant antigen-independent nonspecific insult, inevitably linked to organ transplantation. Endothelial glycocalyx is degraded during the earliest events in IRI. Previously we have been able to demonstrate the coating the renal artery surface of procine kidney after brain death with a heparin conjugate during preservation using hypothermic machine perfusion (HMP). The purpose of this study was to assess the impact of this measure on initial renal functional and pathologic parameters in matched pairs of porcine kidneys after a simulated warm reperfusion with whole blood on the circuit.
Method: After induction of brain death in pigs (n=6), both kidneys were preserved for 20 hours by HMP. In total, 50 mg heparin conjugate was added to one of the HMP systems (experimental group). Blood, urine and histological samples were collected during the subsequent 3 hours ex vivo normothermic perfusion with oxygenated autologous blood, using a cardiopulmonary bypass machine to add exogenous creatinine.
Results: The rate of decline of exogenous administered creatinine ($p=0,03$) and the amount of total produced urine at each time point ($p=0,023$) was higher in the experimental group. Histologically, tubular changes were less frequent in the experimental group ($P=0.045$). Thromboelastography showed no increased risk of bleeding.
Conclusions: Perfusion of porcine kidneys with heparin conjugate during HMP ameliorates early IRI and improves organ function soon after reperfusion. No increased risk of bleeding was seen with this treatment. This protective intervention strategy may potentially improve functional capacity of organ transplant in the clinical setting.

	I	II	III	IV	V
HR (bpm)	157.23	185.19	243.87	246.1	249.82
MAP (mmHg)	49.22	27.24	54.95	43.18	82.45
AF (ml/min)	-1.175	-0.61	5.73	3.78	10.42@
CF (ml/min)	2.74	7.38	16.8	5.38	16.58*
CO (ml/min)	2.13	6.77	22.53	9.16	27*

- @ $p<0.05$ vs groups II and IV; # $p < 0.05$ vs group IV; * $p < 0.05$ vs groups I, II and IV

P.1035

Hydrogen sulfide prevents renal ischemia-reperfusion injury in CLAWN miniature swine

Mitsuhiro Sekijima[1], Hisashi Sahara[1], Sayaka Morita[2], Yuichi Ogawa[2], Katsuyuki Miki[2], Takahiro Murokawa[1], Takehiro Iwanaga[1], Yurika Ichinari[1], Jigesh Shah[1], Yusuke Tomita[1], Akira Shimizu[3], Kazuhiko Yamada[1].
[1]Division of Organ Replacement and Xenotransplantation Surgery, Center for Advanced Biomedical Science and Swine Research, Kagoshima University, Kagoshima, Japan; [2] Department of Surgery III, Tokyo Women's Medical University, Tokyo, Japan; [3]Department of Analytic Human Pathology, Nippon Medical School, Tokyo, Japan.

Objective: Prevention of ischemia-reperfusion injury (IRI) is important for both early and long-term allograft function, particularly when using donation after cardiac death (DCD) and extended-criteria donors. Hydrogen sulfides (H_2S) have recently been reported to demonstrate both anti-inflammatory and cytoprotective effects, however, its role in a large animal model has yet to be elicited. Given this we sought to: 1) Determine the role of H_2S in renal IRI, 2) Determine the optimal route of H_2S administration and 3) Evaluate the cytoprotective effects of H_2S.
Methods: Female, MHC-inbred, CLAWN miniature swine (n=8) underwent renal ischemia for 120-minutes by occlusion of the left renal artery and vein. Group 1 animals (n=3) underwent renal ischemia exclusively without any additional treatment. Group 2 recipients (n=2) received 1.1 mg/kg of intravenous sodium sulfide (Na_2S) as H_2S donor 10-minutes prior to kidney reperfusion, followed by an additional 1.1 mg/kg of Na_2S 30-minutes post-reperfusion. Group 3 recipients (n=3) underwent selective renal artery administration of 1.1 mg/kg of Na_2S 10-minutes prior to reperfusion, followed by an additional 1.1 mg/kg of Na_2S 30-minutes post-reperfusion via the supra-renal aorta with concomitant occlusion of the infra-renal aorta, thus allowing for exclusive renal administration. Post-operative renal function was monitored by daily serum creatinine, analysis of circulating cytokine activity (TNF-α, IL-6 and HMGB1) to measure the inflammatory response to IRI and histological evaluation of renal biopsies obtained on post-operative days (POD) 2, 7 and 14.
Results: All animals experienced transient acute kidney injury, achieving a peak serum creatinine level by POD3. Recipients in the untreated group (Group 1) had the highest post-operative serum creatinine level, 9.46 ± 1.12 mg/dL, when compared to the H_2S treated groups. Recipients of selective renal H_2S administration (Group 3) experienced a statistically lower peak serum creatinine level when compared to the intravenous H_2S administration group (Group 2), 5.98±1.06 mg/dL vs. 6.82 ± 1.26 mg/dL, respectively ($p<0.05$). H_2S administration did not result in any adverse side effects in the recipients.
Conclusion: H_2S administration appears to have potential cytoprotective and anti-inflammatory effects following renal IRI. This effect was most profound with selective renal artery administration. Further work investigating the benefits of H_2S for organ procurement and preservation is warranted as this may allow for improved outcomes following renal transplantation.

P.1036

The effects of resveratrol on liver transplantation from non-heart-beating donors in pigs

Kenji Shimizu[1], Shigehito Miyagi[1], Yuta Kakizaki[1], Koji Miyazawa[1], Kai Maida[1], Toshiaki Kashiwadate[1], Yasuyuki Hara[1], Masafumi Goto[2], Naoki Kawagishi[1], Noriaki Ohuchi[1], Susumu Satomi[1].
[1]Division of Advanced Surgical Science and Technology, Graduate School of Medicine, Tohoku University, Sendai, Japan; [2]Division of Transplantation and Regenerative Medicine, Tohoku University School of Medicine, Sendai, Japan.

Introduction: Successful liver transplantation (LTx) from non-heart-beating donors (NHBDs) might be an effective way to enlarge donor source. We have previously reported that resveratrol could reduce inflammatory cytokines on NHBDs and preserved the liver grafts' function from NHBDs in rats' ischemia-reperfusion model. The purpose of this study was to investigate the effects of resveratrol on actual LTx from NHBDs for the clinical application.
Materials and Methods: Pigs were divided into three groups: (1) the heart-beating (HB) group (n = 5), whose livers were retrieved from HB donors; (2) the NHB group (n = 5), whose livers were retrieved under apnea induced NHB conditions; and (3) the resveratrol (RES) group (n = 5), whose livers were retrieved the same manner as the NHB group and pretreated with resveratrol at the perfusion (3 mg/L in UW solution), the cold preservation (3 mg/L in UW solution), and just after reperfusion (0.4 mg/kg). In all groups, those were transplanted after 4 h cold preservation. We examined aspartate transferase (AST), alanine transferase (ALT), lactate dehydrogenase (LDH), and tumor necrosis factor α (TNF-α). We also performed histological examinations.
Results and Discussion: In the HB group, all five recipients survived for more than seven days but in the NHB group, three of five cases died within 24 hours. In the RES group, four recipients survived for more than seven days, and the seven days survival rate was significantly better than that in the NHB group (83% versus 0%, $p < 0.05$, Kaplan-Meier log-rank test). The value of AST, ALT and LDH increased on postoperative day 1, but they decreased with time. The value of LDH in the RES group on postoperative day 1 was significantly lower than those in the NHB group. The histology revealed numerous well-preserved sinusoidal structures in the RES group, the same as observed in the NHB group.
Several studies reported that resveratrol has the cytoprotective effects. Our large animal model study suggests that resveratrol might be effective in improving the viability of marginal donor grafts, including NHBDs.
Conclusion: Resveratrol treatment might represent a promising approach for clinical LTx from NHBDs.

P.1037

The renoprotective effects of epigallocatechin gallate and dipeptidyl peptidase IV inhibitor gemigliptin on tacrolimus-induced nephrotoxicity in mice

Byung Chul Shin[1], Hyun Lee Kim[1], Jong Hoon Chung[1], Nam Gyu Choi[2], Seong Pyo Moon[2].
[1]Department of Internal Medicine, Division of Nephroloy, Chosun University Hospital, Gwangju, Korea; [2]Department of Surgery, Chosun University Hospital, Gwangju, Korea.

Background : It has been reported that the proteinuria is an early predictive marker in detection of tacrolimus (TAC) nephrotoxicity. The aim of this study was to investigate the renoprotective effects of eipgallocatechin gallate (EGCG) and dipeptidyl peptidase IV (DPP IV) inhibitor gemigliptin on TAC-induced acute nephrotoxicity in mice.
Methods: The mice (n=20) were divided into 5 groups (n=5/group); control group were intraperitoneally (IP) injected 0.9% saline, TAC group were IP injected TAC 2 mg/kg, DPP IV inhibitor group were given in addition gemigliptin 20 mg/kg (G20) by oral gavage. TAC-EGCG group were given TAC by IP injection and EGCG 100 mg/kg by subcutaneous injection. TAC-EGCG-G20 group were given with same dosages.
Results: The 24 hours urine protein amounts were significantly increased in TAC group (46.1 ± 10.9 mg/day) compared to control group (11.3 ± 4.4 mg/day) and significantly decreased in TAC-EGCG-G20 group (13.1 ± 5.9 mg/day, $P < 0.01$) compared to TAC group. The nitric oxide (NO) production by TAC was significantly suppressed by EGCG and gemiglipin management. Renal tissue malondialdehyde level was significantly increased in TAC group compared to control group and significantly decreased in TAC-EGCG-G20 group compared than that of TAC group. The renal function and antioxidant enzyme activities were significantly suppressed in TAC group compared with control group and restored in EGCG and gemiglipin treatment group.
Conclusion: EGCG and gemigliptin treatment has beneficial antiproteinuric and renoprotective effects on TAC-induced acute renal injury in mice.

References:

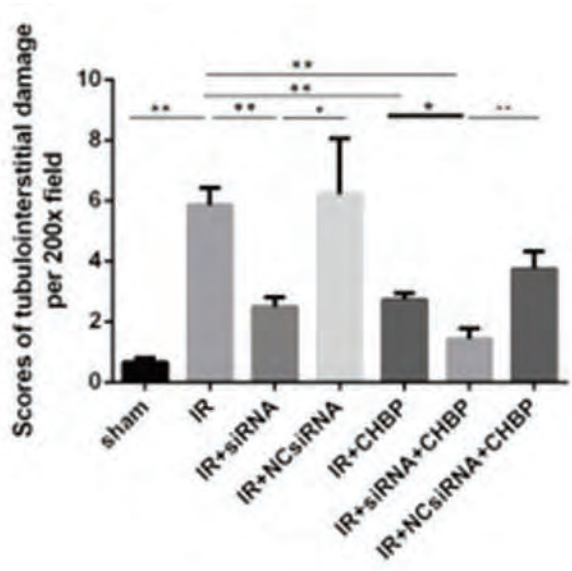
[1] Shin BC, et al: Transplant Proc 2012; 44: 1080-1081
[2] Ryu HH et al: Nephrol Dial Transplant 2011; 26: 1188-1193
[3] Yoon SP, et al: Acta Histochem 2014; 116: 1210-1215
[4] Back JH et al, Transplant Proc 2015; 47: 2032-2034
[5] Jin L et al. PLoS One. 2014 Jun 24;9(6):e100798. doi: 10.1371/journal.pone.0100798. eCollection 2014.

P.1038

CHBP and caspase-3 SiRNA ameliorate mouse renal Ischemia reperfusion injury

Weiwei Chen[1], Cheng Yang[5], Yuanyuan Wu[3], Yufang Zhang[2], Aifen Liu[2], Yaqiu Long[2], Tongyu Zhu[4,5], Yaping Fan[1], Bin Yang[1,2,7,8].
[1]Nephrology, Affiliated Hospital of Nantong University, Nantong, People's Republic of China; [2]Basic Medical Research Centre, Medical School of Nantong University, Nantong, People's Republic of China; [3]Pathology, Medical School of Nantong University, Nantong, People's Republic of China; [4]Urology, Zhongshan Hospital of Fudan University, Shanghai, People's Republic of China; [5]Shanghai Key Laboratory of Organ Transplantation, Zhongshan Hospital of Fudan University, Shanghai, People's Republic of China; [6]Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, People's Republic of China; [7]Renal Group, University Hospitals of Leicester, Leicester, United Kingdom; [8]Infection, Immunity and Inflammation, University of Leicester, Leicester, United Kingdom.

Introduction: Renal ischemia/reperfusion (IR) is a main cause of acute kidney injury (AKI) in native and transplant kidneys alike. We have demonstrated up-regulated caspase-3, inflammation and apoptosis in renal IR injury, which could be reversed by a novel cyclic helix B peptide (CHBP) and small interfering RNA (siRNA) targeting caspase-3. However, its underlying mechanisms have not be fully understood, which were further explored in this mouse renal IR model.
Methods: Bilateral renal occlusion for 30 min followed by 48 h reperfusion was performed in male C57BL/6 mice. CHBP (24 nmol/kg) was injected intraperitoneally after reperfusion. The siRNA (0.03 mg/kg) was injected intravenously 2 h before ischemia. The serum samples and bilateral kidneys were collected for renal function, histology and molecular biology analyses.
Results: The level of serum creatinine was increased by IR and decreased by the treatment of CHBP and/or caspase-3 siRNA ($P<0.01$). The score of tubulointerstitial damage in H&E staining sections was also significantly increased by IR, but reduced by CHBP and/or caspase-3 siRNA. In addition, the number of active caspase-3+ cells and apoptotic cells was raised by IR, reversed by CHBP and/or caspase-3 siRNA ($P<0.01$). The expression of 17 kD active caspase-3 was upregulated by IR and down-regulated by CHBP and/or caspase-3 siRNA ($P<0.01$).



Conclusions: CHBP and caspase-3 siRNA ameliorated IR injury, both of which might have certain synergistic effects. CHBP might reduce caspase-3 synthesis and activation, subsequently improve cellular apoptosis, renal function and structure.

Affiliated Hospital of Nantong University; Medical School of Nantong University; Zhongshan Hosptial of Fudan University; Shanghai Institute of Materia Medica in Chinese Academy of Sciences; The National Natural Science Foundation of China; University Hospitals of Leicester; University of Leicester

References:

[1] Yang C. Research highlight, Asian Journal of Urology 2016;3:4-5.
[2] Yang C. Transplantation Direct 2015; 1:1-9.
[3] Yang C. J Transl Med. 2015;13:39
[4] Yang C. Mol Ther. 2014;22:1817-28.

P.1039

A study of oxidative stress biomarkers in renal allograft transplant recipients

Sunil Kumar[1], Mukut Minz[1], Ujjawal Sharma[3], Rajendra Prasad[2], Ashish Sharma[1], Sarbpreet Singh[1], Deepesh Bkenwar[1], Ravi Dhital[1]. [1]Renal Transplant Surgery, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India; [2]Biochemistry, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India; [3]BIiotechnology and Experiminetal Medicine, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India.

Background: Renal transplantation is associated with a period of ischemia starting at the recovery of organ from the donor until it's reperfusion in the recipient. This ischemia reperfusion injury of the allograft induces oxidative stress in the recipient which is defined as an imbalance between the formation of reactive oxygen species and the total antioxidant capacity of the individual. This study was conducted with the aim to evaluate the oxidative stress biomarkers in renal allograft transplant recipients before, during and after transplant.
Methodology: Eighteen end stage renal disease patients undergoing ABO compatible living or deceased donor renal allograft transplant in our institute were included in the study as subjects. Venous blood samples (*5.0 ml) were drawn from the patients and were processed for measuring following biomarkers of oxidative stress:

• 8-iso-PGF2α (8-iso-prostaglandin F2 α) – marker of lipid peroxidation

• AOPPs (advanced oxidation protein products) – marker of protein oxidation

• 8-OHdG (8-hydroxydeoxyguanosine) – marker of oxidative DNA/RNA damage

• 3-NT (3-Nitrotyrosine) – marker of nitrosative stress

• IL-1β (Interleukin 1 beta) – marker of inflammation

These measurements were made at different times: before the transplant (baseline values), 5 minutes after reperfusion, 10 minutes after reperfusion, 30 minutes after reperfusion and 2 weeks after the transplant. The results were statistically analyzed. ANOVA and 2-sided Dunnett test were used to analyze the results of the biomarkers. All data were expressed as mean ± SD and p values less than 0.05 were considered significant.
Results: Out of the 18 patients included, there were 78% males and 22% females. The patients were in the age group of 10-60 years with the mean age of 32.2 ± 14.9 years. The basic disease in all the patients was Glomerulonephritis. As compared to the baseline value of 590.99 ± 218.07 pg/ml, 8-iso-PGF2α levels decreased slightly at 5 minutes after reperfusion to 533.10 ± 284.96 pg/ml; $p>0.05$ and then progressively increased till two weeks to 680.32 ± 121.62 pg/ml; $p<0.05$. The levels of AOPP at baseline were 187.3 ± 144.5 ng/ml, which decreased at 5 minutes after reperfusion to 173.8 ± 135.6 ng/ml; $p>0.05$ and then increased significantly at two weeks to 201.9 ± 138.9 ng/ml; $p<0.05$. The levels of IL-1β at baseline were 36.2 ± 12.2 pg/ml, which increased at 10 minutes after reperfusion to 43.5 ± 16.1 pg/ml; $p<0.05$ and then decreased at two weeks to 28.8 ± 10.4; $p<0.05$. 8-OHdG and 3-NT did not show any significant change in the levels.
Conclusion: In renal transplantation, oxidative stress starts after reperfusion and is reflected by immediate rise in the inflammatory biomarker i.e IL-1β in the early period. Over the next two weeks, there is increased oxidative stress which is implied by increase in the markers of lipid peroxidation (8-iso-PGF2α) and protein oxidation (AOPP).

References:

[1] Snoeijs MG, Van Heurn LW, Buurman WA. Biological modulation of renal ischemia–reperfusion injury. Curr Opin Organ

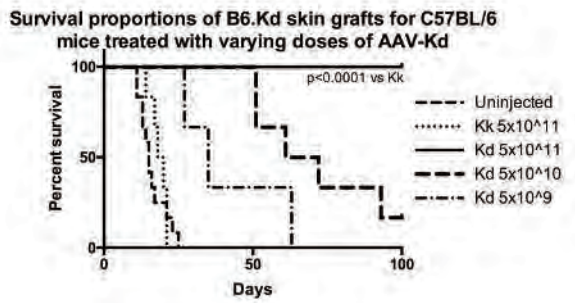
Transplant 2010;15(2):190–9
[2] Walker LM, York JL, Imam SZ, et al. Oxidative Stress and Reactive Nitrogen Species Generation during Renal Ischemia. Toxicol Sci 2001;63:143–8
[3] Matsumoto S, Hanai T, Matsuura T,et al. Can monitoring of serum 8-OHdG level for 2 hours after renal transplantation predict prognosis of the graft? Transplant Proc 2006;38: 2014 –15
[4] Montuschi P, Barnes P J, Roberts L J. Isoprostanes: markers and mediators of oxidative stress. FASEB J 2004;18:1791–1800
[5] Roberts LJ and Morrow JD. Measurement of F2-isoprostanes as an index of oxidative stress in vivo. Free Radic. Biol. Med. 2000;28:505–13.
[6] Witko-Sarsat V, Friedlander M, Capeillere-Blandin C, et al.Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kidney Int 1996;49:1304-13
[7] Patel PR, Bevan RJ, Mistry N, et al. Evidence of oligonucleotides containing 8-hydroxy-2'-deoxyguanosine in human urine. Free Radic Biol Med. 2007;42(4):552-8.
[8] Ischiropoulos H, Zhu L, Chen J, et al. Peroxynitrite-mediated tyrosine nitration catalyzed by superoxide dismutase. Arch Biochem Biophys. 1992;298(2):431-7.

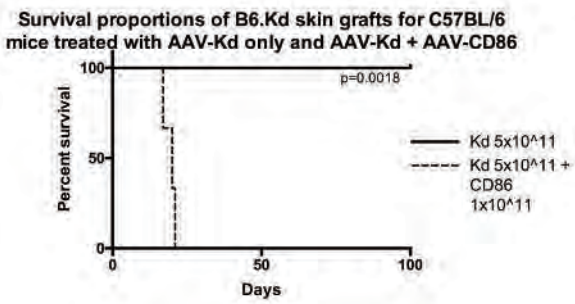
P.1040

Polymicrobial oroesophageal infection presenting with dysphagia in a renal allograft transplant recipient

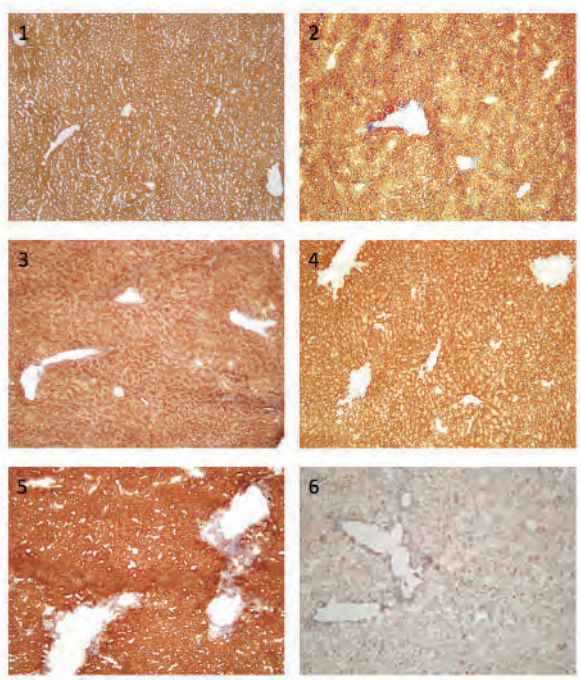
Sunil Kumar[1], Mukut Minz[1], Saroj Ksinha[2], Kim Vaiphei[3], Ashish Sharma[1], Sarbpreet Singh[1], Deepesh Bkenwar[1].
[1]Renal Transplant Surgery, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India; [2]Gastroenterology, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India; [3]Histopathology, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India.

Background: Although coninfection of candida albicans, herpes simplex virus (HSV) and cytomegalovirus (CMV) result in infectious esophagitis in immunocompromised individuals, their association with esophageal tuberculosis has never been reported in literature. We report a renal allograft transplant recipient with polymicrobial infection of the upper gastrointestinal tract who presented with dysphagia at 8 months.
Case: A 57 year old man with diabetes mellitus and end stage renal disease received a renal allograft from a living donor. He was discharged on tacrolimus, mycophenolate mofetil (MMF) and prednisolone with a serum creatinine of 1.07 mg/dl. Eight months later he presented with dysphagia, odynophagia, retrosternal heaviness, hiccough and vomiting. He had oropharyngeal candidiasis and was started on fluconazole but symptoms persisted. Upper gastroesophageal endoscopy (UGIE) showed multiple esophageal ulcers of variable size (5-30mm).





Serum creatinine increased from a baseline of 1.0 mg/dl to 2.6 mg/dl. Blood and urine cultures were sterile, chest x-ray was normal and CMV in blood was negative. Sputum for acid fast bacilli (AFB) done thrice was negative. Histopathology of the gastroesophageal junction showed numerous epithelioid cell granulomas along with Langhan's type giant cells and lamina propria inflammation. Ziehl Neelsen stain showed many positive bacilli.



IHC staining for Kd in: **1**-C57BL/6 mouse liver d7 post inoculation with AAV-Kd $5x10^{11}$ **2**-C57BL/6 mouse liver d14 post inoculation with AAV-Kd $5x10^{11}$ **3**-C57BL/6 mouse liver d7 post inoculation with AAV-Kd $5x10^{11}$ + AAV-CD86 $1x10^{10}$ **4**-C57BL/6 mouse liver d14 post inoculation with AAV-Kd $5x10^{11}$ + AAV-CD86 $1x10^{10}$ **5**-C57BL/6 mouse liver d7 post inoculation with AAV-Kd $5x10^{11}$ + AAV-CD86 $1x10^{11}$ **6**-C57BL/6 mouse liver d14 post inoculation with AAV-Kd $5x10^{11}$ + AAV-CD86 $1x10^{11}$

Many of the nuclei of squamous lining of the esophagus and gastric glands were enlarged and hyperchromatic with irregular nuclear membrane. Immunohistochemistry staining for HSV was positive. He was started on antitubercular therapy (ATT) comprising of rifampicin, isoniazid, ethambutol, and pyrazinamide along with valacyclovir and fluconazole. Within one week, patient started taking normal diet and was discharged with a serum creatinine of 1.5 mg/dl. At a follow up of three months he was asymptomatic and serum creatinine was 1.2 mg/dl.

Discussion: Symptomatic candidiasis usually improves within a few days of therapy, although in resistant cases coinfection with CMV and HSV must be ruled out. The reported incidence of tuberculosis in the transplant population ranges from 0.2% to 15% with primary esophageal tuberculosis being a rare entity. In the present case the symptoms were initially thought to be related to oral candidiasis and patient was treated with fluconazole. However there was no response and on further evaluation a coinfection with HSV and tuberculosis was documented. Esophageal tuberculosis was not anticipated in the absence of constitutional symptoms.

Conclusion: Renal transplant recipients are at risk of developing opportunistic infections. In a tuberculosis endemic area like India these infections might have coinfection of tuberculosis. Early diagnosis and treatment can lower the morbidity and mortality and improve the outcomes of kidney transplantation.

References:

[1] Rosołowski M, Kierzkiewicz M. Etiology, diagnosis and treatment of infectious esophagitis. Prz Gastroenterol 2013;8(6):333-337.
[2] Marshall JB. Tuberculosis of the gastrointestinal tract and peritoneum. Am J Gastroenterol 1993;88:989-999.
[3] Patel NC, Caicedo RA. Esophageal infections: an update. Curr Opin Pediatr. 2015;27(5):642-648.
[4] Eymard D, Martin L, Doummar G, Piché J. Herpes simplex esophagitis in immunocompetent hosts. Can J Infect Dis 1997;8(6):351-353.
[5] Samhan M, Al-Mousawi M, Halim M, Nampoory MRN. Tuberculous tracheo-esophageal fistula in a renal transplant patient. Saudi J Kidney Dis Transplant 2005;16(2):198-200.
[6] Koseoglu F, Emiroglu R, Karakayali H, et al. Prevalence of mycobacterial infection in solid organ transplant recipients. Transplant Proc 2001;33:1782-1784.
[7] Jha V, Chugh KS. Posttransplant infections in the tropical countries. Artif Organs 2002;26(2):770-777.
[8] Harish K, Gokulan C. Primary oesophageal tuberculosis: a rare entity. Trop Gastroenterol 2007;28:178-179.
[9] Nagi B, Lal A, Kochhar R, et al. Imaging of esophageal tuberculosis: a review of 23 cases. Acta Radiol. 2003;44:329-333.
[10] Koseoglu F, Emiroglu R, Karakayali H, et al. Prevalence of mycobacterial infection in solid organ transplant recipients. Transplant Proc 2001;33:1782-4.

P.1041

Intra-peritoneal administration of sodium acetate improves renal function and prolongs survival in a murine model of renal allograft transplantation

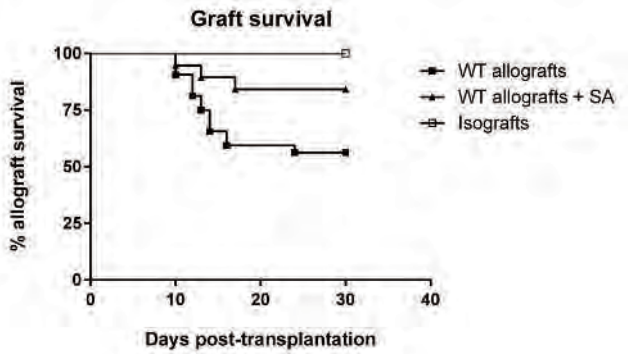
Tony Kwan[1], Yik Wen Loh[1], Jin Ma[1], Stephen Alexander[1,3], Huiling Wu[1,2], Steve Chadban[1,2].
[1]Charles Perkins Centre, University of Sydney, Sydney, Australia;
[2]Renal Medicine, Royal Prince Alfred Hospital, Sydney, Australia;
[3]Centre for Kidney Research, The Children's Hospital at Westmead, Sydney, Australia.

Aim: To determine whether administration of sodium acetate (SA) will attenuate renal allograft rejection in a fully MHC-mismatched murine model

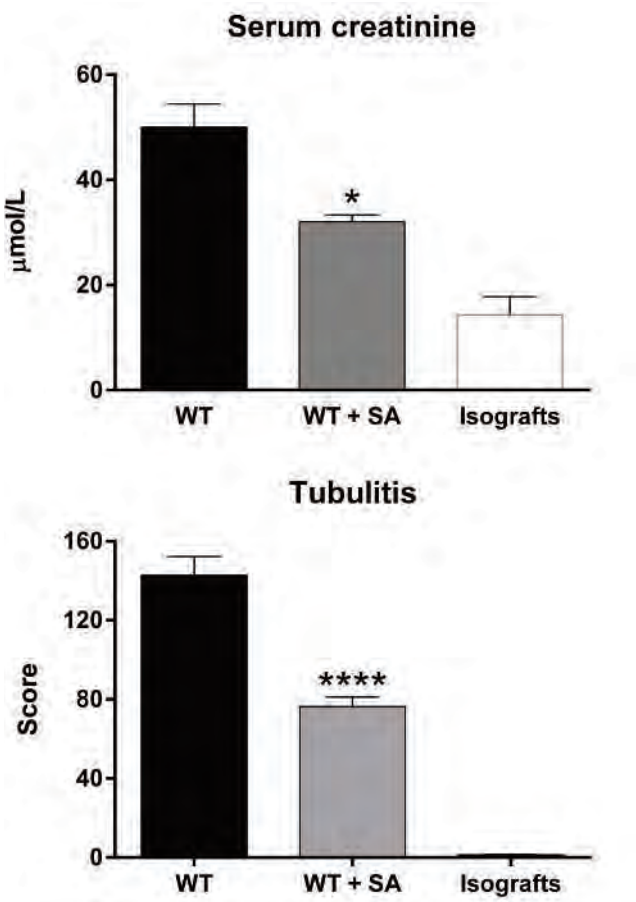
Background: Short-chain fatty acids (SCFAs) are microbial metabolites generated from fermentation of dietary fibre in the gut where they contribute to the microbiome and modify immune responses through local and systemic effects. SCFAs delivered by diet or intra-peritoneal (ip) injection have been found to attenuate the development of autoimmune disorders and various forms of AKI via induction of Tregs. Here, we examined whether this could be applied to attenuate alloimmunity in a transplantation model.

Methods: Life-sustaining BALB/c kidney allografts were transplanted into nephrectomised C57BL/6 mice. The intervention group received 200mg/kg ip SA for 14 days post-transplantation, followed by oral 150mM SA solution (WT+SA allografts, n=9), whilst the control group received neither (WT allografts, n=14). A third group of C57BL/6 mice received C57BL/6 kidney isograft as controls (n=6). Mice were sacrificed on day 14 for assessment of tubulitis, serum creatinine and graft infiltration of various cells using immunohistochemistry. The remainder were observed for survival up to 100 days.

Results: Increased survival of the WT+SA allografts (16/19) compared to WT allografts (18/32) was apparent by 30 days post-transplantation (Figure 1, $p<0.05$).



At day 14, WT+SA allografts demonstrated significant reductions in serum creatinine (32.0±3.94 vs 50±19.2µmol/L, $p<0.05$) and tubulitis scores (76.33±14.4 vs 142.7±35.3, $p<0.0001$) compared to WT allografts (Figure 2).



WT+SA allografts also exhibited increased CD4+ T cell (107.1±20.6 vs 73.1±31.7 cells/HPF, $p<0.05$) and Foxp3+ Treg accumulation (34.3±8.2 vs 22.7±6.3 cells/HPF, $p<0.01$) compared to WT allografts.

Conclusions: Administration of SA was protective against the development of allograft rejection, evidenced by improved renal function and prolonged graft survival. The mechanisms remain under investigation but likely involves increased regulation by Tregs.

P.1042

IL-17 production and inflammatory cascade is decreased by ATG

Dietmar Abendroth[1], Jürgen Kaden[2], Manfred J.Stangl[3].
[1]Center of Surgery, University of Ulm, Ulm, Germany; [2]Clinical Chemistry, Vivantes-Klinikum Friedrichshain, Berlin, Germany; [3]Transplant Center Munich-Großhadern, Ludwig-Maximilians-University, Munich, Germany.

Introduction: Rejection remains the most important cause of allograft failure. Recently, a newly identified CD4+ T cell subset, Th17, distinct from Th1 or Th2, was characterized by the production of interleukin-17A (IL-17A). It has been shown that Th17 expression was markedly increased in inflamed transplants. There is evidence that a low inflammatory response is leading to better long-term outcome. In a retrospective analysis we determined IL-17A production in setup of two different immunosuppressive regimens: ATG-F induction vs. triple drug in comparison to a sensitive inflammatory marker kynurenine (earlier in the cascade) and IL-33.
Material and Methods: By using a commercial ELISA (Cusabio™) we determined IL-17A, IL-33 and with a colorimetric assay kynurenine (9th-11th and 28 – 30th day post transplantation; 46% f/54%m). In gr.I (n=38, ATG-F-induction; CsA, MMF, MP) and gr.II (n=36, CsA, MMF, MP). A third group III (n=23) with rejection episodes (BPAR) in different stages were used for comparison reasons. All patients with immediate graft function.
Results: There was a significant difference between the three groups. For IL-17 we estimated 21,2±3 pg/ml / 22,8±23,1 pg/ml (gr.I); 116±45 pg/ml / 89±26 pg/ml (gr.II) and 271±87 pg/ml / 233±76 pg/ml (gr.III). Values for kynurenine were 5,4±2,5 / 4,7±1,8 nmol/ml (I), 6,9±3,2 / 5,5±2,8 (II); 12,3±4,9 / 13,4±4,1 (III). IL-33 was significant elevated in the rejection group. There was a significant benefit concerning a lower inflammatory response in the group with induction treatment even after 28 days post induction treatment. The benefit was higher in patients treated with CMV-hyperimmunoglobuline. Kynurenine as parameter showed an early initial increase followed by IL-17.
Conclusion: The significant difference between the IL-17 production in patients treated with ATG could be one explanation for the better long term outcome. ATG is leading to a lower inflammatory potential with an added effect by hyperimmunoglobuline. This was also proven by kynurenine. IL-33 data are not able to predict the long term run. There is evidence that the low IL-17 and low kynurenine production predicts the better long-term outcome.

P.1043

Hypothermic machine perfusion oxigenation. A real step forward? Tissue and perfusate mRNA expression

Victoria Gomez Dos Santos[1], Vital Hevia[1], Macarena Rodríguez-Serrano[2], Laura García-Bermejo[2], Adolfo Martinez[3], Ana Saiz[4], Victor Diez-Nicolas[1], Francisco Javier Burgos[1].
[1]Urology, Hospital Ramón y Cajal, Madrid, Spain; [2]Biomarkers and Therapeutic Targets Group, Hospital Ramón y Cajal, Madrid, Spain; [3]Transplant Coordination, Hospital Ramón y Cajal, Madrid, Spain; [4]Pathology Departments, Hospital Ramón y Cajal, Madrid, Spain.

Introduction: The lack of oxygen combined with hypothermia during preservation leads to the reduction of aerobic metabolism, situation considered as protective for the organ. Deceased after cardiac death donors (DCDs) represent a valuable source of organs; however, preventing poor outcome is difficult, even with the use of machine perfusion (MP). Addition of oxygen during MP could allow the graft to maintain a low metabolic aerobic respiration with maintenance of ATP levels which permitted a delay of injury process. microRNAs (miRNAs) can be secreted to body fluids mostly in exosomes, in a GTP and calcium-dependent manner. Our group has demonstrated the feasibility of miRNAs detection in preservation solution during MP. Additionally, tissue miRNAs in pre and post perfusion biopsies could translate transcriptional changes at cellular level induced by oxygenation.
The Aim: of the study was to determine the potential benefit of low flow aerobic machine preservation through miRNAs expression. The experimental protocol was reviewed and approved by the Ethics Committee for Animal Experimentation.
Material and Methods: A porcine orthotopic transplantation model mimicking type III DCD conditions was developed in commercial farm pigs. Cold preservation was performed by conventional non-oxygenated MP in Life-Port™ device or oxygenated MP by a continuous oxygenation flow (PO2 > 500 mmHg). Evaluation included miRNAs in preservation solution and in pre and post MP biopsies. miRNAs were determined by qRT-PCR after RNA extraction and results were expressed as differential of DCTs. (DCT in biopsies = CT miRNA- CT (RNU6B and 5S as internal controls; DCT in preservation solutions = CT miRNA- CT UniSP2 as external control).
Results: Nine female commercial farm pigs 3 to 6 months were randomized. Oxygenated and non-oxygenated grafts exhibited similar miRNAs expression levels in kidney biopsies.

	non OXIGENATED ANIMALS. POST-PERFUSION - PRE-PERFUSION DCTs								
	miR126	miR127	miR10a	miR146a	miR210	miR26b	miR29a	miR27a	miR101
	-0,87	-0,97	-0,81	-1,10	-0,89	-0,62	-0,85	-0,98	-0,97
	1,06	1,04	1,01	0,96	1,72	3,07	0,96	1,46	1,65
ANIMAL 7									
ANIMAL 8									
ANIMAL 5									
ANIMAL 6									
ANIMAL 9									

On the contrary, oxygenated grafts showed higher levels of miRNAs expression in preservation solutions, therefore strongly suggesting that oxygenation of preservation solution could allow more efficient secretion of miRNAs

	DCTs OXIGENATED ANIMALS								
	FINAL - INITIAL DCTs PRESERVATION SOLUTION								
	miR126	miR127	miR10a	miR146a	miR210	miR26b	miR29a	miR27a	miR101
ANIMAL 1	-1,93	-2,01	-2,98	-2,43	-2,30	-1,42	-2,10	-1,98	-1,82
ANIMAL 5	-1,25		10,28	3,87	6,87	5,91	7,98	7,78	9,78
ANIMAL 6	-3,92	0,17	-2,87	-1,51	-2,58	-5,02	-2,22	-2,78	-2,30
ANIMAL 9	-1,95	-0,67	0,19	8,61	0,19	0,02	-2,98	-1,95	-5,35
	DCTs non OXIGENATED ANIMALS								
	FINAL - INITIAL DCTs PRESERVATION SOLUTION								
	miR126	miR127	miR10a	miR146a	miR210	miR26b	miR29a	miR27a	miR101
ANIMAL 2	-1,66	-1,93	-1,74	-1,08	-1,94	-0,43	-0,99	-0,64	0,66
ANIMAL 3	0,78	3,59	0,25	1,77		0,34	-1,11	1,80	1,63
ANIMAL 4	-2,35	-2,85	-3,73	-2,97	-1,60	-3,98	-5,58	-6,18	-1,06
ANIMAL 7	-3,84	-4,26	1,04	1,09	1,08	-2,87	-2,76	-1,48	-1,77
ANIMAL 8	-2,89	-0,07	-0,43	-0,02	-1,77	-3,12	-1,20	-1,20	-2,00

Conclusions: Preservation solution oxygenation did not seem to modify kidney tissue miRNAs expression. However oxygenation could modulate miRNAs secretion to preservation solution, most probably trough alterations in energetic kidney status and intracellular calcium, among other mechanisms.

P.1044

MicroRNAs panel validation in kidney graft hipothermic machine perfusion fluid as novel biomarkers for graft disfunction

Victoria Gomez Dos Santos[1], Macarena Rodríguez-Serrano[2], Laura García-Bermejo[2], Edurne Ramos[2], Victor Diez-Nicolas[1], Sara Alvarez[1], Vital Hevia[1], Laura Martinez[1], Ana Fernandez[4], Adolfo Martinez[3], Ana María Torres[5], Francisco Javier Burgos[1].
[1]Urology, Hospital Ramón y Cajal, Madrid, Spain; [2]Biomarkers and Therapeutic Targets Group, Hospital Ramón y Cajal, Madrid, Spain; [3]Transplantation Coordination, Hospital Ramón y Cajal, Madrid, Spain; [4]Nephrology, Hospital Ramón y Cajal, Madrid, Spain; [5]Biobank, Hospital Ramón y Cajal, Madrid, Spain.

Introduction: Delayed graft function (DGF) is a common complication after deceased donor kidney transplantation (KT) which affects short and long-term outcome. Currently available biomarkers in perfusate lack sensitivity in predicting graft outcome. Our group has already set up a protocol to determine miRNAs in preservation solution during graft perfusion as well as in sera from KT recipients. Discovery phase included a screening of 768 miRNAs in 3 kidney donors and their recipients (Poster 82. 2015 EAU Congress. Madrid). A miRNAs panel was selected based on modulation of expression and functional relevance. The aim of the study was to validate the selected miRNAs in a larger cohort of grafts and recipients.
The Ramón y Cajal University Hospital Research Ethics Board approved the current study.
Material and Methods: We conducted a prospective cohort study of graft dysfunction in KT from ECD. Ethical approval was obtained from Ethics Review Board. A screening experiment led to a panel of miRNAs in preservation solution and post-KT recipient sera. As validation cohort we analysed samples from 12 donors and their actual 18 recipients.
After total RNA extraction miRNAs were detected and quantified by Real-Time PCR performed using SYBR Green and specific probes for each miRNA of interest, as well as exogenous Spike- in as technical control. miRNA expression values are expressed as Differential Cross-Threshold (DCTs) from the following formula DCT= miRNA CT - Spike In CT.
Results: The 8 miRNAs panel selected from preservation solution and the 13 miRNAs selected from recipient sera in discovery phase was validated by individual qRT-PCR, since they exhibited amplification curves and CTs technically appropriated. miRNAs expression in the initial sample was different among all grafts although all reached similar expression in final sample thus suggesting that change measure between initial and final sample should be the parameter to be correlated with clinical evolution.
Functional analysis of miRNas predicted targets showed their involvement in epithelial-mesenchymal transition process, nitric oxide pathway, pluripotencial cells maintenance, ischemic injury and induced renal damage.
Conclusions: Selected miRNAs in discovery phase were technically validated in preservation solution and recipient sera in a cohort of expanded criteria donors. miRNAs in graft preservation fluids are promising biomarkers for predicting outcome prior to KT. This may have clinical impact for graft reconditioning strategies.

P.1045

Reduced plasmalogens and increased ceramides in the sera from acute hepatic allograft rejection

Nayoung Kim[1], Hyun Ju Yoo[1], Young-In Yoon[2], Su Jung Kim[1], Gi-Won Song[2], Shin Hwang[2], Sung-Gyu Lee[2].
[1]Asan Institute for Life Sceinces, Asan Medical Center, Seoul, Korea; [2]Division of Liver Transplantation and Hepatobiliary Surgery, Department of Surgery, Asan Medical Center, Seoul, Korea.

Background: Development of liquid chromatography (LC)-mass spectrophotometer (MS) and gas chromatography (GS)-MS with high sensitivity and specificity has enabled metabolomics in human diseases. Since the current diagnostic measures have limitations in the early prediction of rejection and complications, we aimed at finding the novel biomarkers for acute/chronic rejection and complications in the organ transplantation by targeted metabolomics, in particular, lipidomics. It is known that the immune responses involve the changes in lipids
Patients and Methods: Sera were collected from the liver transplant patients every week for 1 month post-op from Feb. 2012 to Dec. 2012 (IRB Approval No. 2011-0898), and stored at -80 degree until use. The rejection and control groups were selected 6 months later. Among approximately 300 patients enrolled, 52 patients with rejection and 71 patients as control were selected for analysis. The quantitation of target metabolites were performed with LS-MS/MS (Agilent1290/Qtrap5500), based on the profiles of the standard molecules.
Results: We found 8 plasmalogens (C18 (Plasm) LPC, C18 (Plasm) LPE, C18 (Plasm) 18:1 PC, C18 (Plasm) 18:1 PE, C18 (Plasm) 20:4 PC, C18 (Plasm) 20:4 PE, C18 (Plasm) 22:6 PC, and C18 (Plasm) 22:6 PE) were down-regulated and 3 ceramides (C16, C18, and C18:1 ceramide) were up-regulated in the sera from patients with rejection. The results were most distinctive post-op 3 weeks and statistically significant.
Conclusion: The results suggest plasmalogen and ceramide could be potential biomarkers for acute hepatic allograft rejection. We are currently investigating which immune and metabolic factors affect the lipid profiles in the organ transplant rejection. (The results are applied for patent.)
This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (Grant No 2015R1A2A2A04007141)

P.1046

A simple and precise LC/MS method to quantitate 6 beta-hydroxycortisol and cortisol in human urine: Use of the 6 beta-hydroxycortisol to cortisol ratio as an indicate of the remnant-liver function in a donor for liver transplantation

Satoshi Kishino[1], Kunihiro Matsumoto[1], Keiko Ohno[1], Tsuyoshi Shimamura[2], Satoru Todo[3].
[1]Department of Medication Use Analysis and Clinical Research, Meiji Pharmaceutical University, Tokyo, Japan; [2]Division of Organ Transplantation, Hokkaido University Hospital, Sapporo, Japan; [3]Department of Surgery, St. Mary's Hospital, Kurume, Japan.

Introduction: Liver regeneration after extensive liver resection is a serious clinical problem. The remnant-liver volume of donors may play an important role in the long-term as well as early postoperative course. The aims of this study were to develop a method to quantitate 6 beta-hydroxycortisol and cortisol in human urine and to clarify the change in the urinary ratio of 6 beta-hydroxycortisol to cortisol (6 beta-OHF/F), the use this ratio being considered to be the simplest and most practical method for estimation of hepatic cytochrome P450 3A4 (CYP3A4) activity, during liver regeneration and to determine whether the urinary 6 beta-OHF/F ratio can be used to estimate the remnant-liver function in a donor for liver transplantation.
Methods: Five liver transplant donors (3 males and 2 females) and 8 healthy volunteers (5 males and 3 females) were enrolled in this study. In donors 1 to 5, 70.5, 58.8, 37.2, 31.0 and 23.5% of the preoperative whole livers, respectively, were resected. The urine concentration of 6beta-hydroxycortisol and cortisol were measured by the high performance liquid chromatography with mass spectrometry (LC/MS), and the urinary 6beta-OHF/F ratio was calculated.
Results and Discussion: We have developed a simple, selective and precise method to quantitate 6 beta-hydroxycortisol and cortisol in human urine. The lower quantitation limits were 1.75 ng/mL for 6 beta-OHF and 2 ng/mL for cortisol in urine. In the liver transplant donors, the urinary 6 beta-OHF/F ratio in the immediate postoperative period showed great variability between donors, values ranging from 1.10±0.67 to 3.85±0.98. The mean urinary 6 beta-OHF/F ratios in two donors, whose excision rates were 70.5% and 58.8%, were remarkably low but increased to within the normal range within 2 weeks after liver resection. There was no notable difference among the mean urinary 6 beta-OHF/F ratios of the donors in the postoperative period (6-14 days). In the another donor, whose excision rate was 37.2%, the urinary 6 beta-OHF/F ratio in the immediate postoperative period was also low, but there was no notable difference between the ratio in the immediate postoperative period and that at 2 weeks after partial hepatectomy.
Conclusions: The present study provides a reliable and practical method for the simultaneous determination of 6 beta-hydroxycortisol and cortisol in urine with good accuracy and precision. The urinary 6 beta-OHF/F ratio is a useful probe for estimating the variability of CYP3A4 activity and for predicting restoration of liver function. Hepatic CYP3A4 activity of donors who underwent liver excision of 50% or more decreased remarkably in the immediate postoperative period but increased to within the normal range within 2 weeks after liver resection.

P.1047

Budget Impact Analysis of Quantiferon-CMV Assay for High-risk Kidney Transplant Recipients

Camille N Kotton[1], Joanne Wu[2], Frank K Kuhr[3], Michael B Nichol[2].
[1]Transplant and Immunocompromised Host Infectious Diseases, Infectious Diseases Division, Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States; [2]Sol Price School of Public Policy, University of Southern California, Los Angeles, CA, United States; [3]Medical Affairs, Molecular Diagnostics, North America, QIAGEN, Inc., Germantown, MD, United States.

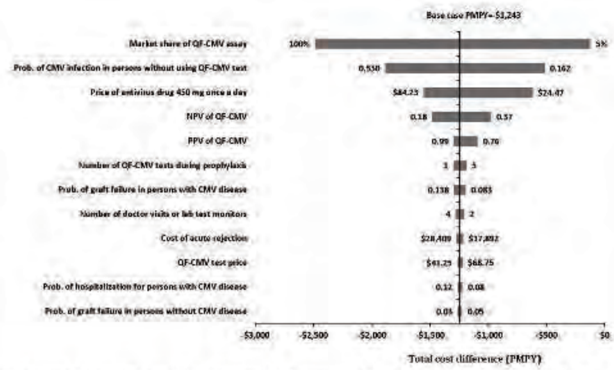
Introduction. The Quantiferon-CMV (QF-CMV) test is an assay of cytomegalovirus (CMV) cell-mediated immunity, and may help clinicians predict the risk of CMV infection after transplantation. This study evaluates the budget impact of the QF-CMV assay for high-risk kidney transplant recipients.
Materials and Methods. We constructed a decision model based on the updated international consensus guidelines and other published literature on the management of CMV in solid-organ transplantation to evaluate the budget impact of QF-CMV assay in the first year after transplant for a hypothetical health plan with 10,000 high-risk (CMV D+R-) kidney transplantation recipients. The model probabilities data related to CMV disease, QF-CMV assay test results, and graft failure were derived from published literature. Probability of hospitalization for patients with CMV disease was based on expert estimation. The costs of doctor visit and laboratory tests were based on Centers for Medicare and Medicaid Services fee schedule. The cost of hospitalization due to CMV infection was obtained from Healthcare Cost and Utilization Project (HCUP). The cost of treatment of acute rejection was obtained from published literature. The budgetary impact on the expected total costs for management of CMV infection was calculated and defined as the cost difference between the strategies using and without using QF-CMV assay.
Results and Discussion. Using the QF-CMV assay in 50% market share, the number of patients with CMV infection was reduced by 20%; the number of patients with graft failure was reduced 8% as comparing to not using QF-CMV assay to predict the risk of CMV infection. Using the QF-CMV assay generated $8,050,000 (or $805 per-member-per-year (PMPY)) savings on extended prophylaxis, $4,007,889 ($401 PMPY) savings on treatment of CMV disease, and $1,199,187 ($120 PMPY) savings on cost associated with graft failure. The savings on expected 1-year total costs for post kidney transplantation for management of CMV infection were $12,432,076 ($1,243 PMPY). The savings were generated from reduced number of CMV diseases due to extended prophylactic treatment or shorter period prophylactic treatment based on the QF-CMV assay test results on predicting the risk of CMV infection. One-way sensitivity analysis revealed that market share of the QF-CMV assay, probability of CMV infection in patients without using QF-CMV assay, and the price of antivirus drug were the most notable variables that significant affected the expected budget.
Conclusion. This budget impact analysis indicated that use of the QF-CMV assay to predict the risk of CMV infection results in clinically and economically favorable for the high-risk kidney transplant recipients in post transplantation management. The QF-CMV assay may be helpful for monitoring the risk of CMV infection during the prophylactic treatment and reducing the costs associated with CMV infection.
Research funding for this study was provided by QIAGEN, Inc.

References:
[1] Kotton CN, Kumar D, Caliendo AM, Asberg A, Chou S, Danziger-Isakov L, Humar A; Transplantation Society International CMV Consensus Group. Updated international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation. *Transplantation.* 2013 Aug 27;96(4):333-60. doi: 10.1097/TP.0b013e31829df29d.
[2] Humar A, Lebranchu Y, Vincenti F, Blumberg EA, Punch JD, Limaye AP, Abramowicz D, Jardine AG, Voulgari AT, Ives J, Hauser IA, Peeters P. The efficacy and safety of 200 days valganciclovir cytomegalovirus prophylaxis in high-risk kidney transplant recipients. *Am J Transplant.* 2010 May;10(5):1228-37. doi: 10.1111/j.1600-6143.2010.03074.x
[3] Luan FL1, Kommareddi M, Ojo AO. Universal prophylaxis is cost effective in cytomegalovirus serology-positive kidney transplant patients. *Transplantation.* 2011 Jan 27;91(2):237-44. doi: 10.1097/TP.0b013e318200000c.
[4] Gheorghian A1, Schnitzler MA, Axelrod DA, Kalsekar A, L'italien G, Lentine KL. The implications of acute rejection and reduced allograft function on health care expenditures in contemporary US kidney transplantation. *Transplantation.* 2012 Aug 15;94(3):241-9. doi: 10.1097/TP.0b013e318255f839.
[5] Matas AJ1, Smith JM, Skeans MA, Thompson B, Gustafson SK, Stewart DE, Cherikh WS, Wainright JL, Boyle G, Snyder JJ, Israni AK, Kasiske BL. OPTN/SRTR 2013 Annual Data Report: kidney. *Transplantation.* 2012 Aug 27;94(4):369-76. doi: 10.1097/TP.0b013e318259407f.
[6] Manuel O, Husain S, Kumar D, Zayas C, Mawhorter S, Levi ME, Kalpoe J, Lisboa L, Ely L, Kaul DR, Schwartz BS, Morris MI, Ison MG, Yen-Lieberman B, Sebastian A, Assi M, Humar A. Assessment of cytomegalovirus-specific cell-mediated immunity for the prediction of cytomegalovirus disease in high-risk solid-organ transplant recipients: a multicenter cohort study. *Clin Infect Dis.* 2013 Mar;56(6):817-24. doi: 10.1093/cid/cis993
[7] Mauskopf JA, Sullivan SD, Annemans L, Caro J, Mullins CD, Nuijten M, Orlewska E, Watkins J, Trueman P. Principles of good practice for budget impact analysis: report of the ISPOR Task Force on good research practices—budget impact analysis. *Value Health.* 2007 Sep-Oct;10(5):336-47.
[8] Healthcare cost and utilization project (HCUP) http://www.ahrq.gov/research/data/hcup/index.html Accessed March 30, 2016

Table 1 Base case budget impact analysis results

Parameters	Reference scenario (A)	New scenario (D)			Budget Impact
	Usual care (A)	Usual care (B)	QF-CMV (C)	Total (D=B+C)	(D-A)
Market Share	100%	50%	50%	100%	
QF-CMV test results and CMV infection status					
Number of positive QF-CMV tests	0	0	1,250	1,250	2,500
Number of negative QF-CMV tests	0	0	3,750	3,750	[illegible]
Number of patients with CMV infection in positive QF-CMV tests	0	0	87	87	175
Number of patients with CMV infection in negative QF-CMV tests	0	0	1,013	1,013	2,025
Number of patients with CMV infection	1690	[illegible]	[illegible]	[illegible]	[illegible]
Number of patients had hospital stay due to CMV infection	368	184	116	294	-74
Total number of patients had graft failure	658	329	273	605.8	-51.5
Number of patients had graft failure due to CMV infection/disease	405	202	121	323.4	-81.4
Number of patients had graft failure in no CMV infection/disease	253	126	156	282.4	29.6
Budget component					
1) Costs of first 100 days prophylaxis ($)	**$64,400,000**	**$32,200,000**	**$32,200,000**	**$64,400,000**	**$0**
1a) Costs of extended prophylaxis*	**$64,400,000**	**$32,200,000**	**$24,150,000**	**$56,350,000**	**-$8,050,000**
2) Costs of QF-CMV test ($)	**$0**	**$0**	**$825,000**	**$825,000**	**$825,000**
3) Total cost of treatment for CMV infection ($)	**$19,931,123**	**$9,965,561**	**$5,957,673**	**$15,923,234**	**-$4,007,889**
Valganciclovir 900 mg twice daily for 21 days	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Lab / doctor visit	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
CMV viral load check	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Monitoring CHEM20	$116,803	$58,432	$34,914	$93,516	-$23,431
Monitoring CBC	$116,803	$58,432	$34,914	$93,516	-$23,431
Cost of hospital stay	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
4) Cost associated with graft failure	**$15,223,657**	**$7,611,829**	**$6,412,641**	**$14,024,470**	**-$1,199,187**
5) Total costs ($)	**$163,934,780**	**$81,977,390**	**$69,545,314**	**$151,522,704**	**-$12,432,076**
Total cost presented as per-member-per-year (PMPY)	**$16,395**	**$8,198**	**$6,955**	**$15,152**	**-$1,243**

Abbreviations: CMV cytomegalovirus; QF-CMV Quantiferon-CMV assay; CHEM chemistry panel; CBC complete blood count.
Note: * Extended prophylaxis was applied to all patients without QF-CMV assay test, or all patients with positive QF-CMV test result.



Abbreviations: CMV= cytomegalovirus, QF-CMV= Quantiferon-CMV; Prob= probability; NPV= negative predict value; PPV= positive predict value.
Figure 1: Tornado Diagram of One-way Sensitivity Analysis. The data displayed outside the bars were the input data values. Tornado diagram showing a series of one-way sensitivity analysis at the decision node based on the budget impact in per-member-per-year (PMPY).

P.1048

Impact of circulating exosomes on the development and recurrence of hepatocellular carcinoma in rats

Toshiaki Nakano[1,2], I-Hsuan Chen[1,2], Shigeru Goto[2,3,4], Chia-Yun Lai[2], Hui-Peng Tseng[1,2], I-Ying Kuo[2], Chih-Che Lin[2], King-Wah Chiu[2], Yu-Fan Cheng[2], Chao-Long Chen[2].
[1]Graduate Institute of Clinical Medical Sciences, Chang Gung University College of Medicine, Kaohsiung, Taiwan; [2]Liver Transplantation Program, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan; [3]Fukuoka Institution of Occupational Health, Fukuoka, Japan; [4]Department of Nursing, Josai International University, Togane, Japan.

Introduction: The recurrence of hepatocellular carcinoma (HCC) after surgical resection or transplantation is one of big issues reflecting the higher mortality in HCC. This study aimed to explore the impact of circulating exosomes for HCC in a rat orthotopic liver tumor model.
Materials and Methods: Buffalo rat-derived hepatoma cells (McA-RH8994: $1.5x10^6$ cells) were injected to naïve rats through the right portal vein, and HCC serum was obtained from the HCC-bearing rats at 5 to 7 weeks after cell injection. To explore the impact of circulating exosomes, 500 µl of HCC serum or exosome-free HCC serum was transfused to naïve rats through the penile vein. MicroRNA microarray was performed for global evaluation of differentially expressed microRNAs in HCC serum exosomes.
Results: One-shot transfusion of HCC serum to naïve rats increased alpha-fetoprotein level, but failed to induce HCC by exosome-free HCC serum. Microarray revealed 25 down-regulating and 23 up-regulating microRNAs in HCC serum exosomes and miR-92b was selected as one of highly expressing microRNAs. Database search revealed that miR-92b regulates CD69, activation marker for T and NK cells, and *in vitro* study demonstrated that HCC serum inhibited the induction of splenic CD69 expression by Concanavalin A stimulation, while exosome-free HCC serum enhance splenic CD69 expression.
Discussion: We have confirmed the impact of circulating exosomes for HCC development and recurrence, and miR-92b in HCC serum exosomes may induce the impairment of host immune surveillance mediated by CD69-positive cytotoxic T cells and/or NK cells in the course of HCC.
Conclusion: MiR-92b in the serum exosomes may be one of potential targets for diagnosis and therapeutics in HCC development and recurrence.
This work was supported by the Ministry of Health and Welfare (MOHW103-TD-B-111-07 to C-L.C.: health and welfare surcharge of tobacco products)

P.1049

Association of delayed graft function (DGF) with serum level of soluble fibrinogen-like protein 2 (sFGL2) in patients with kidney transplantation

Hassan Nikoueinejad[1], Alireza Soleimani[2], Ali Salmani[4], Mojtaba Sehat[3].
[1]Nephrology & Urology Research centre, Baqiyatallah University of Medical Sciences, Tehran, Iran (Islamic Republic of); [2]Internal Medicine, Kashan University of Medical Sciences, Kashan, Iran (Islamic Republic of); [3]Community Medicine, Kashan University of Medical Sciences, Kashan, Iran (Islamic Republic of); [4]Student Research Centre, Kashan University of Medical Sciences, Kashan, Iran (Islamic Republic of).

Introduction: Delayed graft function (DGF) is one of the most common complications of transplantation which means needing Dialysis throughout the first week after transplantation. Considering the effects of regulatory T cells on DGF, we aimed to ascertain the relationship between soluble FGL-2, as their secretory factor[1], and DGF after transplantation.
Materials and Methods: This Nested case-control study was done in a 6-month period among 2 groups of 58-member of transplanted patients with and without DGF. The control group included the patients who were operated in 2015 and didn't show DGF symptoms. Then sFGL-2 serum level in all blood samples was measured by Elisa. Evaluating the impact of confounding factors, we measured the variables in both groups using logistic regression analysis of uni-variate and multi-variate.
Results: Serum level of sFGL2 in recipients with DGF was significantly higher than that of those without DGF ($P<0.001$). Also, there was a strong meaningful relationship between the serum level of sFGL2 and the risk of GDF ($P<0.001$), while none of the variables: rejection, HLA mismatch, type of medication, family relation between recipient and donor, age, and sex did not have any effects on the mentioned relation.
Conclusion: Secreting sFGL2, regulatory T cells may try to suppress immune responses leading to DGF. Such concept may introduce sFGL2 as a diagnostic as well as prognostic marker of DGF in renal transplants.
References:
[1] Shalev I, Liu H, Koscik C, Bartczak A, Javadi M, Wong KM, et al. Targeted deletion of fgl2 leads to impaired regulatory T cell activity and development of autoimmune glomerulonephritis. The Journal of Immunology. 2008;180(1):249-60

P.1050

CD28-positive cytomegalovirus specific cytotoxic T lymphocytes as a novel biomarker associated with cytomegalovirus viremia in kidney allorecipients

Maciej Zieliński[1], Agnieszka Tarasewicz[2], Hanna Zielińska[1], Magdalena Jankowska[2], Grażyna Moszkowska[1], Alicja Dębska-Ślizień[2], Bolesław Rutkowski[2], Piotr Trzonkowski[1].
[1]Department of Clinical Immunology and Transplantology, Medical University of Gdansk, Gdansk, Poland; [2]Department of Nephrology, Transplantology and Internal Diseases, Medical University of Gdansk, Gdansk, Poland.

Introduction: CMV infection remains major complication after kidney transplantation, thus diagnostics tools that would improve identification of individuals at risk of development of CMV – related complications are useful. For this reason, searching for proper immunological biomarkers candidates gives hope to individualize antiviral therapy and minimize side effects of antiviral drugs.
Materials and Methods: In the study, immunological markers of CMV viremia were assessed in 52 kidney transplant recipients during two years lasting follow – up. Immunological markers associated with viral infection, like lymphocytosis, cytotoxic T lymphocytes (CTL) and serum cytokines levels were compared with less common immunological assays, like activated T lymphocytes, CMV-specific CTL stratified according to naïve/memory phenotype. The test to assess expression of CD28 antigen on CTL, as a possible additional marker of CMV-specificity, was developed.
Results: CD28-positive CMV-specific CTL have been found the most useful marker for CMV viremia prediction. Tested value of 3 cells/µl was found to be most suitable for CMV activation assessment with sensitivity 93,55% [95 % confidence interval (CI) 0.786–0.992] and 94,07% specificity [95 % confidence interval (CI) 0.904–0.966]. The estimated AUC was 0,9717 [95 % confidence interval (CI) 0.9450–0.9877 ; p<0,0001].
Discussion: Among tested biomarkers, CD28-positive CMV-specific CTL should be put at the first line, as the most promising tool for this application. CD28-positive CMV-specific CTL, can be therefore considered as "truly active" lymphocytes involved in antiviral immunity.
Conclusion: A set of biomarkers associated with activation of immune system has been reviewed in search of parameters that might be useful for prediction of CMV viremia. Among tested biomarkers, CD28-positive CMV-specific CTL should be put at the first line, as the most promising tool for this application. The major advantage of this assay is virus specificity with existing relationship to the functionality of cells by the CD28 molecule.

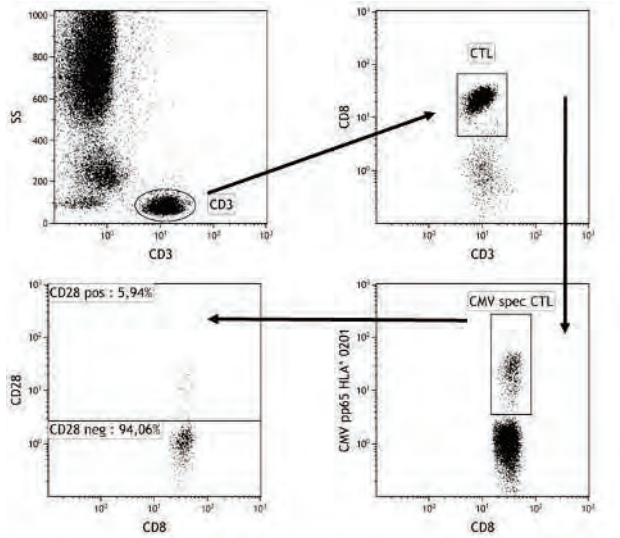


Figure 1: Enumeration of CMV specific CTL and CD28 positive CMV specific CTL

This research was funded by the National Science Centre (grants nr NN402420738 and NN402 562440) and the National Centre for Research and Development, Poland (grant no STRATEGMED1/233368/1/NCBR/2014)

References:

[1] Griffiths P, Baraniak I, Reeves M. The pathogenesis of human cytomegalovirus. *J Pathol* 2015; 235 (2): 288.
[2] Kotton CN, Kumar D, Caliendo AM, et al. Updated international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation. *Transplantation* 2013; 96 (4): 333.
[3] Kotton CN. CMV: Prevention, Diagnosis and Therapy. *Am J Transplant* 2013; 13 Suppl 3: 24.
[4] Bataille S, Moal V, Gaudart J, et al. Cytomegalovirus risk factors in renal transplantation with modern immunosuppression. *Transpl Infect Dis* 2010; 12 (6): 480.
[5] Rowshani AT, Bemelman FJ, van Leeuwen EM, van Lier RA, ten Berge IJ. Clinical and immunologic aspects of cytomegalovirus infection in solid organ transplant recipients. *Transplantation* 2005; 79 (4): 381.
[6] Allice T, Cerutti F, Pittaluga F, et al. Evaluation of a novel real-time PCR system for cytomegalovirus DNA quantitation on whole blood and correlation with pp65-antigen test in guiding pre-emptive antiviral treatment. *J Virol Methods* 2008; 148 (1-2): 9.
[7] Goossens VJ, Blok MJ, Christiaans MH, Sillekens P, Middeldorp JM, Bruggeman CA. Early detection of cytomegalovirus in renal transplant recipients: comparison of PCR, NASBA, pp65 antigenemia, and viral culture. *Transplant Proc* 2000; 32 (1): 155.
[8] Blok MJ, Lautenschlager I, Christiaans MH, et al. Sensitive detection of cytomegalovirus infection in transplant recipients using nucleic acid sequence-based amplification. *Transplant Proc* 2000; 32 (1): 149.
[9] Hirsch HH, Lautenschlager I, Pinsky BA, et al. An international multicenter performance analysis of cytomegalovirus load tests. *Clin Infect Dis* 2013; 56 (3): 367.
[10] Pang XL, Fox JD, Fenton JM, et al. Interlaboratory comparison of cytomegalovirus viral load assays. *Am J Transplant* 2009; 9 (2): 258.
[11] Crough T, Khanna R. Immunobiology of human cytomegalovirus: from bench to bedside. *Clin Microbiol Rev* 2009; 22 (1): 76.
[12] Sester U, Presser D, Dirks J, Gärtner BC, Köhler H, Sester M. PD-1 expression and IL-2 loss of cytomegalovirus- specific T cells correlates with viremia and reversible functional anergy. *Am J*

Transplant 2008; 8 (7): 1486.
[13] Ferrando-Martínez S, Ruiz-Mateos E, Casazza JP, et al. IFNγ-TNFα-IL2-MIP1α-CD107a+PRF1+ CD8 pp65-Specific T-Cell Response Is Independently Associated With Time to Death in Elderly Humans. *J Gerontol A Biol Sci Med Sci* 2014.
[14] Abate D, Saldan A, Mengoli C, et al. Comparison of cytomegalovirus (CMV) enzyme-linked immunosorbent spot and CMV quantiferon gamma interferon-releasing assays in assessing risk of CMV infection in kidney transplant recipients. *J Clin Microbiol* 2013; 51 (8): 2501.
[15] Brooimans RA, Boyce CS, Popma J, et al. Analytical performance of a standardized single-platform MHC tetramer assay for the identification and enumeration of CMV-specific CD8+ T lymphocytes. *Cytometry A* 2008; 73 (11): 992.
[16] Gratama JW, Brooimans RA, van der Holt B, et al. Monitoring cytomegalovirus IE-1 and pp65-specific CD4+ and CD8+ T-cell responses after allogeneic stem cell transplantation may identify patients at risk for recurrent CMV reactivations. *Cytometry B Clin Cytom* 2008; 74 (4): 211.
[17] Gratama JW, Boeckh M, Nakamura R, et al. Immune monitoring with iTAg MHC Tetramers for prediction of recurrent or persistent cytomegalovirus infection or disease in allogeneic hematopoietic stem cell transplant recipients: a prospective multicenter study. *Blood* 2010; 116 (10): 1655.
[18] Cwynarski K, Ainsworth J, Cobbold M, et al. Direct visualization of cytomegalovirus-specific T-cell reconstitution after allogeneic stem cell transplantation. *Blood* 2001; 97 (5): 1232.
[19] Gratama JW, van Esser JW, Lamers CH, et al. Tetramer-based quantification of cytomegalovirus (CMV)-specific CD8+ T lymphocytes in T-cell-depleted stem cell grafts and after transplantation may identify patients at risk for progressive CMV infection. *Blood* 2001; 98 (5): 1358.
[20] Engstrand M, Tournay C, Peyrat MA, et al. Characterization of CMVpp65-specific CD8+ T lymphocytes using MHC tetramers in kidney transplant patients and healthy participants. *Transplantation* 2000; 69 (11): 2243.
[21] Christmas SE, Halliday D, Lawton N, et al. Cytomegalovirus-specific CD8+ T cells do not develop in all renal transplant patients at risk of virus infection. *Transpl Immunol* 2009; 22 (1-2): 99.
[22] La Rosa C, Krishnan A, Longmate J, et al. Programmed death-1 expression in liver transplant recipients as a prognostic indicator of cytomegalovirus disease. *J Infect Dis* 2008; 197 (1): 25.
[23] Moss P, Khan N. CD8(+) T-cell immunity to cytomegalovirus. *Hum Immunol* 2004; 65 (5): 456.
[24] Trzonkowski P, Debska-Slizień A, Jankowska M, et al. Immunosenescence increases the rate of acceptance of kidney allotransplants in elderly recipients through exhaustion of CD4+ T-cells. *Mech Ageing Dev* 2010; 131 (2): 96.
[25] Desai R, Collett D, Watson CJ, Johnson PJ, Moss P, Neuberger J. Impact of Cytomegalovirus on Long-term Mortality and Cancer Risk After Organ Transplantation. *Transplantation* 2015; 99 (9): 1989.
[26] Fishman JA, Emery V, Freeman R, et al. Cytomegalovirus in transplantation - challenging the status quo. *Clin Transplant* 2007; 21 (2): 149.
[27] Cantisán S, Torre-Cisneros J, Lara R, Zarraga S, Montejo M, Solana R. Impact of cytomegalovirus on early immunosenescence of CD8+ T lymphocytes after solid organ transplantation. *J Gerontol A Biol Sci Med Sci* 2013; 68 (1): 1.
[28] Pawelec G, Derhovanessian E. Role of CMV in immune senescence. *Virus Res* 2011; 157 (2): 175.

P.1051

Human blood cell-free circulating DNA (cfDNA) and miRNA as biomarkers of liver and kidney antibody mediated rejection (AMR) or cellular allograft rejection (ACR). Pilot study

Piotr Mieczkowski[1], Ewa Malc[1], Paula Steele[2], Tomasz Kozlowski [2].
[1]Department of Genetics, University of North Carolina, Chapel Hill, NC, United States; [2]Department of Surgery, University of North Carolina, Chapel Hill, NC, United States.

Rationale: cfDNA is detectable in organ injury as nonspecific marker[1]. miRNA functions as cell-to-cell communication mediators. It is possible that both molecules may serve as early marker of graft (gft) damage and miRNA will help differentiate early stage causes of gft impairment (tacrolimus toxicity/rejection) in organ recipients (rpts).
Hypothesis: It is possible to detect the presence of cfDNA/miRNA in organ rpts independent of ongoing treatment including pheresis (PP). Variable unique trends of miRNA expression can be identified during rejection/gft injury/recovery phase using diagnostic criteria[2].
Methods: Serum samples of liver or kidney rpts diagnosed with AMR (liver n=2, kidney n=1), ACR (liver n=1) or with stable kidney function (n=2) were analyzed. Rpts diagnosed with AMR/ACR underwent treatment with a variety of regimens including PP for AMR. Samples were collected during rejection and at gft stability. Plasma extracted cfDNA(Quick-cfDNA™S/PKit-ZymoResearch) was used for library (KAPABiosystem) preparation and capture of selected exons (SureSelect XT-Agilent–UNC cfDNA panel). Exon-enriched cfDNA libraries were analyzed for DNA sequencing using Illumina technology (HiSeq2500). DNA sequencing data generated from enriched cfDNA libraries were analyzed using CLC(Qiagen) software. Only common Single Nucleotide Variants from dbSNP database and targeted region were used for AlleleVariantFrequency analysis. The miRNA analysis was performed using HTG miRNA System. Digital count of 2000 units was normalized for final miRNA analysis.
Results: ***cfDNA:*** Homo-, heterozygous and low frequency somatic and gft variants were detected. Large release of foreign cfDNA from livers after 2-3 days post-surgery was evident. (It may indicate ischemia reperfusion injury or AMR.) Kidney rpts did not show a similar strong effect on release of gft cfDNA. Rejection treatment stabilized the release of liver cfDNA (Fig.1).

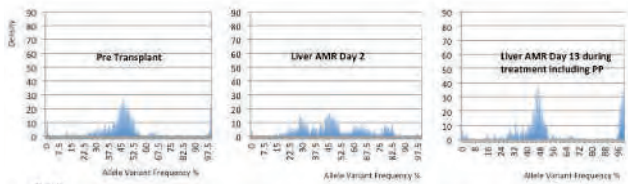
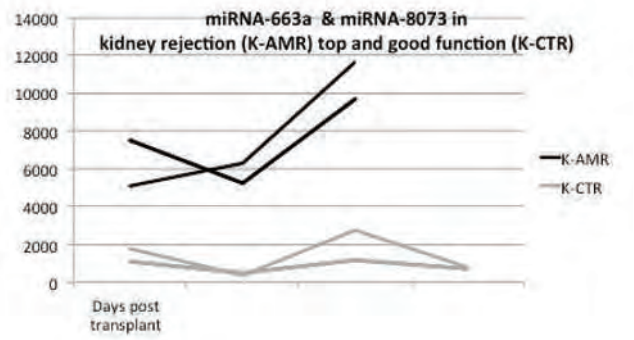


Fig.1.
Variant Allele Frequency in fcDNA in Liver patient diagnosed with Liver antibody mediated rejection. Stabilization of the signal when treatment initiated.

miRNA: Using a 2243 particle panel in each rpt we detected approximately 650 miRNA molecules. Arbitrary selection reduced the number of molecules for this analysis to 50. Liver and kidney AMR patients shared a similar profile of miRNA. Liver transplant patients with ACR detection shared a similar panel of miRNA expression. In kidney rpts with long-term stable gft function only half of (n=25) miRNA particles was detected. On occasion signal for the same molecule in stable gft was stronger then in gfts with rejection. Fig.2 represents miRNA-663a/miRNA-8073 in kidney rpts.



Stronger detection was seen in AMR when compared to control (CTR). miRNA were detectable on comparable levels also after PP.

Conclusions: Immunosuppressive treatment & PP did not affect miRNA detection. Variability of signal allows for differentiation between rejection and stability. cfDNA pattern is consistent with tissue damage and stabilizes after treatment. Several miRNA molecules can be identified as variable between rejection and stability. It is unclear whether miRNAs originate from either the recipient or donor cells or both. miRNA molecules have the potential to become a noninvasive marker of gft damage. Further investigation is indicated.

Dr. David Gerber is acknowledged for critical review of the abstract

References:

[1] KP Daly, Annals of Transl Med 2015 Mar 3(4):47
[2] T Kozlowski, Liver Transplantation 17:35-368, 2011

P.1052

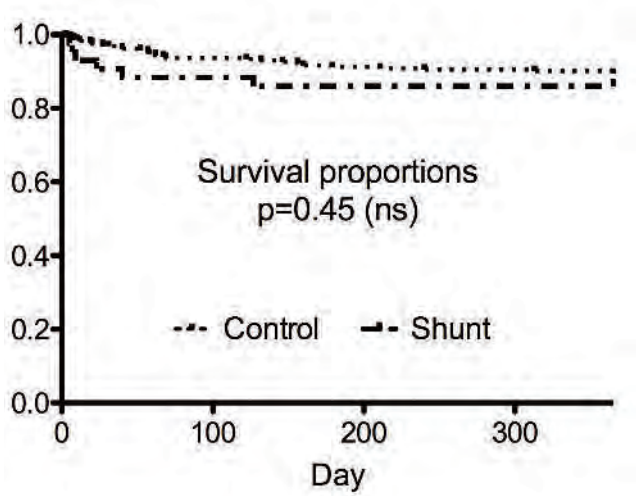
Natural history of spontaneous porto-systemic shunt (PSs) and transplant outcomes in liver allograft recipients

Philip Pepple, David Gerber, Tomasz Kozlowski.
Surgery, University of North Carolina, Chapel Hill, NC, United States.

Rationale: Portal hypertension leads to formation of PSs venous connections presenting as peri-splenic, spleno-renal shunts (SRs), recanalized umbilical vein and/or esophageal varicosities. Often, large spleno-renal shunts (SRs) form as a specific type of PSs. PSs can divert blood from the portal system and potentially affect function of a the liver allograft. There is a paucity of information and controlled studies to clarify whether preexisting excessive PSs can negatively impact recipients (rpts) in long/short term follow up, and whether correction/closure of the PSs is mandatory. Hypothesis. PS/SRs do not negatively affect liver transplant (Tx) outcomes in the vast majority of patients.

Methods: A single center retrospective analysis of adult liver Tx between 2005-13. Control group (CTRg) was SRs free. Shunt group (SNTg) was identified as patients with any PSs including SRs. Rpts had abdominal MRI before and after Tx. Presence of the PSs was defined before surgery. CTRg/SNTg were followed routinely with physical exam, blood testing, and serial Doppler exams, at least immediately and at 12 months (mo) after Tx. Unpaired t tests and survival log-rank (Mantel-Cox) tests were used to assess difference ($p<0.05$) between groups (Prism v.5.x).

Results: We analyzed 329 adult rpts. In SNTg (n=42, 14.6%), 40 rpts had MRI confirmed SRs, two had large caliber coronary to renal vein or IMV to gonadal vein shunt. These PSs were identified with co-existing recanalized umbilical veins or, perispenic/esophageal/retroperitoneal varices. Only 5 rpts of SNTg were identified pre-Tx with portal thrombosis. Calculated MELD for CTRg&SNTg were 24±7 and 22±9 (ns), age 54±8 and 53±12 years (ns) respectively. In CTRg, 30 died (10.4%) compared to 7 in SNTg (16.6%) rpts within 12 mo of Tx. Survival curves are statistically not different ($p=0.45$)(Fig.1).



Mean time to death was 82±15 days (range 0-313, median 58) for CTRg and 32±17 days (range 7-127, median 23) for CTRg and for SNTg correspondingly ($p=0.13$). All survivors in both groups, except 2 in the SNTg, had Doppler hepatopetal flow in portal/splenic veins. In SNTg, 35 rpts were observed for 12 mo and 20 of them had MRI at that time with proven persistence of the SR-PSs. 3 rpts in SNTg experienced complications related to portal vein steal syndrome. 2 died of liver

necrosis and neurological complications days after initial Tx. 1 suffered encephalopathy that resolved after PSs occlusion 8 mo after Tx. This patient had hepatofugal flow in splenic but hepatopetal flow in portal vein. Other causes of mortality in SNTg; hepatic art. thrombosis (n=1), infection (n=3), GVHD (n=1). 1 of 2 rpts suffering graft necrosis had intraoperative attempts to ligate multiple PSs veins, and the second underwent immediate postoperative endovascular PSs occlusion. Both had evidence of steal syndrome on postoperative Doppler and venogram. Remaining SNTg rpts had well-functioning allograft.
Conclusion: Liver-Tx was successful in rpts with PSs-SRs. A small fraction experienced (>5%) graft necrosis during the perioperative period despite efforts to correct PSs. PSs can persist for more than a year after Tx and have no negative impact on long-term allograft function in the majority of rpts.

P.1053

Development of de-novo donor specific antibody (DSA) and vitamin d status after renal transplantation

Sonia Mehrotra, Raj Kumar Sharma, Mahabaleshwar Mayya, Manas R.Patel, Narayan Prasad, Amit Gupta, Dharmendra S.Bhadauria, Anupama Kaul.
Nephrology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India.

Introduction: Mineral bone disorders related to chronic kidney disease persist after kidney transplantation[1]. Vitamin D also has immuno-modulator properties.Vitamin D deficiency has been reported to be an independent risk factor for acute cellular rejection[2]. There are no studies correlating mineral metabolic disturbances after renal transplantation and development of de-novo donor specific antibody (DSA).
Material and Methods: Fifty two chronic kidney disease patients on dialysis going for transplantation were prospectively studied before and after renal transplantation. FGF23, Vitamin- D receptor (VDR), 25 (OH) D, 1-25 (OH)2 D, PTH, serum Ca, serum Po4, e-GFR and vitamin- D status was evaluated and correlated with development of de-novo DSA.
Results and Discussion: The prevalence of hypo-phosphatemia after transplantation was 32.0%, hypercalemia 12.0%, elevated intact PTH levels at 3 months was seen in 66.7% and in 30.8 % patients at 6 months after transplant. FGF-23 levels were high in 72.5% of patients before transplant (495.94±690.68 pg/ml) and decreased to normal levels at 3 month post-transplant (31.63±14.17 pg/m) (control 32.07±9.78 pg/ml). 25-(OH) D levels showed Vit D deficiency in 25.0% before transplant (26.09±12.19 ng/ml),in 48.1% at 6 months post transplant (23.36±15.11ng/ml) .1-25 (OH)2 D levels before transplant were 102.37±108.44 pmol/L which was less than control (143.30±108.0 pmol/L) and decreased further to 46.20±42.11 pmol/ml at 3 months but increased 78.37±60.12 pmol/ml at 6 months post transplantation. VDR activity before transplant was (15.41±31.41 ng/ml) similar to control group (13.24±9.78 ng/ml) and showed increase at 3 months 21.91±38.80 ng/ml and 6 months after transplantation (26.03±53.90 ng/ml). There was difference in the 12 months eGFR in post-transplant patients with or without vit D deficiency (MDRD eGFR 63.95±22.50 ml/min and 71.03±23.87 ml/min). Serum intact PTH levels were 379.54±281.27 pg/ml before transplant and came down to 103.96±68.34 at 3 months, 69.87±116.03 at 6 months post transplantation. Post-transplant de-novo donor specific antibody (DSA) developed more significantly in patients with vit D deficiency (30.7%) group vs 5.5% in group without deficiency ($p<0.048$). There was trend of higher e-GFR at one year post transplant in patients without vitamin D deficiency (levels $\geq$ 20 ng/ml). Post transplant 1-25(OH) D deficiency was associated with increased incidence of urinary tract, fungal and gastrointestinal infections.
Conclusions: In this study there was trend of increased de-novo DSA development in post-transplant period associated with vitamin D deficiency which could have a detrimental effect on graft outcome as indicated by decreased e-GFR at 12 months post transplant. The dysregulated mineral metabolism continues after kidney transplant despite improvement in renal function and normalization of FGF- 23.[1] Though improved, 1-25(OH)2 D levels after transplantation were still less than normal healthy controls. This may be due to reduced renal mass and 1-alpha hydroxylase activity as compared to healthy controls with two kidneys as compared to transplant patients who have only one functioning kidney. Vitamin-D as an immuno modulator has shown expansion of CD4+ and CD25+ Tregs with the calcitriol treatment. But such reports are lacking in transplant recipients.
References:
[1] Frank Bienaimé, Delphine Girard, Dany Anglicheau, Guillaume Canaud, Jean Claude Souberbielle, Henri Kreis, Laure Hélène Noël, Gérard Friedlander, Caroline Elie, Christophe Legendre, and

Dominique Prié,Vitamin D Status and Outcomes After Renal Transplantation J Am Soc Nephrol 2013;24: 831-841
[2] John R. Lee, Darshana Dadhania, Phyllis August, Jun B. Lee, Manikkam Suthanthiran, and Thangamani Muthukumar.Circulating Levels of 25-Hydroxyvitamin D and Acute Cellular Rejection in Kidney Allograft Recipients. Transplantation 2014;98: 292-299

P.1054

De-novo donor specific antibody (DSA) against HLA-DQ antigens by lysate based cross match on Luminex platform resulting in acute antibody mediated rejection in a renal transplant patient

Raj Kumar Sharma, Sonia Mehrotra, Narayan Prasad, Amit Gupta, Dharmendra S.Bhadauria, Anupama Kaul, Mahabaleshwar Mayya.
Nephrology, Sanjay Gandhi Post Graduate Medical Institution, Lucknow, India.

Introduction: A highly sensitive single antigen bead assay (SAB) by luminex is a very useful tool for post-transplant monitoring of HLA antibodies for diagnosing anti body mediated rejection ABMR. Lysate based crossmatch may not pick up anti DP, DQ antibodies due to their poor capture in assay.
Material Methods: A 46 year old male with diabetes mellitus and renal calculus disease and hypertension presented with acute graft dysfunction about six month after renal transplantation. He underwent live unrelated ABO compatible transplant on 09-04-2014 by kidney paired donation. His complement mediated cytotoxicity (CDC) cross match was negative before kidney transplant. At the time of transplantation antithymocyte globulin(ATG) induction (3 doses) was given. His serum creatinine was 1.4mg % with a nadir s.creatinine of 1.1mg%. He was on triple drug immunosuppression ; tacrolimus, mycophenolate mofetyl (MMF) and prednisolone.
Result and Discussion: First post transplant admission was on 11-sept-2014 with acute graft dysfunction, serum creatinine increased from 1.1 mg% to 1.8mg% on 11-09-15 ; tacrolimus blood level (C0) was 6.4ng/ml. He had leukopenia ; total leukocyte count (TLC) being 900/cmm. Renal allograft biopsy report showed mild to moderate acute tubular necrosis with mild interstitial infiltrate. C4d staining was negative. Patient was given 3 doses of intravenous methyl prednisolone. Serum cretinine decreased to 1.56 mg %.DSA for class ll was positive by lysate based cross match on Luminex platform. A repeat anti HLA donor specific antibody by SAB assay on Luminex showed antibodies against donor DQB1* 02:01 and DQB1* 03:02. There were no anti HLA- A, B, DR antibodies in the recipient.
Whether this graft dysfunction is the result of donor specific antibody and should this patient undergo treatment for ABMR (C4d -), could only be decided after doing HLA-DP and DQ typing of the donor. The donor had the HLA DQB1*02 and 03 alleles confirming that recipient has developed donor specific antibody directed only against DQ antigens of the donor ,in the setting of acute graft dysfunction and C4d negative graft biopsy. Renal allograft biopsy showed changes of acute tissue injury as indicated by acute tubular necrosis, evidence of recent antibody interaction with endothelium-(moderate micro vascular inflammation), and serological evidence of donor specific antibody against DQ antigens of donor. The patient was treated with 7 sessions of plasmapharesis and high dose intravenous immunoglobulin infusion therapy. Patient showed improvement in graft function (serum creatinine came down from 1.8mg% to 1.5mg%). His serum creatinine on last follow up was 1.5mg%, 3 months after treatment of ABMR.
Conclusion: SAB technology has increased ability to identify antibody specificity. For this information to translate into an accurate virtual cross match(VXM), the complete donor HLA typing must be known, including HLA A, B, Cw, DR, DQ, and DP. Post-transplant demonstration of development of the de-novo DSA by SAB assay on Luminex platform is of value for monitoring and diagnosis of ABMR.Lysate based cross match on luminex is cheaper than SAB assay and can pick up anti DQ antibodies and could be used for monitoring.

P.1055

Association between donor toll-like receptors-4 polymorphism and acute rejection after liver transplantation in Belarus

Denis Efimov[1], Alijaksei E.Shcherba[1], Alexander M.Dzyadzko[2], Alla I.Kireeva[3], Svetlana Krasutskaja[1], Sergey Korotkov[1], Oleg O.Rummo[1].
[1]Transplantation Department, RSP Center for Organ and Tissue Transplantation, Minsk, Belarus; [2]Anesthesiology and ICU Division, RSP Center for Organ and Tissue Transplantation, Minsk, Belarus; [3]Institute of Genetics and Cytology National Academy of science, Minsk, Belarus.

Introduction: It has been shown that TLR-4 have a key regulatory role in allograft rejection and tolerance in liver transplantation[1]. Moreover a significant association between some single nucleotide polymorphisms (SNPs) within the deceased donor TLR-4 gene and liver graft failure (LGF) was reported[2]. But there is no prospective study to assess association between TLR-4 polymorphism and acute rejection after liver transplantation. The aim of the study was to assess influence of the donor TLR-4 polymorphism on the acute rejection incidence after liver transplantation in Belarusian population.
Materials and Methods: 53 sequential DBD adult liver transplants from standard criteria donors were included in the prospective case-control study. Liver graft biopsy taken at procurement operation were analyzed and three SNPs (rs11536865 (G/C), rs5030717(A/G) and rs913930 (T/C) as previously described to be associated with LGF) within the TLR-4 gene were sequenced (3500 Genetic Analyzer, «Life technologies», USA). Acute rejection was assessed with standard Banff criteria on graft biopsy (BPAR). The Fisher exact test and regression analysis were used to assess association between BPAR and SNPs.
Results: The overall BPAR incidence was 26,4% (14/53). In given cohort the rs11536865 was monoallelic (GG genotype); both rs5030717 and rs913930 had 3 genotypes (AA, AG, GG and TT, TC, CC consequently). The trend toward protective effect of minor C allele within rs 913930 was revealed (BPAR incidence in CC and TC genotypes vs TT genotype was 11,1% (2/18) vs 34,2% (12/35), p=0,07, $\chi 2 = 2,95$). There were no association between rs 5030717 SNPs and the risk of BPAR (p=0,5).
Conclusion: The results of this pilot study showed the potential association of donor TLR-4 polymorphism within rs 913930 and the risk of BPAR in Belarusian population. The study is ongoing to clarify further the role of donor TLR-4 polymorphism and liver transplantation outcomes.
References:
[1] Jessica Howell, Paul Gow, Peter Angus, Kumar Visvanathan. Role of toll-like receptors in liver transplantation. *Liver transpl.* 2014; 20,3: 270–280.
[2] William S. Oetting et al. Donor Polymorphisms of TLR4 Associated with Graft Failure in Liver Transplant Recipients. *Liver Transpl.* 2012; 18(12): 1399–1405

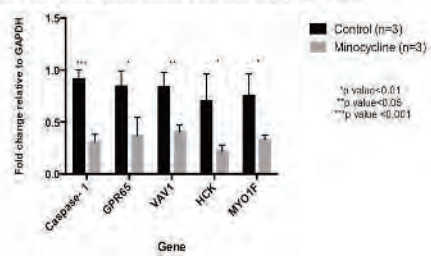
P.1056

Minocycline targets multiple key driver genes in a model of acute allograft rejection

Karen L. Keung[1,3], Zhengzi Yi[2], Chengguo Wei[1], Li Li[2], Madhav C.Menon[1], Weijia Zhang[2], Barbara Murphy[1].
[1]Renal Division, Icahn School of Medicine at Mount Sinai, New York, NY, United States; [2]Department of Genetics and Genomics, Icahn School of Medicine at Mount Sinai, New York, NY, United States; [3]Centre for Transplant and Renal Research, Westmead Millenium Institute, University of Sydney at Westmead Hospital, Sydney, Australia.

Introduction: A novel strategy of identifying potential therapeutic targets for acute allograft rejection is to evaluate the transcript expression profile from biopsy tissue obtained at the time of rejection.
Methods: Our group has identified a 14- key driver meta-gene set associated with acute rejection from six renal allograft biopsy transcript expression datasets. Using the Connectivity Map (CMAP) database for drug repurposing, minocycline was identified as one of the drugs targeting this metagene set. We investigated the effects of minocycline in acute allograft rejection in a heterotopic murine heart transplant model. The hearts of six 10 week-old BALB/c males donors were transplanted into six C57BL/6 male recipients of similar age. Drug-treated mice (n=3) received IP injections twice daily at 50mg/kg/dose from 36 hours prior to surgery to 72 hours following. Control mice (n=3) received twice daily intra-peritoneal injections of phosphate-buffered saline. Mice were sacrificed on day 4 when all hearts transplants were still viable and the allografts were harvested. Quantitative real-time polymerase chain reaction (RT-qPCR) was used to assess the relative transcript levels of the 14 key driver genes normalized to GAPDH in the control and treatment groups. RT-qPCR was also performed to evaluate the relative expression of inflammatory cytokines TNF-alpha, CCL2, CCL3, CCL5, IL-6 and IL-8 which may reflect downstream effects.
Results: RT-qPCR results are shown in Figure 1. There were statistically significant differences in relative mRNA expression in 5 of the 14 key driver metagenes- Caspase 1, GPR65, VAV1, HCK, and MYO1f- in the minocycline-treated mice compared to controls. The most significant difference between the minocycline and control groups was the relative transcript expression levels of Caspase- 1. Of the inflammatory cytokines, significant reductions in the relative mRNA expression of CCL2, CCL3, CCL5 ($p<0.10$) and TNF-α ($p<0.05$) were seen.
Discussion: Caspase-1, a protease that cleaves IL1b, has been well studied and known to be inhibited by Minocycline. The other aforementioned key driver meta-genes are not as well described. Further animal and functional studies will be performed to verify our preliminary findings, and to confirm if inhibition by Minocycline alters the histological evolution of acute allograft rejection, and ultimately allograft survival.
Conclusion: Molecular expression profiling can lead to the identification of potential targets for therapy in the setting of acute allograft rejection. Minocycline inhibits the transcription of a number of genes in an AR-associated key driver metagene set, but whether this translates to prolonged graft survival requires further study.

Figure 1. Relative mRNA expression levels of 5 key driver meta-genes in RT-qPCR



P.1057

Association between interleukin 17A and IL17RA gene polymorphisms and acute rejection following kidney transplantation

Hong joo Lee[1], Dong young Lee[4], Jin Sug Kim[2], Da Rae Kim[2], Su Woong Jeong[2], Su Woong Jeong[2], Ji Hoon Lee[2], Chun Gyoo Ihm[2], Yang Gyun Kim[2], Ju Young Moon[2], Sang Ho Lee[2], Sun Woo Kang[3], Tae Hee Kim[3], Yeong Hoon Kim[3], Kyung Hwan Jeong[2].
[1]Department of Nephrology, Red Cross Hospital, Seoul, Korea;
[2]Department of Nephrology, Kyung Hee University, Seoul, Korea;
[3]Department of Nephrology, Inje University, Seoul, Korea;
[4]Department of Nephrology, Seoul Veterans Hospital, Seoul, Korea.

Acute rejection (AR) after kidney transplantation resulting from alloimmune responses has a negative effect on graft survival. IL17, the hallmark cytokine of the newly defined Th17 cell subset, might have a role for the inflammatory response during allograft rejection. IL17RA mainly reacts with IL17A and elicit signal transduction. Many reports showed that single nucleotide polymorphisms (SNPs) of these cytokines can affect the occurrence of AR.
Therefore, we have evaluated 332 renal allograft recipients to investigate the relationship between IL17A and IL17RA polymorphisms and AR in Korean population. All 332 renal transplant recipients were divided into 198 men with mean age (± standard deviation) 39.5 (± 11.7) years and 134 women, mean age 41.0 (± 10.9) years. The mean follow up duration after kidney transplantation was 74.4 months and the overall number of AR group was 58. We selected 6 SNPs of IL17A (rs3819024, rs2275913) and IL17RA (rs879575, rs879577, rs2229151, rs4819554) gene and genotyped them to evaluate the association of SNPs and AR. Recipient gender (p=0.039), initial antimetabolite (p = 0.001), injection of antibody (basiliximab) (p = 0.001) and duration after kidney transplantation (p = 0.001) represented significant differences between 2 groups. Genotyping data demonstrated that rs879577 for IL17RA was significant association between SNPs and AR by logistic regression analysis after adjustment for age, gender, immunosuppressive therapies (antimetabolite, and basiliximab). Codominant model of rs879577 (p= 0.004, odds ratio 0.11, 95% confidence intervals 0.02-0.86) was significant. To demonstrate pair-wise linkage disequlibrium (LD), no LD block was created using the Gabriel method
In conclusion, these results suggest that the IL17RA gene polymorphism may be related to the development of AR in kidney transplant recipients.

P.1058

Association between Interleukin 7 receptor α gene polymorphism and chronic rejection after renal transplantation in Korean patients

Hong joo Lee[1], Jin Sug Kim[2], Da Rae Kim[2], Su Woong Jeong[2], Ji Hoon Lee[2], Chun Gyoo Ihm[2], Yang Gyun Kim[2], Ju Young Moon[2], Sang Ho Lee[2], Sun Woo Kang[3], Tae Hee Kim[3], Yeong Hoon Kim[3], Kyung Hwan Jeong[2].
[1]Department of Nephrology, Seoul Red Cross Hospital, Seoul, Korea;
[2]Department of Nephrology, Kyung Hee University, Seoul, Korea;
[3]Division of Nephrology, School of Medicine Inje University, Busan, Korea.

Chronic renal allograft loss is still an unsolved problem in kidney transplantation. Although recently developed immunosuppressive drugs have substantially decreased the incidence of rejection, inhibiting memory T-cell expansion in order to control chronic rejection remains a challenge in transplantation. IL-7/IL-7Rα is most importantly identified as key regulator of homeostatic T-cell expansion. Consequently, IL-7Rα may be a good candidate gene to evaluate in kidney transplantation rejection. In this study, we investigated whether polymorphisms of the IL-7Rα gene were associated with susceptibility to kidney transplantation rejection.
Chronic rejection was defined by positive transplant biopsy pathology or clinical renal function deterioration that recovered after intensive immunosuppressant treatment, and developed after six months of kidney transplantation.
We genotyped three selected SNPs (rs 1494558, rs2172749 and rs 1494555) in the IL-7Rα gene using direct sequencing in 335 renal transplant recipients. In order to evaluate the association of IL-7Rα SNPs with chronic rejection, patients were also divided according to the development of rejection after six months post-transplant. Chronic rejection developed in 42 patients. There were no significant differences in follow up duration, sex, number of HLA mismatches, cause of renal failure, immunosuppressant regimen, and number of patients with previous transplants between the chronic rejection and non-chronic rejection groups. Of the SNPs examined, one (rs1494555) showed a statistical association with chronic rejection (p=0.026, odds ratio (OR)=0.44 95% confidence interval (CI)=0.21-0.90 in the dominant model).
One IL-7Rα gene polymorphism was associated with susceptibility to chronic rejection in kidney transplantation in Korean patient.

P.1059

Biomarker discovery of delayed graft function from perfusate proteome

Somchai Chutipongtanate[1], Arpa Chutipongtanate[2], Channarong Changtong[1], Atiporn Inksathit[3], Nutkridta Pongsakul[1], Chagriya Kitiyakara[3], Bunyong Phakdeekitcharoen[3], Surasak Kantachuves[3], Vasant Sumethkul[3], Nuttapon Arpornsujaritkun[4], Piyanut Pootracool[4], Wiwat Tirapanich[4], Sopon Jirasiritham[4], Siriwan Jirasiritham[2], Churat Weeraphan[5], Daranee Chokchaichamnankit[5], Chantragan Srisomsap[5], Jisnuson Svasti[5,6].
[1]Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; [2]Department of Anasthesiology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; [3]Department of Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; [4]Department of Surgery, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; [5]Laboratory of Biochemistry, Chulabhorn Research Institute, Bangkok, Thailand; [6]Applied Biological Science Program, Chulabhorn Graduate Institute, Bangkok, Thailand.

Background: Delayed graft function (DGF) is a common complication after decease donor kidney transplant which associated with poor long-term graft survival. Identifying biomarkers for early recognition of DGF may be beneficial to improve postoperative outcomes.
Objective: This study aimed to identify novel biomarkers of DGF from perfusate using gel-based proteomic approach.
Methods and Materials: Perfusate specimens were collected after removal of decease donor kidney from static cold storage-kidney transporter. Syringe-push membrane absorption (SPMA) method[1] was adopted to prepare perfusate proteins. Differentially expressed proteins between DGF group and immediate graft function group were analyzed by two-dimensional differential in-gel electrophoresis (2-D DIGE) (n=6 each). Differential expressed proteins were submitted to mass spectrometric protein identification. Western blot analysis was performed to confirm expression data. *P*-value <0.05 was considered statistically significant.
Results: SPMA method provided a good quality of perfusate proteome as shown by 2-D gel electrophoresis. Comparative proteomic analysis using 2-D DIGE revealed that several perfusate proteins, for example, fibrinogen beta chain, lumican, alpha-1 microglobulin and haptoglobin alpha-1 chain, were differentially expressed between groups (p <0.05). Of these, fibrinogen beta chain was selected to validate its expression by Western blot analysis. The result confirmed the expressional data, in which fibrinogen beta chain was lower in DGF group.
Conclusions: Alterations in fibrinogen beta chain is a candidate biomarker of DGF, which can be detected during perioperative period. Further validation in larger number of patients is required to clarify its benefits. In addition, this study supported intragraft coagulation as a pathophysiologic process of DGF in decease donor kidney transplant.
References:
[1] Chutipongtanate S, *et. al.* Syringe push membrane absorption as a simple rapid method of urine preparation for clinical proteomics. *Clinical Proteomics* 2015; 12: 15

P.1060

The FokI vitamin D receptor polymorphism is associated with biliary complications after living donor liver transplantation

Adianto Nugroho, Kwang-Woong Lee, Kyung Chul Yoon, Suk Kyun Hong, Hyo-Sin Kim, Hyeyoung Kim, Nam-Joon Yi, Kyung-Suk Suh[1].
Department of Surgery, Seoul National University College of Medicine, Seoul/Jongno-gu, Korea.

Introduction: Biliary complications are still considered as the Achilles Heel in Liver Transplantation. It has an important impact on graft survival, hospital readmissions, need for re-intervention, and overall cost. Several genetic polymorphisms are known to have a relation with biliary problems, including Vitamin D receptor (VDR) polymorphism with its immunomodulatory properties to cause biliary problems in non-transplant setting. We aimed to verify whether VDR polymorphisms, both in donor and recipient, might affect the incidence of biliary complications in Living Donor Liver Transplantation.
Methods: Eighty-nine patients underwent Living Donor Liver Transplantation at Seoul National University Hospital from April 2014 to September 2015 was prospectively studied. Recipient and Donor genotyping for VDR polymorphism sites, including FokI A>G (rs2228570), BsmI A>G (rs1544410), TaqI C>T (rs731236), ApaI A>C (rs7975232) was performed. Cox Regression was performed to analyze the biliary complications free survival.
Results: Liver cirrhosis associated with HBV was the most common indication for liver transplantation (58.4%) in this series. HCC was found in 58 patients (65.2%). The incidence of biliary complication was 18%, with the median follow up time of 12 (3-21) months. The frequency of ApaI genotypes CC were 15.7% in donors and 55.1% in recipients, BsmI genotypes GG were 89.9% in donors and 91 in recipients, TaqI genotypes TT were 89.9% in donors and 92.1% in recipients, and FokI genotypes GG were 41.6% in donors and 37.1% in recipients. Biliary complications were associated with the presence of Donor and recipient BMI > 23 kg/m2, HCC and ABO incompatibility. Donors with FokI GG genotype, has a lower incidence of biliary complications, while donor BMI > 23 kg/m2, HCC and ABOi is associated with higher incidence, as suggested in the multivariate analysis. Furthermore, complication free survival in the donor FokI genotype was better.
Conclusion: VDR polymorphisms were observed in both liver transplant recipients and donors in Korean population, and one of its SNPs (FokI) could possibly serve a protective effect from biliary complications.

P.1061

Hepatitis B virus immunoglobulin is internalized in hepatocytes via endocytosis and induce auto-phagosome

Kwang-Woong Lee, Sooin Seo, Seung Cheol Oh, Min Young Park, Sohee Kim, Nam-Joon Yi, Kyung-Suk Suh.
Department of Surgery, Seoul National University College of Medicine, Seoul/Jongno-gu, Korea.

Background and Aim: Hepatitis B immunoglobulin (HBIG) is used long time for prevention of hepatitis B virus (HBV) recurrence after liver transplantation. The HBIG is thought to bind and neutralize with virions or particles which have hepatitis B virus surface antigen (HBsAg) in serum. But according to more recent studies, investigated in vitro HBsAg specific immunoglobulin G (IgG) is internalized in hepatocytes. And HBIG can clearance to HBV with endocytosis in Fc receptors for IgG(FcRn) expression cell lines. The aim of this study was to investigate further mechanism of intracellular action of intravenouse human hepatitis B virus immunoglobulin (I.V. Hepabig) and Hepabig-gene in sense of more specific interaction with HBsAg. With respect to its mechanism of action, Hepabig or Hepabig-gene can effectively promote passive immunization for individuals exposed to the HBV by binding to HBsAg and reducing rate of replication.
Methods: The cell lines used in this study were: Huh7, HepG2, HepG2.2.15 (HBV-positive, HBsAg-positive) and PLC/PRF/5(HBsAg positive). Human primary hepatocytes were isolated from resected partial liver. A variety of cell lines and isolated hepatocytes were exposed to 1) I.V.Hepabig, 2) recombinant Hepabig-gene and 3) Fab portion of Hepabig-gene for 1 hour. Confocal fluorescence microscopy was used to localize HBsAg specific IgG. Western blot analysis for the level of endogenous LC3 and HBsAg proteins was performed to identify autophage..
Results: HBsAg was colocalized with I.V. Hepabig, Hepabig-gene or Fab type in the cytoplasm as a punctate pattern of immunofluorescence in HBsAg expression cell lines (HepG2.2.15 and PLC/PRF/5). I.V. Hepabig also localized in cytoplasm with HBsAg in isolated primary hepatocyte from HBsAg positive human liver tissue. Western blot analysis proved that I.V. Hepabig and Hepabig-gene treated hepatocytes accumulated more intracellular HBsAg than control, but not in Fab type of Hepabig-gene treated hepatocytes. Especially, LC3-II which is lipidation of LC3-I form was detected with just Hepabig-gene treatment samples.
Conclusions: These results suggest that I.V. Hepabig and Hepabig-gene are present in FcRn expression hepatoma cell lines and primary hepatocytes by endocytosis and colocalized with HBsAg in the cytoplasm. Furthermore, the immunoglobulin-sAg complex induced autophgosom in the cytoplasm.

P.1062

Multiple bile duct: Does it impact on recipient outcomes?

Kyung Chul Yoon, Kwang-Woong Lee, Hyo-Sin Kim, Suk Kyun Hong, Hyeyoung Kim, Nam-Joon Yi, Kyung-Suk Suh.
Department of Surgery, Seoul National University College of Medicine, Seoul/Jongno-gu, Korea.

Introduction: The variation of multiple bile ducts in a living donor graft can be encountered frequently but whether or not this leads to biliary complications has not been known yet. we want know the overall risk factors of biliary stricture and the distance between the bile duct and the number of biliary anastomosis in the graft with multiple bile duct openings after living donor liver transplantation using right hemiliver graft with duct to duct anastomosis
Method: We retrospectively reviewed 317 patients who underwent living donor liver transplantation from 2011 to 2013 and excluded 57 patient who were received re-LT, Hepaticojejunostomy, were padiatric patient, were used Lt graft and lost to follow up. Among them only 5 patient had leakage and one patient without stricture was excluded.
Result: A total of 259 patients were enfolled and 81 patients had more than 2 bile duct openings. Fifty eight patients(22.4%) developed biliary stricture needing ERCP or PTBD. Multiple bile duct openings was not a risk factor comparing the graft with single bile duct opening, and recipient BMI and bile duct leakage were significant in multivariate analysis. Portal vein thromobosis and MELD score greater than 25 were significant in the graft with multiple bile duct openings. The number of anastomosis and distance of the bile duct openings were not significant but distance of the bile ducts less than 5mm could be performed single anastomosis in 90% patients.
Conclusion: It was controversial that the graft with multiple bile duct openings can be a risk factor or not in the previous studies. But in our study, Multiple bile duct openings was not a risk factor for biliary stricture and the duct distances and the number of anastomosis were also not a risk factors in the graft with multiple duct openings. Portal vein thrombosis as a recipient factors can be a risk factor which can cause portal hypertensive biliopathy.

P.1063

Coronary vein to portal vein anastomosis in a small portal vein with a large shunt in living donor liver transplantation

Kwang-Woong Lee, Kyung Chul Yoon, Suk Kyun Hong, Hyo-Sin Kim, Hyeyoung Kim, Nam-Joon Yi, Kyung-Suk Suh.
Department of Surgery, Seoul National University College of Medicine, Seoul/Jongno-gu, Korea.

Background and Aims: Chronic liver disease can lead to portal hypertension due to high resistance to portal outflow, which can cause several shunts and a decrease in the size of the portal vein. There are several methods to overcome the small portal vein with a large shunt, such as renal vein ligation, portal vein reconstruction using another vein graft, portal vein angioplasty and shunt to portal vein anastomosis. We will introduce one of the methods to overcome the small portal vein with a large coronary shunt: coronary vein to portal vein anastomosis.
Patient and Video Contents: A 58-year old male patient suffered from alcoholic liver cirrhosis with repeated PSE. His liver function was Child A and MELD score was 12. His donor was his younger brother who was 53 years old and right GRWR was 0.97.
We will introduce coronary vein to portal vein anastomosis in a small portal vein with a large coronary vein and changes of the pre and post-operative CT findings.

P.1064

Extracellular matrix extract as a therapeutic approach for ischemic liver in mice

Kamal Hussein[1,2], Kyung M.Park[1,2], Yun S.Lee[1], Kyung S.Kang[3], Heung M.Woo[1,2,4].
[1]Stem Cell Institute, Kangwon National University, Chuncheon, Korea; [2]College of Veterinary Medicine, Kangwon National University, Chuncheon, Korea; [3]College of Veterinary Medicine, Seoul National University, Seoul, Korea; [4]Harvard Stem Cell Institute, Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, United States.

Introduction: Ischemic injury of the liver is a major complication of liver resection, transplantation and hypovolemic shock. To replace the necrotic hepatocytes and damaged ECM, liver tissue engineering approaches have become a major research focus for treatment of liver ischemia. The aim of this study was to prepare an injectable liver matrix extract from decellularized mouse liver, analyze the *in vitro* properties and the *in vivo* potential to mimic the natural hepatic extracellular environment.
Methods: Mouse liver was decellularized and processed to form a hepatic matrix with the ability to gel *in vitro*. We tested the ability of the matrix to enhance the migration of hepatocytes and endothelial cells. Then, the matrix gel was injected into liver parenchyma in mouse after induction of liver ischemia.
Results: The resulting liver matrix maintained a complex composition, including glycosaminoglycan, collagen, elastin and growth factors content. Hepatocytes and endothelial cells were shown to migrate towards the liver matrix *in vitro*. The matrix was delivered successfully in a minimally invasive procedure. The results of *in vivo* tests showed that the matrix gel was able to enhance the neovascularization and cell migration toward the ischemic regions.
Conclusion: We have demonstrated that liver matrix gel could be utilized as an injectable scaffold for liver tissue engineering to promote neovascularization and to reduce the ischemic environment.
This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ0110022015)", Rural Development Administration, Republic of Korea

P.1065

Optimization of a therapeutic protocol of human mesenchymal stem cells in mice heart transplant model

Longshan Liu[1], Feng Wang[1], Xiaoyong Chen[2], Yanwen Peng[3], Jun Li[1], Peng Xiang[2,3], Changxi Wang[1,4,5].
[1]Organ Transplant Center, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, People's Republic of China; [2]Center for Stem Cell Biology and Tissue Engineering, Key Laboratory for Stem Cells and Tissue Engine, Ministry of Education, Sun Yat-Sen University, Guangzhou, People's Republic of China; [3]Cell-gene Therapy Translational Medicine Research Center, The Third Affiliated Hospital, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, People's Republic of China; [4]Guangdong Provincial Key Laboratory on Organ Donation and Transplant Immunology, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, People's Republic of China; [5]Institution of Organ Donation and Transplant Immunology, Sun Yat-sen University, Guangzhou, People's Republic of China.

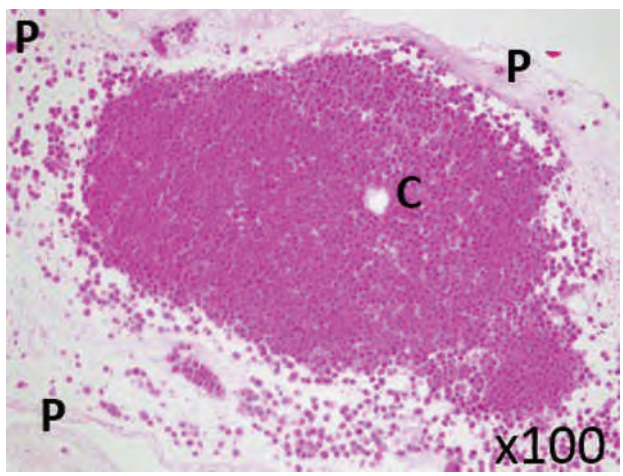
Background: Many studies have demonstrated mesenchymal stem cells (MSCs) could inhibit transplant rejection. However, the agreement on the correlation of transfused time or dose with its effect has not been reached. The aim of this study was to investigate the correlation of transfusion timing and dose of MSCs with its effect on inhibition of transplant rejection.
Methods: Human MSCs (hMSCs) were intravenously administered to Balb/c recipients at gradient doses, 1×10^6 (Low dose), 2×10^6 (Medium dose) and 5×10^6 cells (High dose)/mouse, 24 h before or after cardiac transplantation from C57BL/6 donors. Allograft survival was compared, and T cells and B cells were monitored 7 days after transplant.
Results: Pre-transplant infusion of hMSCs prolonged graft survival to 10 days at high dose, while low or medium dose didn't show beneficial effect. Post-transplant infusion of hMSCs prolonged graft survival to 9.5 (low dose), 12 (medium dose) and 21 days (high dose) respectively. Post-transplant MSCs enhanced longer graft survival than pre-transplant MSCs at high dose (21 vs 10 days, $p<0.05$). Lymphocyte infiltration to cardiac grafts was reduced by hMSCs treatment, especially in the post-transplant high dose group. Compared to the control group, $CD4^+$ and $CD8^+$T cells in the post-transplant high dose group respectively decreased from 20.2% to 11.5% and from 10.6% to 6.3% in spleens ($p<0.01$), and T cells activation ($CD25^+$ expression) was also inhibited from 4.0% to 1.6% ($p<0.01$). Moreover, CD4+Foxp3+regulatory T cells increased by 10% in spleens and 25% in draining lymph nodes. Of note, CD5+CD19+B cells also increased by 20-25% in spleens.
Conclusions: Transfusion time and dose of hMSCs did affect transplant outcome in mice. hMSCs may take effect through inhibition of Th1 immune response and increase of regulatory T and B cells, while the very primary initiative mechanism deserves further investigation.

P.1066

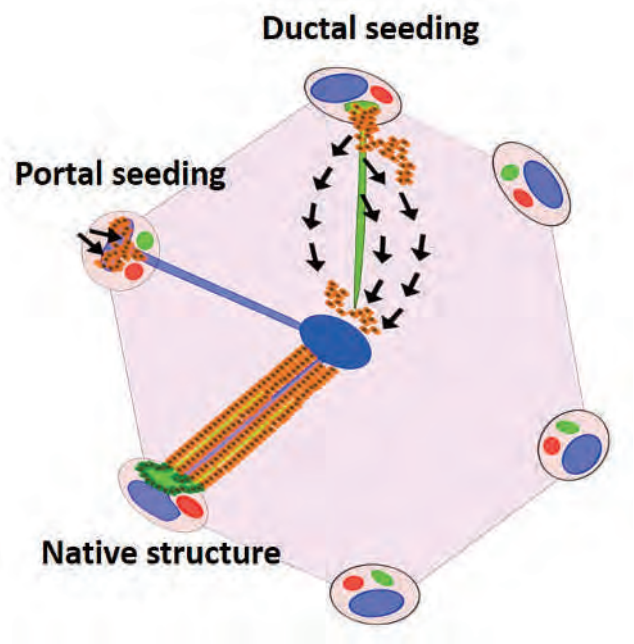
Biliary tree as a window for repopulation of the decellularized liver scaffold

Satoshi Ogiso, Kentaro Yasuchika, Ken Fukumitsu, Takamichi Ishii, Hidenobu Kojima, Yuya Miyauchi, Ryoya Yamaoka, Hokahiro Katayama, Takayuki Kawai, Elena Yukie Yoshitoshi, Sadahiko Kita, Katsutaro Yasuda, Shinji Uemoto.
Department of Surgery, Kyoto University, Kyoto, Japan.

Introduction: Construction of a transplantable liver has been desired, but the lack of adequate scaffolds has impeded the whole liver engineering. Recently, the decellularization technique, which removes cells from the extracellular matrix of a native organ, has been successfully adapted to generate a three-dimensional scaffold. Indeed the decellularized liver seems a suitable scaffold for the whole liver regeneration, retaining liver-specific ultrastructures, intact vascular networks, and various kinds of growth factors, the task of effective recellularization remains. For the complete whole liver regeneration, the parenchyma, vasculature, and support components must be reestablished prior to implantation. The present study investigated the efficacy of recellularization via the biliary tree to establish an efficient seeding method of the parenchyma.
Materials and Methods: Mice adult hepatocytes were seeded in a decellularized rat liver via the portal vein or via the biliary duct and cultured in a customized organ chamber with perfusion of the whole liver through the portal vein. Distribution and viability of repopulated cells in recellularized livers were compared between the two seeding routes. Liver-specific functions of repopulated cells were evaluated.
Results: Engraftment ratio was over 95 % in livers recellularized both via the portal vein and via the bile duct. The viability of repopulated cells was also over 95 % in both groups of recellularized livers at two days of perfusion culture. The majority of hepatocytes seeded via the portal vein remained within portal branches in macroscopic and microscopic assessment at two days after seeding and the distribution ratio to the parenchyma was 7.5 % in histological evaluation. In contrast, when seeded via the biliary duct, massive cell migration was observed out of the biliary tree and the distribution ratio to the parenchyma was 87.3 %. Furthermore, biliary ductal seeding deposited cells in the peri-portal area and also in the peri-central area.



Discussion: The anatomical relations would explain for the higher efficiency of biliary ductal seeding; the biliary canaliculi is directly in contact with the parenchyma as contrasted with the portal vein which is separated from the parenchyma by the pace of Disse. Cell migration from the peri-portal to the peri-central area by the biliary seeding suggest its capability of repopulating the whole parenchyma.



In addition, biliary seeding does not cause portal clogging, which would facilitate portal seeding of liver endothelial cells.
Conclusions: Seeding via the biliary duct is a promising approach allowing efficient parenchymal repopulation from periportal to peri-central area as well as facilitating re-endothelialization of the vasculature via the portal vein and vena cava.
References:
[1] Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix. Uygun BE, et al. *Nat Med* 2010
[2] The use of whole organ decellularization for the generation of a vascularized liver organoid. Baptista PM, et al. *Hepatology* 2011
[3] A whole-organ regenerative medicine approach for liver replacement. Soto-Gutierrez A, et al. *Tissue Eng Part C Methods* 2011

P.1067

Efficacious combination of substrate and medium for feeder-free culture of naïve porcine iPS

Shiori Waki[1], Hisashi Sahara[1], Kaoru Mitsui[2], Mitsuhiro Sekijima[1], Takahiro Murokawa[1], Takehiro Iwanaga[1], Yurika Ichinari[1], Kenichiro Kosai[2], Kazuhiko Yamada[1].
[1]Division of Organ Replacement and Xenotransplantation Surgery, Center for Advanced Biomedical Science and Swine Research, Kagoshima University, Kagoshima, Japan; [2]Department of Gene Therapy and Regenerative Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan.

Background: MHC-defined CLAWN miniature swine have great advantages for translational research because of their uniform immunological background. We have recently established porcine iPS cells using CLAWN miniature swine. Because traditional methods of porcine iPS cell culture require the use of mouse or human fibroblast feeder layers, feeder-free culture of porcine iPS cells is required to minimize several problems such as labor-intensive and difficulty to maintain in undifferentiated state, feeder-free method of porcine iPS cells culture, especially for naïve type iPS cells is required. In this study, we attempted to identify an efficacious combination of substrate and medium to establish feeder free culture method for naïve state of porcine iPS cells.
Methods: To evaluate the ability of feeder-free culture on proliferation, undifferntiation, and pluripotency of porcine iPS cells, we tested the combination of two substrates, Matrigel and Synthemax and five media. As a control group, iPS cells were maintained with pig iPS medium containing fetal bovine serum (BS) with feeder cells, MSTO. As feeder free group, iPS cells were maintained with either pig iPS medium containing fetal BS (BS group) or normal porcine serum (PS group) without feeder cells. Also, conditioned medium which was supernatant of the co-culture with MSTO and pig iPS medium (CM group), and human mTeSR1 as serum free culture (mTeSR group) was included. To maintain the naïve state of pig iPS cells, porcine LIF was added in all of these media.
Results: (1) Proliferative capacity: The increase ability of Matrigel-BS group, Matrigel-PS group, and Matrigel-CM group was approximately similar to or even better than that of Matrigel-control group. On the other hand, iPS cells increased very slowly or did not increase in Synthemax. (2) Undifferentiated ability: Undifferentiated ability of iPS indicated by positive alkaline phosphatase staining was extremely maintained in Matrigel-CM group and Synthemax-BS group compared to control group. (3) Pluripotency: Embryoid body was successfully induced in BS group or CM group regardless of the type of substrate. Embryoid body was not formed in PS group or mTeSR1 group. Moreover, all embryoid body expressed gene markers of all three germ layers confirmed by RT-PCR (Pax6, Nestin, ACTC1, GATA6). All these results taken together indicate that combination of Matrigel and CM medium is preferable for porcine iPS cell culture.
Conclusion: In terms of proliferative capacity, undifferentiated ability and maintenance of pluripotency, combination of Matrigel and CM medium is preferable for porcine iPS cell culture. Further studies will be required for the establishment of clinically-applicable xeno-free culture system.

P.1068

Immunodepletion and hypoxia preconditioning of mouse compact bone cells as a novel protocol to isolate highly immunosuppressive mesenchymal stem cells

Kisha Sivanathan[1,2,3], Darling Rojas-Canales[1,2], Stan Gronthos[4,5], Shane Grey[6], Patrick T.Coates[1,2,3,4,7].
[1]Centre for Clinical and Experimental Transplantation, Royal Adelaide Hospital, Adelaide, Australia; [2]School of Medicine, Faculty of Health Sciences, University of Adelaide, Adelaide, Australia; [3]Centre for Stem Cell Research and Robinson Institute, School of Medical Sciences, University of Adelaide, Adelaide, Australia; [4]Central Northern Adelaide Renal Transplantation Service (CNARTS), Royal Adelaide Hospital, Adelaide, Australia; [5]Mesenchymal Stem Cell Group Laboratory, School of Medicine, Faculty of Health Sciences, University of Adelaide, Adelaide, Australia; [6]Transplant Immunology Group, Garvin Institute of Medical Research, New South Wales, Australia; [7]South Australia Health and Medical Research Institute, Adelaide, Australia.

Mouse mesenchymal stem cells (mMSC) are traditionally isolated from the bone marrow (BM) and are a rare population amongst hematopoietic cell lineages and other non-MSC like cells that co-exist in the BM niche. The heterogeneity of BM mMSC cultures, therefore, represents a major obstacle for research aiming to examine *in vivo* function, optimize therapeutic strategies and address safety concerns of human BM MSC therapy. In this study, we aimed to investigate the use of mMSC harvested from the compact bones (CB) as they are recently known to be major reservoirs of mMSC. We performed collagenase type II digestion (3mg/ml, 1hr) of BM depleted CB to liberate cells attached to the bone surfaces. Immunodepletion was then performed based on the expression of CD45 (lymphocyte common antigen) and TER119 (erythroid lineages) to eliminate hematopoietic cells. The negative cell fraction was cultured under hypoxia (5% O2) conditions to establish primary mMSC cell lines. Fetal-bovine serum (FBS) for CB mMSC cultures, CB collagenase digestion protocol and mMSC establishment culture condition (hypoxia versus normoxia) were pre-optimized in our study. Higher yields of CB mMSC were obtained based on this isolation protocol with ability to differentiate into adipocytes and osteoblasts. These cells also expressed the standard mMSC associated markers (>90% Sca-1, CD29, CD44, CD90) with no contaminating cells when sub-cultured to passage 3 (<1% CD11b, CD31, CD34, CD45). Culture expanded CB mMSC were able to potently suppress total T cells and subset (CD4+ and CD8+) T cell proliferation in a DC allostimulatory 1-way mixed lymphocyte reaction (MLR), that mimic an allograft rejection response *in vivo*. Preconditioned with IL-17A (mMSC-17), mMSC-17 unlike human MSC-17 showed no superior suppression of T cells compared to UT-mMSC[1].
In conclusion, CB mMSC without IL-17A preconditioning may represent a new source to isolate mouse MSC with high purity and potent immunosuppressive properties that would be beneficial in a context of allotransplantation rejection.

References:

[1] Sivanathan KN, Rojas-Canales D, Hope CM, et al. Interleukin-17A induced human Mesenchymal Stem Cells are superior modulators of immunological function. Stem Cells. 2015.

P.1069

Graft maturation after repopulation of decellularized rat livers

Benjamin Struecker, Antje Butter, Khalid Aliyev, Karl H.Hillebrandt, Nathanael Raschzok, Anja Reutzel-Selke, Andreas Andreou, Johann Pratschke, Igor M.Sauer.
General, Visceral, and Transplantation Surgery, Charité - Universitaetsmedizin Berlin, Berlin, Germany.

Decellularization of livers of different species is a well-established procedure, but data on liver recellularization and implantation are limited to proof-of-concept studies. Information on the functional evolution and re-organization of grafts after repopulation and during ex vivo maturation is not available. We established a novel proprietary bioreactor to repopulate decellularized rat livers (n=21) with primary rat hepatocytes via the hepatic artery to evaluate graft morphology and function during seven days of ex vivo perfusion via the portal vein. Grafts were analyzed at 1 h, 6 h, 12 h, 24 h, 3d, 5 d and 7 d after recellularization (all n=3) by immuno-histologic evaluation, hepatocyte-related enzyme (AST, ALT, LDH) and albumin measurement in the perfusate, and hepatic metabolism gene (albumin, Cyp1A2) expression. We present the first available protocol for repopulation of rat livers via the hepatic artery. Within the first 24 hours after repopulation, the hepatocytes seemed to migrate out of the vascular network and form clusters in the parenchymal space around the vessels. Graft function increased for the first 24 hours after repopulation and then constantly decreased thereafter. However, the hepatocytes were viable even after seven days of perfusion. Our data suggests that the period of ex vivo maturation should be kept as short as possible and that repopulated grafts should be implanted as soon as cell engraftment and graft re-organization are completed."

P.1070

Serum levels of stem cell factor and granulocyte colony-stimulating factor during hepatic regeneration after partial hepatectomy in humans

Laura Tariciotti, Laura Fazzolari, Francesco Santopaolo, Tommaso Maria Manzia, Mario Angelico, Giuseppe Tisone, Leonardo Baiocchi.
Liver Unit, Fondazione Policlinico "Tor Vergata", Rome, Italy.

Background and Aims: Several cytokines contribute to the process of hepatic remodeling during hepatic regeneration and injury repair including Stem Cell Factor and Granulocyte Colony Stimulating Factor. A recent study demonstrated that in rodent the expression of these two molecules is significantly increased after partial hepatectomy. In this study we seek to evaluate, in patients undergoing partial liver hepatectomy, the fluctuations of serum levels of Stem Cell Factor and Granulocyte Colony Stimulating Factor, before and after surgery, as a function of clinical patient features.
Methods: 17 patients (9 male, age 63±22) undergoing liver mass surgical resection were included in the study. Serum samples at baseline, day 1, day 7 and 1 month after surgery were tested for Stem Cell Factor and Granulocyte Colony Stimulating Factor, using commercially available kits. Clinical and biochemical patient variables were recorded.
Results: 11 patients underwent resection for primary malignancy of the liver (hepatocarcinoma) while remaining underwent liver metastasis (4) or giant hemangioma (2) removal. Six patients had liver cirrhosis. While Stem Cell Factor exhibited a non-statistic decrease at day 1 after surgery, it subsequently increased progressively, being significantly over-expressed at 1 month (baseline vs. 1 month: 3.2±3.5 vs. 5.9±3.2 ng/mL; p=0.02). However, Granulocyte Colony-Stimulating Factor serum levels remained unchanged at a constant value of 10 pg/mL. Comparison between patients according to type of tumor showed no differences between these two factors. Conversely when data were analyzed according to liver cirrhosis, a significant increase of Stem Cell Factor from baseline levels was confirmed at 1 month in cirrhotic patients only (baseline versus 1 month: 3.4±0.58 vs. 6.4±2.4 ng/mL; p=0.01). Regarding outcome, a cut-off value <3.5 ng/mL at baseline for Stem Cell Factor (ROC curve analysis) discriminated patients requiring Intensive Care Unit after surgery (sensitivity 83%, specificity 72%, AUC = 0.85).
Conclusions: Serum levels of Granulocyte Colony-Stimulating Factor do not exhibit changes after partial hepatectomy in humans; conversely, Stem Cell Factor is significantly increased in cirrhotic patients only (at 1 month). Value of this molecule below 3.5 ng/mL at baseline, may predict the necessity for Intensive Care Unit stay after liver resection.

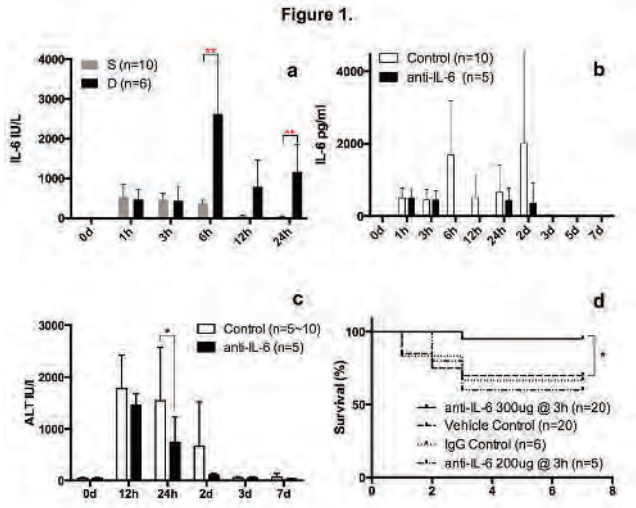
P.1071

IL-6 blockade attenuates posthepatectomy liver injury, accelerates liver regeneration and improves survival

Yinzhe Xu[1,2], Nalu Navarro-Alvarez[1], Jiahong Dong[2], James F.Markmann[1], Heidi Yeh[1].
[1]Transplantation Division, Department of Surgery, Massachusetts General Hospital, Boston, United States; [2]Hepatobiliary Surgery, Chinese PLA General Hospital, Beijing, People's Republic of China.

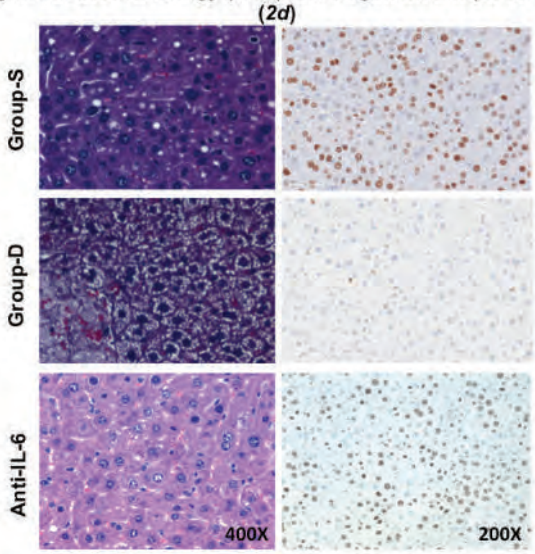
Background: Small-for-size syndrome limits the minimum liver mass required for successful segmental liver transplantation. Maximizing the ability of small segments to regenerate could potentially expand both the living and deceased liver donor pool. We investigated the role of inflammatory cytokines in promoting and impairing liver regeneration in a murine hepatectomy model.
Method: C57BL/6 mice underwent 70% hepatectomy and received supportive treatment only (vehicle control), 200ug anti-IL-6 blocking antibody at 3 hours post-op, 300ug anti-IL-6 Ab at 3h, or 300ug isotype control at 3h (IgG control). Serum IL-6 and ALT levels were measured at baseline and at serial time-points post-op. Liver biopsies were also taken for H&E and Ki-67 staining. Survival was determined both by following animals out to 7 days unless found dead or determined to be moribund and euthanized prior to the end of the study period, and by using a validated body condition scoring system that predicts long term survival with 97.3% accuracy.
Results: Serum IL6 levels increased in all mice up to 3h post-op. However, in mice that died, IL6 levels rose to 7-fold higher levels at 6h and remained elevated until death, while in mice that survived, IL6 levels dropped at 6h and returned to baseline by 12h (2251±581 vs. 371±129 pg/ml, P=0.003, fig. 1a).

Figure 1.



These changes preceded serum ALT differences, which did not become significantly higher in dying animals until 24h post-op. Unsurprisingly, histology of livers from dying animals showed steatosis, vacuoles, necrotic patches, and low levels of Ki-67 compared to those from animals that survived. 300ug anti-IL6 blocking antibody, but not 200ug, resulted in decreased IL-6 (fig. 1b) and ALT levels (fig. 1c), improved survival (fig. 1d), increased hepatocyte replication (Ki-67+ cells, 67.9% vs 27.1% on 2d, p=0.03) and reversed the histologic changes (fig. 2).

Figure 2. Liver Histology (H&E) and Regeneration (Ki-67 IHC) (2*d*)



Conclusion: Although IL-6 is crucial for initiating hepatocyte replication, prolonged elevation of IL-6 is associated with continued liver injury, poor regeneration, and death from liver failure. Delayed blockade of IL-6 activity decreased liver injury, and increased hepatocyte replication and survival and may be useful in promoting liver regeneration after small segment liver transplantation.

P.1072

The property of modulatory T cells generated by mixed lymphocyte culture with CD154 blockade

Kiyohiko Hotta, Tetsu Oura, Benedict Cosimi, Tatsuo Kawai.
Center for Transplantation Science, Massachusetts General Hospital, Boston, United States.

Background: Regulatory T cell (Treg) immunotherapy has been applied to minimize the immunosuppressive drugs in clinical transplantation. To avoid contamination of CD4+CD25- T cells, only highly purified Tregs have been infused to the patients. In this study, we investigated immunological properties of CD4+CD25- T cells after mixed lymphocyte culture (MLC) with a costimulatory blockade.
Method: Isolated CD4+ T cells (Responder A) from human blood buffy coat were cultured with irradiated allogeneic PBMCs (Stimulator B) for 14 days in the presence or absence of anti-human CD154 mAb. These primed CD4+ T cells were then added as a modulator (Modulator A) to a newly prepared (second) MLC with Responder A and irradiated original stimulator (Simulator B) or third party stimulator (Stimulator C) for 5 days.
Results: There was no significant difference in the percentage of Tregs induced after MLC with or without aCD154mAb. However, Modulator A generated after MLC with aCD154mAb significantly more suppressed the second MLC than Modulator A generated without aCD154mAb. These modulators generated with aCD154mAb also displayed antigen-specific suppression as Modulator A generated by Stimulator B with anti-CD154 mAb in the first MLC significantly more suppressed the second MLC with Stimulator B than with Stimulator C (Fig. 1). To identify which T cell subset possesses suppressive function, CD4+CD25highFoxP3+ cells (Treg) and CD4+CD25-FoxP3- cells (non-Treg) were sorted after the first MLC. Treg isolated after the first MLC displayed significant suppressive function of the second MLC. Interestingly, although suppressive function was not prominent as Treg, non-Treg isolated after the first MLC also displayed suppressive function (Fig. 2).
Conclusion: These data indicate that suppressive function can be expected even with antigen-primed non-Treg. In vivo studies to evaluate a suppressive function of these non-Tregs are currently underway.

Figure 1. MLC with alloantigen primed CD4 + T cells

First MLC (Modulator)			Second MLC	
Res	Stim	aCD154	Res	Stim
A	B	+	A	B
A	B	-	A	B
A	B	+	A	C
A	B	-	A	C

Res: Responder, Stim: Stimulator

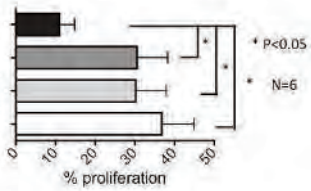
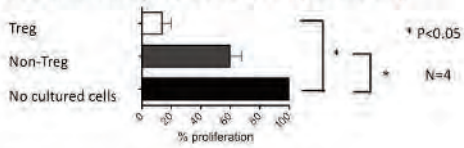


Figure 2. MLC with alloantigen primed Non-Treg cells or Treg



P.1073

Targeting histone deacetylase in renal tubular epithelial cells inhibits amplification of Th1 cell-mediated inflammation

Sun-Woo Kang[1], Yeong-Hoon Kim Kim[1], Su-Kil Seo[2].
[1]Nephrology, Inje University, Busan, Korea; [2]Microbiology and Immunology, Inje University, Busan, Korea.

Objective: More studies are focusing on renal tubular epithelial cells (RTECs) as a new target to restore inflammatory environment as clarifying their immune regulatory function. Here, we investigated whether histone deacetylases (HDACs) are activated in RTECs during T cell-mediated inflammation and their blockade is able to reduce the inflammatory responses.
Methods: Human renal proximal tubular epithelial cell line HK-2 was cultured in the presence or absence of recombinant IFN-g (200 U/ml) plus TNF-a (5 ng/ml). The HDAC activity was determined on the expression levels of acetylated H3 and a-tubulin by immune blot assay. To determine the functional activity of HDAC inhibitor SB939, we analyzed the immune stimulatory phenotype of HK-2 cells such as class II MHC molecule, CD80, CD86, and CD40 by flow cytometry. In addition, the culture supernatants were used for measuring cytokines and chemokines by ELISA assay.
Results: We found that HDAC activity was markedly increased in HK-2 cells by treatment of IFN-g/TNF-a within 12 h. Treatment of pan-HDAC inhibitor SB939 in HK-2 cells completely prevented HDAC activity increased by IFN-g treatment. SB939 treatment predominantly inhibited up-regulating CD40 expression but not MHC class II, CD80, and CD86. In addition, MCP-1 was significantly inhibited more than IL-6 and TNF-a by SB939 treatment.
Conclusion: Our results demonstrate that 1) HDAC activity is increased in RTECs in response to IFN-g, 2) which further facilitates T cell-mediated inflammatory responses through CD40 and MCP-1. Therefore, our study suggests that HDAC inhibitor has a therapeutic potential for the treatment of acute renal inflammatory diseases such as allograft rejection in transplantation.

P.1074

Expression of endoplasmic reticulum-mediated stress proteins in FK506-treated T lymphocytes

Ho kyun Lee, Soo Jin Na Choi, Sang Young Chung.
Surgery, Chonnam National University Medical School, Gwangju, Korea.

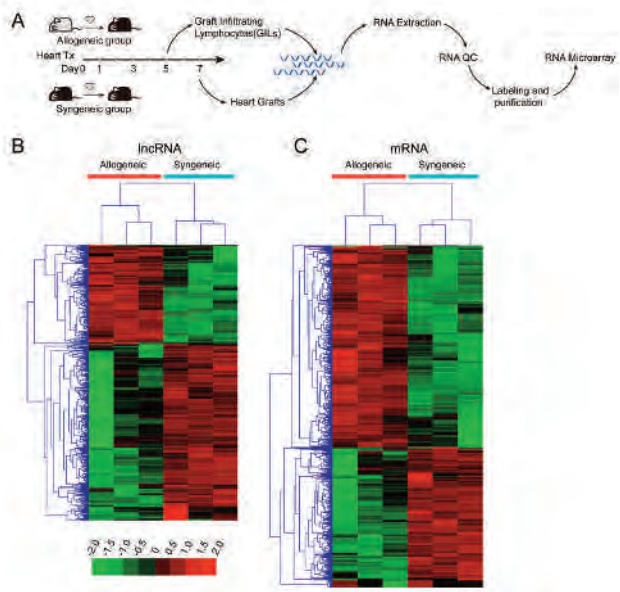
FK506 induced apoptotic stress protein expression was investigated in Jurkat human T lymphocytes. The effect of FK506 on apoptosis and cell viability were examined by western blotting of double-stranded RNA-dependent protein kinase (PKR)-like ER kinase (PERK), phosphor-PERK, CHOP, Grp78/BiP, Grp94, inducible nitric oxide synthase (iNOS), Bcl-2, and Bak. A flow cytometry analysis was performed after DAF-DA or DCF-DA staining. FK506-induced apoptosis was confirmed by nuclear fragmentation after DAPI staining. Grp78/BiP expression was increased 6 hours after FK506 treatment, and Grp94 expression increased after 36 hours. Increased phospho-PERK expression was observed 6 hours after FK506 treatment and peak activation of phospho-PERK was observed at 36 hours. CHOP/GADD153 expression was increased 48 hours after FK506 treatment. Expression of iNOS following FK506 treatment began to increase at 12 hours, peaked at 24 hours, and decreased after 36 hours. FK506 increased nitric oxide production through iNOS induction. Generation of reactive oxygen species (ROS) in FK506-treated Jurkat cells began increasing at 24 hours. Bak protein expression increased after 12 hours in a time dependent manner. From these results, we confirmed that FK506 induces apoptosis and acts dose-dependently to decrease the viability of Jurkat cells through activation of apoptosis signaling and expression of apoptotic stress proteins.

P.1075

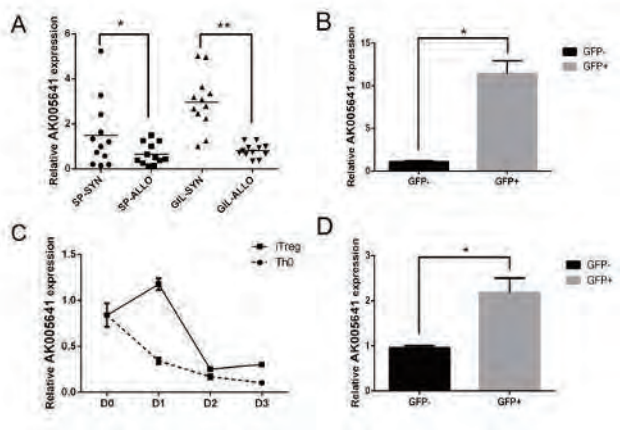
Expression of lncRNA-AK005641 in murine heart allograft acute rejection model and Treg differentiation

Cheukfai Li, Zhiyong Guo, Dongping Wang, Guangxiang Gu, Xiaoshun He.
Organ Transplant Center, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, People's Republic of China.

Long noncoding RNAs(lncRNAs) have been proved to mediate T cell activation and differentiation. Less is known about their function in allograft acute rejection. As shown in Figure 1, We first set up the heterotopic heart transplantation model, divided into allogeneic group (BALB/c→C57BL/6) and syngeneic group (C57BL/6→C57BL/6). Graft infiltrating lymphocytes and heart grafts were harvested at day 5 and day 7 post transplantation, respectively. LncRNAs and mRNA expression were measured through microarray analysis. (Fig1A) The strong differential expressed lncRNAs and mRNAs (fold change >= 2) in graft infiltrating lymphocytes from microarray were hierarchical clustered and shown in heat map (Fig1B).



Among them, lncRNA-AK005641 was selected for further expression validation according to bioinformatics analysis. The expression of lncRNA-AK005641 was significantly downregulated in both heart grafts and graft infiltrating lymphocytes, which was consistent with the microarray data. (Fig2A) Next we desired to know the relevance of lncRNA-AK005641 in various lymphocyte subsets. Firstly, $CD4^+Foxp3^+$ T cells, regarded as nTreg, were sorted from Foxp3/GFP transgenic mice splenocytes. The expression of lncRNA-AK005641 was significantly higher in nTreg comparing with $CD4^+Foxp3^-$ T cells.(Fig1B) Subsequently, Th0 and iTreg polarization condition culture were performed. In the period from naïve T cell differentiated into iTreg, the expression of lncRNA-AK005641 was upregulated when compared with Th0 culture in each time point. (Fig2C) On day 3, the $CD4^+$ $Foxp3^+$ T cells and $CD4^+$ $Foxp3^-$ were sorted from iTreg culture. The expression of lncRNA-AK005641 was significantly upregulated in iTreg. (Fig2D).



Taken together, the result indicated that lncRNA-AK005641 had a high expression in both nTreg and iTreg. Our results obtained dramatically elevated expression of lncRNA-AK005641 in nTregs and in the period of iTreg differentiation in vitro, suggesting that lncRNA-AK005641 may regulate part of T cell differentiation transcriptional program. Our work indicated a novel lncRNA correlated with acute rejection and Treg polarization. We expect that the further characterization of the lncRNA-AK005641 identified in our data could uncover the functional mechanism in immune response during acute rejection.

P.1076

Anti-CD272 antibody (6B2) induced prolongation of fully MHC-mismatched murine cardiac allograft and generation of Foxp3+ regulatory T cells

Masateru Uchiyama[1], Enzhi Yin[1], Xiangyuan Jin, Takako Yanangisawa[1], Satoko Mizuno[2], Hideo Yagita[3], Masanoti Niimi[1].
[1]Surgery, Teikyo University, Tokyo, Japan; [2]Neurology, Saitama International Medical Center, Saitama Medical College, Saitama, Cambodia; [3]Immunology, Juntendo University Hospital, Tokyo, Japan.

Background: The co-inhibitory receptor B and T lymphocyte attenuator (BTLA; CD272) has been implicated in the regulation of autoimmune and may potentially play an important role in alloimmune responses. We investigated the effect of anti-BTLA monoclonal antibody (6B2) in the survival of fully MHC-mismatched murine cardiac allograft transplantation.
Methods: CBA mice (H2k) underwent transplantation of C57BL/6 (B6, H2b) hearts and received a single dose of anti-BTLA monoclonal antibody (6B2) by intraperitoneal injection on the day of transplantation and for 3rd, 6th and 9th day thereafter. Adoptive transfer study, flow cytometry study and immunohistochemical (IHC) study were performed to determine whether Foxp3+ regulatory T cells were generated. Histologic, cell proliferation and cytokine assessments were performed.
Result: Untreated CBA mice rejected B6 cardiac grafts acutely (median survival time [MST], 7 days). When CBA mice were treated with 6B2 on the day of transplantation and for 3rd, 6th and 9th day thereafter, the allograft survival was significantly prolonged to MST, >100 days and had more Foxp3+ cell in IHC study. Secondary CBA recipients given whole splenocytes from primary 6B2-treated CBA recipients with B6 cardiac allografts 30 days after grafting had prolonged B6 allograft survival (MST, >30 days). Cell proliferation of splenocytes of 6B2-treated mice was suppressed compared with that of untreated recipients. Flow cytometry studies showed an increased CD4+CD25+Foxp3+ cell population in splenocytes from 6B2-treated mice. HE staining showed that cardiac allografts from primary 6B2-treated CBA recipients had sparse cell infiltration and only slight myocardial damage.
Conclusion: Anti-CD272 monoclonal antibody (6B2) could induce hyporesponsiveness of fully MHC-mismatched cardiac allografts and generation of CD4+CD25+Foxp3+ regulatory T cells.

P.1077

Screening of medical supplements to prevent and ameliorate non-alcoholic steatohepatitis (NASH)

Li-Wen Hsu[1,3], Shigeru Goto[1], Toshiaki Nakano[1,2], Chia-Yun Lai[1], Yu-Fan Cheng[1], Chao-Long Chen[1].
[1]Liver Transplantation Program, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung City, Taiwan; [2]Graduate Institute of Clinical Medical Sciences, Chang Gung University College of Medicine, Kaohsiung City, Taiwan; [3]Department of Medical Research, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung City, Taiwan.

Introduction: Non-alcoholic steatohepatitis (NASH) with a high risk of subsequent liver cirrhosis and hepatocellular carcinoma is the most serious form of non-alcoholic liver disease (NAFLD). In living donor liver transplantation, NASH has become a great concern for both donors and recipients. For the past three years, we have extensively screened antioxidant derivatives, which also improve lipid metabolism. Finally, we have demonstrated that vitamin D_3 and a novel antioxidant alpha-lipoic acid derivative (DHL-HisZn) have the beneficial effect on NASH. In this study, we explored the therapeutic potential of vitamin D_3 and DHL-HisZn in *in vivo*/*in vitro* NASH models.
Materials and Methods: After a 1-week acclimation period on a standard diet, LEW rats were switched to a choline-deficient and iron-supplemented l-amino acid-defined diet for 6 weeks to develop NASH livers. For in vitro NASH model, human hepatoma cell line, HepG2 cells were cultured with oleic acid-BSA complex and the lipid droplets were labeled with BODIPY 493/503 lipid probe.
Results: Both vitamin D_3 and DHL-HisZn inhibited inflammatory cytokines such as IL-6, decreased inflammatory large/hypertrophic adipocytes, and inhibited activation of hepatic stellate cells, which play a crucial role in the fibrotic response. Our preliminary study revealed that vitamin D3 treatment increased the distribution of mitochondria-relevant UCP-1-positive brown adipocytes in *in vitro*/*in vivo* NASH models. Although vitamin D_3 is currently set for clinical trials (phase II) in Europe, we confirmed that vitamin D_3 alone is not sufficient to ameliorate liver steatosis. On the other hand, DHL-HisZn attenuates fatty acid uptake and lipid accumulation through the downregulation of CD36 expression, which involves the fatty acid uptake in HepG2 cells.
Discussion: We realized that novel anti-oxidant alpha-lipoic acid derivative (DHL-HisZn) in addition to active form vitamin D_3 is necessary to consistently prevent NASH and liver fibrosis.
Conclusion: The optimal combination of DHL-HisZn and vitamin D_3 may lead to prevent and ameliorate NASH and liver fibrosis for future clinical applications in transplant donors and recipients.
Ministry of Science and Technology (MOST 104 - 2314 - B - 182A - 018)

P.1078

Pre-clinical insight into the use of dendritic cells as cellular therapies in inducing tolerance to liver allografts

Muhammad Atif.
Vascular Surgery, University of Birmingham, Birmingham, United Kingdom.

The search for novel solutions to inducing allograft tolerance is now firmly focussed on cellular therapies. In this review, we consider the evidence base over the past 5 years supporting the use of dendritic cells (DCs) in inducing tolerance to liver allografts. We highlight pre-clinical data ranging from in vitro, ex vivo to in vivo in small animal models.
DCs are antigen presenting cells (APCs) with the ability to recognise pathogen-associated molecular patterns (PAMPs) and promote a cellular/humoral response or a regulatory response. In vitro methods at modulating DCs have involved culturing with diltiazem to polyclonal T-cells. There is data to support that host regulatory DCs are the main regulators of the immune response in vivo as any infused donor regulatory DCs are processed in secondary lymphoid organs. As for biomarkers, there has also been an association in the paediatric liver transplant population that patients with operational tolerance have higher levels of pre-plasmacytoid DCs and high B7-H1:CD86 ratio.
The data indicates that hepatic DCs can be influenced via multiple modalities (genetic, cellular and cytokine) with view to inducing a state of tolerance. There is scope for work in large animal models to provide the basis for a Phase 1 study.

References:

[1] Will be listed on poster

P.1079

High peripheral transitional B cells and high expression of B cell associated genes in peripheral blood at 3 months after kidney transplantation: Identification of low risk phenotype

Petra Hruba[1], Veronika Svachova[2], Zdenek Krejcik[3], Viktor Stranecky[4], Alena Sekerkova[2], Ilja Striz[2], Eva Honsova[5], Ondrej Viklicky[1,6].
[1]Transplant Laboratory, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [2]Department of Clinical and Transplant Immunology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [3]Department of Genetics, Institute of Hematology and Blood Transfusion, Prague, Czech Republic; [4]Institute of Inherited Metabolic Disorders, Prague, Czech Republic; [5]Department of Clinical and Transplant Pathology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [6]Department of Nephrology and Transplant Center, Institute for Clinical and Experimental Medicine, Prague, Czech Republic.

Background: Higher numbers of transitional B cells together with B cell signature are known to be present in operationally tolerant patients. The aim of the study was to compare transcriptome of kidney recipients with low and high absolute numbers of transitional B cells at 3 months.
Methods: Whole blood transcriptome of patients with low (n=15) and high (n=17) absolute numbers of transitional B cells at 3 months was compared using Illumina Human HT-12 v4 Expression BeadChips. The cut-off for absolute number of transitional B cells was defined by ROC analysis for the risk of rejection in our previous study. Differentially expressed genes were defined as those with fold change ≥ 2 and $p<0.05$. The enrichment of deregulated genes in biological processes was analyzed using DAVID database.
Results: The annotation enrichment analysis for genes up-regulated in patients with high transitional B cells at 3 months revealed among the most significant GO terms: intrinsic to plasma membrane (p=8.1E-03), integral to plasma membrane (p=9.9E-03) and plasma membrane part (p=1.2E-02). As patients at 3 months differed also in the number of plasmablasts, subgroups divided according to absolute numbers of transitional B cells and plasmablasts were analyzed separately. Among the most significant upregulated signaling pathways in patients with both high transitional B cells and high plasmablasts compared to patients with low transitional B cells and high plasmablasts were found: B cell receptor complex (p=1.1E-02) and B-cell receptor signaling pathway (p=1.4E-02). Up-regulated genes were previously described as operationally tolerant (CD79A, CD79B, IGLL1, PNOC, FCRLA). When groups of patient with high transitional B cells and high or low plasmablasts were compared, patients with high plasmablasts had higher expression of defensins. This group had also the highest expression of IGLL1 and FCRLA tolerance transcripts.
Conclusions: Microarray analysis confirmed higher expression of B cell associated tolerance genes in kidney recipients under standard immunosuppression with high transitional B cells at 3 months.
Supported by IGA N14102/2013 and MZO 00023001

P.1080

Heart en bloc thymus transplantation permits long-term, acute rejection-free cardiac allograft survival in nonhuman primates (NHPs)

Makoto Tonsho, Philip J Spencer, Ruchao Yu, Kyu-Ha Huh, Aseda Tena, Timothy Millington, Ashok Muniappan, Rex N Smith, Robert B Colvin, James S Allan, Joren C Madsen.
Center for Transplantation Sciences, Massachusetts General Hospital, Boston, MA, United States.

Background: The role of vascularized thymus on tolerance induction has been well studied in swine recipients of kidney allografts, but not in NHP recipients of heart allografts. Here, we investigate whether co-transplantation of vascularized donor thymus would prolong the survival of allogeneic cardiac allografts without chronic immunosuppression.
Methods: MHC-mismatched heart en bloc thymus allografts were transplanted into thymectomized cynomolgus macaques treated with equine ATG (days -2, -1, 0), anti-IL-6R mAb (days 0, 7), and conventional immunosuppression, consisting of tacrolimus, mycophenolate mofetil, and methylprednisolone. Starting on POD 166, the immunosuppressive drugs were slowly weaned; and by POD 207, all drugs were stopped. Thymopoiesis was monitored with T cell receptor excision circles (TREC), as well as phenotypic markers of peripheral recent thymic emigrants. The alloreactive T cell and B cell responses were serially assessed, and the grafts were examined by serial biopsies.
Results: The first animal treated this way has achieved acute rejection-free cardiac survival with preserved cardiac function for over 537 days. Thymopoiesis by the donor thymus was detected early after transplantation and donor-specific T cell hyporesponsiveness was observed in γ-IFN ELISPOT. The recipient developed alloantibody and allograft vasculopathy by day 300, but never demonstrated evidence of acute cellular rejection. Two other recipients are currently >97 and >57 days posttransplant without signs of acute rejection.
Conclusion: This is the first demonstration of long-term cardiac allograft survival induced by the co-transplantation of vascularized donor thymus in nonhuman primates. The apparent split tolerance achieved by this strategy may be overcome by adding a B cell depleting agent to the protocol.

P.1081

NKT cells in the liver are important for peripheral tolerance induction in mice

Zhanpeng Wang[1], Hongyu Liu[1], Zhuonan Li[1], Jorge D.Reyes[2], James D.Perkins[2], Wei Li[1].
[1]Hepatobiliary-Pancreatic Surgery, China-Japan Union Hospital of Jilin University, Changchun, People's Republic of China; [2]Surgery, Division of Transplantation, University of Washington Medical Center, Seattle, United States.

Introduction: NKT cells are mainly located in the liver in mice. The role of liver inherent NKT cells are still unclear.
Materials and Method: In this study, we employed mouse orthotopic liver transplantation and heterotopic heart transplantation models to critical examine the role of liver NKT cells in liver and peripheral tolerance induction.
Results: NKT cells were increased in the tolerated liver grafts which expressed higher levels of CD95L and PD-L1, while the CD4+CD25+Foxp3+ regulatory T cells (Treg) were markedly increased in the both liver and spleen at day 7 post transplantation. The heart allograft survival were prolonged significantly in the recipient which accepted donor spleen cells by portal vein (p.v.) injection in contract to the recipient which received the donor spleen cells by tail vein (i.v.) injection. In vitro immunological assay revealed that the number of NKT cells in the liver was increased and Treg were increased in both liver and spleen from the p.v. treated mice significantly. The IL-2 expression was decreased, IL-4 and IL-10 were increased. Further, significantly increased IL-4, IL-10, and IFN-γ production was detected from liver NPCs under y "α-GalCer"/"alpha-GalCer".
Conclusion: NKT cells are important to liver transplant tolerance induction appear to play a key role in down regulation of peripheral immune responses and facilitate CD4+CD25+Foxp3+ Treg induction.

P.1082

In vitro immune cell monitoring as a guide for long-term immunosuppression in adult liver transplant recipients

Eunkyoung Jwa, Shin Hwang, Sung-Gyu Lee, Tae-Yong Ha, Chul-Soo Ahn, Gil-Chun Park, Jee-Yeon Kim, Min-Kyu Sung.
Division of Hepatobiliary Surgery and Liver Transplantation, Asan Medical Center, SeouL, Korea.

Background: We evaluated the clinical usability of immune cell monitoring in adult liver transplantation (LT) recipients.
Methods: This study was composed of two parts as using calcineurin phosphatase (CNP) activity assay and ImmuKnow assay independently as in vitro monitoring tools of immune cell function in adult LT recipients.
Results: There was a rough correlation between CNP activity and tacrolimus concentration in 33 patients. This association was evident in patients who were only administered tacrolimus, but disappeared after the co-administration of mycophenolate. In 118 healthy individuals, the mean proportion of helper T-cells was 37.4 8.1%. According to ImmuKnow assay, their immune responses were strong in 12 patients (10.2%), moderate in 92 patients (78.0%), and low in 14 patients (11.9%). In 85 patients waiting for LT, there was a rough correlation between the ImmuKnow ATP level and age. Their immune responses were strong in 0 patients (0%), moderate in 8 patients (9.4%), and low in 77 patients (90.6%). There was a difference in the ImmuKnow ATP levels between healthy individuals and patients with liver disease. In 137 LT recipients, there was no correlation between the ImmuKnow ATP levels and tacrolimus concentration. This trend did not change after grouping the patients according to co-administration with mycophenolate. Eight recipients experienced acute rejection, but none showed strong immune response.
Conclusions: We think that both CNP activity assay and ImmuKnow assay are too limited to objectively determine the level of immunosuppression. Further studies should be performed to identify other methods for immune function monitoring.

P.1083

Survival outcomes of combined hepatocellular carcinoma-cholangiocarcinoma following liver resection and liver transplantation

Eunkyoung Jwa, Shin Hwang, Chul-Soo Ahn, Tae-Yong Ha, Gil-Chun Park, Min-Kyu Sung, Jee-Yeon Kim, Sung-Gyu Lee.
Division of Hepatobiliary Surgery and Liver Transplantation, Asan Medical Center, SeouL, Korea.

Background: Combined hepatocellular carcinoma and cholangiocarcinoma (cHCC-CC) is rare. This study investigated the clinicopathological features of cHCC-CC following liver resection (LR) and liver transplantation (LT).
Methods: Between January 2000 and September 2012, 53 patients with cHCC-CC underwent LR, accounting for 1.1% of LR for primary liver malignancies. At the same period, we identified 18 LT recipients showing cHCC-CC at the explant livers.
Results: In LR group, mean tumor diameter of cHCC-CC group was 5.52.9 cm and single tumor was identified in 50. Pathological classification included combined (n=41), mixed (n=11), and double (n=1) tumors. The 1, 3 and 5-year tumor recurrence rates were 60.8%, 71.8% and 80.7%, respectively. The 1, 3 and 5-year overall survival rates were 73.3%, 35.6% and 30.5%, respectively. Median survival after recurrence was 6 months. Tumor recurrence and patient survival did not differ significantly according to AJCC tumor staging and histological type (all p0.2). In LT group, Milan criteria were met in 13 patients. A single tumor was identified in 10 and multiple lesions in 8 patients. The maximal tumor diameter was 2.9 1.7 cm. Seven patients experienced tumor recurrence, in which 6 recurred within the first 12 months. The 1, 3 and 5-year tumor recurrence rates were 40.0%, 46.7% and 46.7%, respectively. All of the patients who experienced recurrences died at a median 4 month-survival after that diagnosis. The 1, 3 and 5-year overall survival rates were 66.7%, 60.0% and 60.0%, respectively.
Conclusions: Patients with cHCC-CC showed poorer long-term survival outcome after LR due primarily due to more advanced tumors. In contrast, post-recurrence survival was much poorer after LT. Further evaluation of differences in tumor characteristics and tumor biology is necessary to accurately predict the prognosis of patients with cHCC-CC.

P.1084

High concentrations of CXCL9 and CXCL10 chemokines but not CXCL8 (IL-8), IL-6, TNF or IFN- g in biopsy tissue are associated with pathological staging of rejection after kidney transplantation

Christine Falk[1,6], Christine Neudörfl[1], Kerstin Daemen[1], Jana Keil[1], Frank Lehner[5], Hermann Haller[2], Jessica Schmitz[3], Cornelia Blume[4], Jan-Hinrich Bräsen[2].
[1]Institute of Transplant Immunology, IFB-Tx, Hannover Medical School, Hannover, Germany; [2]Department of Nephrology and Hypertension, Hannover Medical School, Hannover, Germany; [3]Department of Pathology, Hannover Medical School, Hannover, Germany; [4]Institute of Technical Chemistry, Hannover Medical School, Hannover, Germany; [5]Department of Visceral and Transplantation Surgery, Hannover Medical School, Hannover, Germany; [6]DZIF, German Center for Infectious Diseases, Hannover Medical School, Hannover, Germany.

Background: Extensive expression profiling efforts of biopsy tissue after kidney transplantation indicate that rejection, especially antibody-mediated rejection (AMR) can be defined by distinct signatures differing from of tissue injury and other renal dysfunctional stages. Based on the pathological evaluation as T cell-mediated (TCMR), AMR or borderline rejection, we hypothesized that the presence of immune cells within the graft would be associated with a distinct cytokine milieu. Therefore, we determined the protein microenvironment in biopsies in kidney capsule vs. cortical and medullary regions and associated the concentrations of certain pro-inflammatory cytokines with pathological staging.
Methods: Based on our protocol biopsy program, 37 snap-frozen secondary kidney biopsies were obtained from transplant recipients ranging from 2 months to 20 years after Tx with ethical approval and informed consent. Protein lysates of capsule, cortical and medullary biopsy tissues were analyzed for 50 cytokines and chemokines using multiplex protein arrays. Histopathological evaluation of all primary biopsies was performed according to BANFF-2013 criteria (14 unsuspicious, 4 TCMR, 5 borderline, 14 AMR).
Results: The protein microenvironment differed significantly between kidney caspular, cortical and medullary regions even in unsuspicious biopsies, for example SCGF ($p<0.02$). In contrast, some proinflammatory cytokines like IL-6, IL-8 (CXCL8), IFN-g, TNF-a were hardly detectable and did not differ between regions of unsuspicious and rejection biopsies (AMR 14, TCMR 5, BL 5). However, significantly higher concentrations of chemokines like CXCL9 and CXCL10 were detected in cortical and medullary regions of biopsies in the rejection group ($p<0.01$).
Conclusion: The protein microenvironment of kidney biopsies histologically classified as rejection differs significantly from unsuspicious renal tissue with respect to the chemokine but not the cytokine milieu. Thus, certain chemokines like CXCL9 and 10 confirm their qualification as biomarker candidates also at the protein level while typical T cell-derived cytokines seem to perform rather poorly as biomarkers.
IFB-Tx, German Ministry for Education and Research, BMBF 01EO1302; SFB738, B3, DFG

P.1085

NK cells of kidney transplanted patients display an activated phenotype that is influenced by immunosuppression and pathological staging

Christine Falk[1,5], Ulrike Horrmann[1], Christine Neudörfl[1], Kerstin Daemen[1], Jana Keil[1], Frank Lehner[4], Hermann Haller[3], Cornelia Blume[2].
[1]Institute of Transplant Immunology, IFB-Tx, Hannover Medical School, Hannover, Germany; [2]Institute of Technical Chemistry, Leibniz University, Hannover, Germany; [3]Department of Nephrology and Hypertension, Hannover Medical School, Hannover, Germany; [4]Department of Visceral and Transplantation Surgery, Hannover Medical School, Hannover, Germany; [5]DZIF, German Center for Infectious Diseases, Hannover Medical School, Hannover, Germany.

The impact of NK cells after kidney transplantation (KTx) is discussed controversially, they may play a role in rejection and in tolerance induction. We investigated phenotype and function of peripheral NK cells of KTx patients compared to healthy donors. NK markers (CD16, CD226), activation markers (HLA-DR, CD25) and inhibitory receptors (CD94/NKG2A) were analyzed by flow cytometry. IFN-g production following PMA/Ionomycin (P/I) stimulation in the presence or absence of immunosuppressive drugs was detected by ICS or ELISpot, supernatants were tested for other cytokines by multiplex analyses.
NK cells of KTx patients have significantly reduced CD16 and CD226 surface expression but increased levels of HLA-DR and CD25, indicating an activated phenotype. Upon P/I stimulation, IFN-g production was associated with CD16 down modulation, abrogated by CNI. IFN-g as well as production of other cytokines like TNF-a, IL-10 and IL-31 was decreased by CNI. IFN-g production of stimulated cells in KTx patients was not impaired while other cytokines like IL-13 and IL-31 were produced at significantly lower levels. Cytotoxins were not affected by immunosuppression.
Thus, NK cells of KTx patients are impaired by immunosuppression, especially the NFAT-dependent cytokine production despite IFN-g. Taken together, NK cells may serve as sensor for immunosuppression.
German Ministry for Education and Research, BMBF 01EO1302; SFB738, B3, DFG
References:
[1] Hoffmann et al. 2015,PLOS One, 6;10(7):e0132484

P.1086

Mitochondrial damage-associated molecular patterns (MTDs) released from hepatic ischemia reperfusion induce inflammatory response

Qianni Hu[1], Ananda Venkatachalam[2], Jessica MacLean[2], Ian Alwayn[1,2,3].
[1]Department of Pathology, Dalhousie University, Halifax, NS, Canada; [2]Department of Surgery, Dalhousie University, Halifax, NS, Canada; [3]Department of Microbiology and Immunology, Dalhousie University, Halifax, NS, Canada.

Background: Liver transplantation is the only life-saving treatment for patients with end-stage liver disease. An unavoidable consequence of the transplantation process is ischemia reperfusion injury (IRI), an immune driven inflammatory response provoked by cellular oxygen deprivation. The inflammatory response, which results from acute oxidative stress and consequent hepatocellular death during the early reperfusion phase, causes the release of damage-associated molecular patterns (DAMPs). Recent studies show that the release of mitochondrial DAMPs (MTDs) is directly linked with functionally important immune consequences and trauma. We investigated the potential of MTDs to be used as markers for IRI in vitro and in vivo.
Methods: Rat and human hepatocytes (McA RH-7777 and HepG2) were used in the in vitro models (cold / warm IRI), Lewis Rats (n=3) were used in in vivo model of hepatic IR. Mitochondrial DNA (mtDNA) was extracted and quantified by qPCR. The hepatocytes were co-cultured with different concentrations of MTDs, and cell viability was demonstrated by apoptosis detection kit; the inflammatory cytokines (TNF-α, IL-6, and IL-10) were tested by ELISA.
Results: The MTDs levels were significantly higher in both cold and warm IRI in vitro, and co-culture MTDs with hepatocytes significantly increased the cell death in a dose-dependent manner. The release of MTDs was also detected in vivo, and associated with markedly inflammatory cytokines secretion and severer liver injury compared to the control. MTDs acted as an alarmin after hepatic IRI, which might contribute to the initiation of systemic inflammatory response.
Conclusion: Our results indicate that IRI can elicit a significant increase of MTDs both in vitro and in vivo suggesting that mtDNAs may serve as novel markers in hepatic IRI during liver transplantation.

P.1087

The protein kinase 2 inhibitor tetrabromobenzotriazole protects against renal ischemia reperfusion injury

Hong Pil Hwang[1,4], Hee Chul Yu[1,4,5], Jae Do Yang[1,4], Baik Hwan Cho[1,4,5], Sik Lee[2,4,5], Sung Kwang Park[2,4,5], Byung Hyun Park[3,5], Sun O Ka[3,5].
[1]Surgery, Chonbuk National University Hospital, Jeonju, Korea; [2]Internal medicine, Chonbuk National University Hospital, Jeonju, Korea; [3]Biochemistry, Chonbuk National University Medical School, Jeonju, Korea; [4]Biomedical Research Institute, Chonbuk National University Hospital, Jeonju, Korea; [5]Research Institute of Clinical Medicine, Chonbuk National University, Jeonju, Korea.

Protein kinase 2 (CK2), activated by growth factor receptors, has a profound influence on cell proliferation and survival. Recently, CK2 activation was reported to enhance reactive oxygen species production and activate the nuclear factor κB (NF-κB) pathway. Because oxidative stress and inflammation are critical events for tissue destruction during ischemia reperfusion (I/R), we sought to determine whether CK2 was important in the renal response to I/R. Mice underwent 25 min of renal ischemia and were then reperfused. We confirmed an increased expression of CK2α during the reperfusion period, while expression of CK2β remained consistent. We administered tetrabromobenzotriazole (TBBt), a selective CK2α inhibitor before inducing I/R injury. Mice subjected to I/R injury showed typical patterns of acute kidney injury; blood urea nitrogen and serum creatinine levels, tubular necrosis and apoptosis, inflammatory cell infiltration and proinflammatory cytokine production, and oxidative stress were markedly increased when compared to sham mice. However, pretreatment with TBBt abolished these changes and improved renal function and architecture. Suppression of nuclear factor-κB (NF-κB) and mitogen activated protein kinase (MAPK) pathways might have caused the renoprotective effects of TBBt. Taken together, these results suggest that CK2α mediates proapoptotic and proinflammatory signaling, thus the CK2α inhibitor may be used to prevent renal I/R injuries observed in clinical settings.

References:

[1] Ruzzene, M., Penzo, D. & Pinna, L. A. Protein kinase CK2 inhibitor 4,5,6,7-tetrabromobenzotriazole (TBB) induces apoptosis
[2] Kim, G. S., Jung, J. E., Niizuma, K. & Chan, P. H. CK2 is a novel negative regulator of NADPH oxidase and a neuroprotectant
[3] Li, Y. W. et al. Protective effect of tea polyphenols on renal ischemia/reperfusion injury via suppressing the activation of TLR4/
[4] Schneider, C. C., Hessenauer, A., Gotz, C. & Montenarh, M. DMAT, an inhibitor of protein kinase CK2 induces reactive oxygen

P.1100

Donor's age and Body mass index are independent factors for estimation of liver weight in deceased donor liver transplantation

Gyu-seong Choi, Jae-Won Joh, SungJoo Kim, JongMan Kim, KyeungSik Kim, JongHwan Lee.
Surgery, Samsung Medical Center, Seoul, Korea.

Background: The weight of liver graft is important factor for deceased donor liver transplantation. Sometimes too large grafts make severe problem to recipients for relatively narrow abdominal cavity.
So, to predict the size or weight of the liver graft is important and then usually we can estimate donor liver volume with diverse equation for standard liver volume. Body surface area(BSA) is decisive factor for standard liver volume. In this study, we tried to find for other independent factors to decide the liver graft volume
Method: From January 2010 to March 2014, we reviewed 106 cases deceased donor liver transplantation except split graft. To find independent factors to weight of liver graft, data were analyzed about donor's age, weight, body mass index(BMI), body surface area(BSA), measured graft weight(MGW).
Result: Data for donor were shown that mean age were 48 year old(10-72), MGW mean 1.49kg(0.96-2.4kg) and average BSA 1.69(1.4-2.32m2). MGW to BSA ratio was 0.86. In correlation analysis, MGWs were correlated to donor's BSA(r=0.538) and we can get a strongly correlation between MGWs to BSA by exclusion of data with old age(>60) and high BMI(>23kg/m2). In old age group, relatively low MGW to BSA ratio was shown (0.78), and high BMI group relatively high MGW to BSA ratio was shown (0.91).
Conclusion: To estimate graft weight, donor's body surface area is most decisive factors but, in donors with old age or high BMI, we have to decide more carefully to choose liver graft.

P.1101

Inferior hepatic veins are inherited to offsprings dominantly-lessons learned from donor volumetry

Jae-Won Joh, Gyu-seong Choi.
Surgery, Samsung Medical Center, Seoul, Korea.

Introduction: Anatomic variants of the hepatic vasculature are common, so precise preoperative donor evaluation, including variations in the vasculature, is essential. To find a rule of inheritance of hepatic vasculature, we analyzed the anatomic similarity according to the donor-recipient relationship.
Methods: From September 2011 to January 2015 we selected 50 families that were over 3 members and 2 generations of family to carry out a computed tomography(CT) angiogram of liver. And we reviewed the CT to find any type of anatomic similarity.
Results: There were no significant inhertence of portal vein or hepatic artery. But, in all cases if children had marked inferior hepatic veins identified on CT, one of their parents' CT showed definite inferior hepatic veins(IHVs).
Conclusion: There was no inheritence in the anatomic variations of the hepatic artery and portal vein, but IHVs were inherited to offsprings dominantly. If donor is offspring of recipients, recipients with definite IHVs will give us an important information about donor's IHVs.

P.1102

Aborted liver transplantation – Single center experience

JeeA Lee, Gyu-seong Choi, Jae-Won Joh, Sung Joo Kim, SungJoo Kim, Suk-Koo Lee.
Surgery, Samsung Medical Center, Seoul, Korea.

Liver transplantation (LT) has become a best option for the treatment of end stage liver failure. Recently, LT has been performed earlier than in the past in order to improve the patient's quality of life; living-donor liver transplantation (LDLT) has gained increasing importance. With this movement towards LDLT, more attention has been given to donor safety, but there is no standardized protocol for the pre-operation stage of the donor to guarantee the donor's safety. The aim of this study is to share the findings of our center's cases of aborted hepatectomy during liver transplantation and to help to establish a reasonable protocol for liver transplantation with respect to the distribution of grafts and the selection of an appropriate candidate for living donor.
We reviewed all 1601 liver transplantation cases retrospectively and found 16 (1%) cases of aborted hepatectomy from 1996 to March 2015. 14 (87.5%) of 16 cases were LDLT; 11 (68.8%) were canceled due to causes related to the recipient including peritoneal seeding, hemodynamic instability, disseminated intraabdominal infection and severe adhesions, and 5 (31.2%) were canceled due to steatosis of the donor's liver.
Donor 1 had severe fatty liver on preoperative abdominal ultrasonogram (US)but preoperative biopsy showed macrovesicular and microvesicular steatosis of 30% and 20% respectively. Intraoperational liver biopsy showed macrovesicular and microvesicular steatosis of 40% and 50% respectively. Donor 2 had moderate fatty liver on preoperative US, and showed recent weight loss. Intraoperational liver biopsy showed macrovesicular and microvesicular steatosis of 30% and 40% respectively. Donors 3 and 4 had only mild fatty liver, and Donor 5 had a normal sonogram; however, because of the intraoperational liver biopsy results, the livers of these donors were considered unsuitable for donation.
In conclusion, the only reason for the donors' aborted hepatectomy was steatosis. Ultrasonography or computed tomography may be used to diagnose steatosis of the liver, but they are not useful for precise evaluation of the severity of liver steatosis in living donors. Moreover, no suitable non-invasive investigations are available. Through sharing our center's experience, we aim to establish a standardized protocol for managing living donors that prevents cases of aborted hepatectomy, thereby reducing the economic, psychological, and physiologic burden of living donors.

P.1103

Younger donor age poses a risk for overestimation of graft weight in adult living donor liver transplantation

Toshihiro Kitajima, Toshimi Kaido, Shintaro Yagi, Taku Iida, Yasuhiro Fujimoto, Etsuro Hatano, Hideaki Okajima, Shinji Uemoto.
Division of Hepato-Biliary-Pancreatic and Transplant Surgery, Department of Surgery, Kyoto University, Kyoto, Japan.

Introduction: Accurate preoperative estimation of graft weight (GW) is essential to prevent small-for-size syndrome in living donor liver transplantation (LDLT). The factors associated with GW overestimation in LDLT have not been well investigated in a large-scale study. Our aims are to evaluate the factors associated with GW overestimation and propose a simple and practical formula for estimating GW from estimated GV (EGV) based on the analyzed risk factors.
Materials and methods: From April 2006 to August 2015, 340 living donors (Right lobe: 191, Left lobe: 149) were enrolled and divided into no-overestimate group (n=179) and overestimate group (n=161). We defined the discrepancy between estimated graft volume (EGV) and actual GW (AGW) ([EGV-AGW]/EGV ×100) ≥ 7% as GW overestimation. Donor data were compared and risk factors for GW overestimation were analyzed. Furthermore, we proposed the formula of GW prediction based on the risk factors. The study was approved by the Ethics Committee of Kyoto University and conducted in accordance with the Declaration of Helsinki of 2008. All authors of this manuscript have no conflicts of interest, and no financial support was received from any source for this study.
Results and discussion: The donors in the overestimate group were significantly younger than those in the no-overestimate group (38.0 years vs. 49.0 years, $p < 0.001$) and the median EGV in the overestimate group were significantly higher than that in the no-overestimate group (615 ml vs. 578 ml, $p = 0.012$). The median body weight in the overestimate group tended to be higher than that in the no-overestimate group (62.0 kg vs. 58.2 kg, $p = 0.094$). Multivariate analysis revealed that donor age younger than 45 years old was an independent risk for GW overestimation (OR: 2.380, 95% CI: 1.526-3.712, $p < 0.001$). The coefficient factor between EGV and AGW in younger (< 45 years) donors was 0.909 ($r^2 = 0.992$, $p < 0.001$) while it was 0.948 ($r^2 = 0.990$, $p < 0.001$) in older (≥ 45 years) donors.
Conclusion: Donor age younger than 45 years old was associated with the GW overestimation in LDLT. We believe that donor age should be considered when preoperatively estimating GW in order to avoid small-for-size syndrome among LDLT recipients.

P.1104

Indocyanin green retention rate at 15 minutes of 20% or higher can be a predictive factor of living liver donor with right hepatectomy?

Cheon-Soo Park[1], Shin Hwang[2], Gil-Chun Park[2], Dong-Hwan Jung[2], Gi-Won Song[2], Deok-Bog Moon[2], Chul-Soo Ahn[2], Tae-Yong Ha[2], Ki-Hun Kim[2], Sung-Gyu Lee[2].
[1]Department of Surgery, Gangneung Asan Hospital, University of Ulsan College of Medicine, Gangneung, Korea; [2]Department of Surgery, Division of Hepatobiliary Surgery and Liver Transplantation, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea.

Background: The indocyanine green retention rate at 15 minutes (ICG R15) is a commonly used functional liver reserve test and an important criterion for major hepatectomy. Sometime, living liver donor with ICG R15 >20% could be performed right hepatectomy. We investigated clinicopathologic features of high ICG group (R15 > 20%) compared with low ICG (R15 ≤ 20%) group.
Methods: From January 2006 to December 2015, seventeen high ICG group (R15>20%) had been performed right hepatectomy for living liver donor in our center. We compared high ICG group (20.127% - 80.478%) (n=17) with low ICG group (n=272), who had been performed right hepatectomy for living liver donor from January 2014 to December 2014, for laboratory test, pathologic features, postoperative prognosis and other factors using by Chi-square test and student t-test.
Results: When low ICG group (n=272) compared with high ICG group (n=17), there were no statistical difference in recipients; mean age was 53.20±8.52 vs 50.41±8.25 (p=0.190), sex, BMI, underlying diseases, primary diseases of liver cirrhosis, model for end-stage liver disease (MELD) score, Child-Turcotte-Pugh (CTP) score, presence of hepatoma, and time of operation. In analysis of donor group, there were no difference in both group (respectively mean value); age (years) 27.37±7.73 vs 27.12±6.72 (p=0.895), body mass index (BMI) (kg/m2) 22.75±2.97 vs 22.67±3.28, aspartate aminotransferase (AST) (IU/L) 18.23±4.53 vs 19.00±4.46 (p=0.496), alanine aminotransferase (ALT) (IU/L) 15.97±8.26 vs 14.71±5.15 (p=0.533), preoperative fatty change on needle biopsy (%) 4.18±7.31 vs 4.88±5.94 (p=0.702), fatty change on intraoperative liver biopsy (%) 5.51±8.19 vs 6.71±7.08 (p=0.556) and postoperative hospital day 12.01±2.82 vs 12.71±4.92 (p=0.351). However there were statistical difference in total bilirubin (mg/dl) 0.68±0.31 vs 0.92±0.35 (p=0.002) and time of operation (min) 395.81±68.17 vs 454.71±62.48 (p=0.001).
Conclusions: At times, high ICG (R15 > 20%) level is detected in preoperative examination of living liver donor for right hepatectomy. But ICG R15 value itself cannot be single determinant for major hepatectomy.
Keyword: living liver donor, indocyanine green retention rate at 15 minutes, major hepatectomy
References:
[1] 1. Shin Hwang1, Tae-Yong Ha1, Gi-Won Song1, Dong-Hwan Jung1, Chul-Soo Ahn1, Deok-Bog Moon1, Ki-Hun Kim1, Young-Joo Lee1, Sung-Gyu Lee1. Quantified Risk Assessment for Major Hepatectomy via the Indocyanine Green Clearance Rate and Liver Volumetry Combined with Standard Liver Volume. *J Gastrointest Surg* (2015) 19:1305–1314
[2] 2. Shun-ichi Ariizumi, Masakazu Yamamoto, Ken Takasaki. Right Hepatectomy for Hepatocellular Carcinoma in Patients with an Indocyanine Green Retention Rate at 15 Minutes of 10% or Higher. *Dig Surg* 2009;26:135–142
[3] 3. Atsushi Nanashima, Takafumi Abo, Syuuichi Tobinaga, Takashi Nonaka, Hidetoshi Fukuoka, Shigekazu Hidaka, Hiroaki Takeshita, Terumitsu Sawai, Toru Yasutake, Takeshi Nagayasu, Takashi Kudo. Prediction of Indocyanine Green Retention Rate at 15 Minutes by Correlated Liver Function Parameters before Hepatectomy. *Journal of Surgical Research* 169, e119–e125 (2011
[4] 4. Toru Mizuguchi, Masaki Kawamoto, Makoto Meguro, Yukio Nakamura, Shigenori Ota, Thomas T. Hui, Koichi Hirata. Prognosis and Predictors of Surgical Complications in Hepatocellular Carcinoma Patients With or Without Cirrhosis after Hepatectomy. *World J Surg* (2013) 37:1379–1387

P.1105

Early experiences of adult living donor liver transplantation in single hospital

Cheon-Soo Park[1], Kun-Moo Choi[1], Hyuk-Jai Jang[1], Shin Hwang[2], Gi-Won Song[2], Sung-Gyu Lee[2].
[1]Surgery, Gangneung Asan Hospital, University of Ulsan College of Medicine, Gangneung, Korea; [2]Department of Surgery, Division of Hepatobiliary Surgery and Liver Transplantation, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea.

Since the first human liver transplantation (LT) performed in1963, LT has been most effective option for almost all nonmalignant end- stage liver diseases and for selected patients with hepatic neoplasms. The number of liver transplantation is increasing until now and, also, the number of institution conducted liver transplantation is increasing. Herein we will report early experience of adult living donor liver transplantation (ALDLT) in a single hospital.
We have been operated six ALDLT from June, 2012. In clinical features of patients, mean age was 52.5±6.62 (years), 4 male and 2 female, the causes of LT were 1 alcoholic LC, 2 HBV-LC, HCC and 3 HBV LC. MELD score was 10.7±4.88 (mean), GRWR was 0.96±0.13, mean operative time was 672±65.6 minutes. All transplanted grafts were modified right graft, all bile duct reconstruction was conducted by duct to duct anastomosis. All patients took tacrolimus based immunosuppressive agents and three of them together with mycophenolate mofetil, because of uncontrolled DM and nephrotoxicity of tacrolimus. Mean hospital day was 28.66±6.97 (days), and there was no postoperative in hospital mortality and morbidity. One patient had end stage renal disease by underlying disease, he had undertaken ABO incompatible living kidney transplantation one year after LT, one patient was suspicious acute cellular rejection, took high dose immunosuppressive agents with steroid and then recovered on three months after LT. In features of living donor, mean age was 28.33±8.71 (years), 2 male and 4 female, hospital day was 11.5 days, and there was no morbidity and mortality.
A multidisciplinary approach with surgical, anesthetic and medical teams, and a wide range of ancillary services, which can be provided with institutional and foundational support, is crucial. We thought that the multidisciplinary teamwork including thorough preparation for LT is most important for which first started the liver transplant hospital.

References:

[1] [1]Lee SG. A complete treatment of adult living donor liver transplantation: a review of surgical technique and current challenges to expand indication of patients. Am J Transplant. 2015;15:17–38.
[2] [2]Moon DB, Lee SG, Hwang S. et al.Toward more than 400 liver transplantations a year at a single center.Transplant Proc. 2013 Jun;45(5):1937-41.

P.1106

Protective effect of crepidiastrum denticulatum extract pretreatment against hepatic ischemia-reperfusion injury in mice

Hyuk Jai Jang[1], Chun Soo Park[1], Hwa Mee Lee[2], Ji Hwan Lee[1], Mee Young Oh[1], Seong Soo Kim[2], Ju Won Noh[3], Hee Ju Lee[1].
[1]Surgery, University of Ulsan, Gangneung Asan Hospital, Gangneung, Korea; [2]Anesthesia, University of Ulsan, Gangneung Asan Hospital, Gangneung, Korea; [3]Natural Medical Center, KIST Gangneung, Gangneung, Korea.

Background: Crepidiastrum denticulatum (CD) is a well-known, traditionally consumed vegetable in Korea, which was recently reported to have bioactive compounds with detoxification and antioxidant properties. Ischemia-reperfusion injury (IRI) is a major critical event that commonly occurs after liver transplantation and resection. Furthermore, inflammatory responses to IRI exacerbate the resultant hepatic injury. In this study, we investigated whether CD protects against IR-induced acute liver injury in mice.
Materials and Methods: Partial (70%) hepatic IRI was induced in male C57BL/6 mice by portal triad pedicle occlusion for 90 min followed by reperfusion for 6 h. CD (75 mg/kg body weight [BW], oral) was administered 7 days before the IRI.
Results: Treatment with CD significantly decreased serum alanine aminotransferase (sALT) and serum aspartate aminotransferase (sAST) as well as liver histological changes. CD also prevented hepatic glutathione (GSH) depletion and increased malondialdehyde (MDA) levels induced by IRI.
Conclusion: CD improved the acute hepatic IRI by reducing oxidative demage. These findings suggest that CD is a promising agent against acute IR-induced hepatic damage.

P.1107

Difference in donor complications by types of skin incision in LDLT

Gyu-seong Choi, Jae-Won Joh, JongMan Kim, SungJoo Kim, KyeungSik Kim, JongHwan Lee, Kyowon Lee.
Surgery, Samsung Medical Center, Seoul, Korea.

Background: Because of the shortage of deceased-donor livers for transplantation, living-donor liver transplantation (LDLT) has become an indispensible treatment strategy for end-stage liver disease. The critical prerequisite for LDLT is the maximal safety of healthy donors. But also donors want to get a minimal operation scars.
Methods: From December 2013 to January 2015, a total of 82 completed donor hepatectomy were performed without laparoscopy in our center. We analyzed donor morbidity associated with LDLT by type of skin incision.
Results: Mid-line skin incisions(MS) were given to 49 donors, right subcotal incision with upper mdiline extension(SS) to 31 donors There was no donor mortality. Complications were observed in 4 (8%) donors of MS group and 3(10%) donos of SS groups. Wound complications were most common, occurring in 2 (4%) donors of MS group and 2(6.5%) donors of SS groups. According to a modified Clavien classification, grade IIIa complications were experienced in 2 donors of MS groups(4%) and 1 donors of SS groups(3.5%). Interventional management was successful in all grade IIIa.
Conclusion: This study demonstrates the safety of donor hepatectomy by type of skin incision. Regardless of skin incision type, complications were relatively minor and easily controlled.

P.1108

Living donor liver transplantation using a graft procured from an advanced age-donor

Atsuyoshi Mita, Koichi Urata, Yuichi Masuda, Yasunari Ohno, Yuichi Nakazawa, Akira Kobayashi, Toshihiko Ikegami, Shin-ichi Miyagawa[1].
Department of Surgery, Shinshu University School of Medicine, Matsumoto, Japan.

Backgrounds: Liver transplantation (LT) has dramatically improved the outcome of patients with end-stage liver disease. An advanced age-donor has been accepted step by step due to donor shortage. Advanced age reportedly impairs regenerative capacity of a graft and significantly increases the severity of hepatitis-C recurrence. In living donor liver transplantation (LDLT), donor age criteria has gradually extended also although advanced age-donors have a risk for their own hepatectomy. The aim of this study is to clarify the impact of donor age on the outcome in LDLT.
Patients and Methods: One hundred seventy two adult recipients who had LDLT at our institute from 1993 until 2014, were included in this study. Their indications for LDLT included 42 liver cirrhosis associated to Hepatitis-C (HCV group) and the others (NHCV group). We retrospectively evaluated graft survival rates after LDLT using grafts procured from various age-donors in each group.
Results: Comparing between HCV and NHCV groups, HCV group contained of significantly older recipients ($p<0.0001$), younger donors ($p=0.0143$) and shorter follow-up period ($p=0.0414$). Graft survival rates were comparable in 130 recipients with grafts procured from under 30 (n=37), 30's (n=28), 40's (n=34), 50's (n=24) and 60's (n=7) year-old donors in the NHCV group (5 year 83.4, 88.0, 85.3, 79.2, 85.7%, 10 year 71.8, 82.1, 78.5, 69.9, 85.7%, $p=0.5556$). While the recipients with grafts procured from 40's (n=7) and 50's (n=3) year-old donors showed significantly worse graft survival rates, compared to those with grafts procured from under 30 (n=17), 30's (n=15) year-old donors in the HCV group (5 year 71.4 and 0% vs 88.2 and 86.2%, 10 year 0 and 0% vs 80.2 and 68.9%, $p<0.0001$). The reason of graft loss in HCV group included 2 liver failures due to fibrosing cholestatic hepatitis, 2 recurrences of hepatic cell carcinoma (HCC) and 2 infections in 32 patients aged less than 40, and 2 liver failures due to hepatitis C recurrence, 2 graft failures of other reasons and 2 recurrences of HCC in 10 patients aged equal and more than 40. Donor age was a risk factor for graft loss after LDLT in the HCV group. In 36 patients treated for hepatitis-C recurrence, 14 who had obtained serological variable response (SVR) were significantly younger than 22 without SVR (29.8 ± 9.0 vs 38.1 ± 12.7 years old, $p<0.05$).
Conclusion: Advanced age of living donor showed negative impact on the graft survival rate after LDLT for recipients with liver cirrhosis associated to hepatitis-C although it had no impact on the outcome for recipients with the other liver disease. It might be one reason that SVR rate was lower in treatment for hepatitis-C recurrence after LDLT using a graft procured from an advanced age-donor.

P.1109

Single pass albumin dialysis (SPAD) – Optimizing bridging therapy for liver transplantation in acute liver failure

Rosa Schmuck, Anja Reutzel-Selke, Gesa Nawroth, Panagiotis Fikatas, Johann Pratschke, Igor MSauer.
General, Visceral and Transplantation Surgery, Experimental Surgery and Regenerative Medicine, Charité – Universitätsmedizin Berlin, Berlin, Germany.

Background: Patients with acute liver failure (ALF) awaiting liver transplantation (OLT) may require bridging therapies untill a organ is available for transplantation. This topic is of increasing interest in times of organ shortage. Several artificial liver support concepts have been evaluated and Single Pass Albumin Dialysis (SPAD) has shown to be one of the most feasible and effective methods for removing albumin bound toxins and water-soluble substances. Our aim was to define the optimal conditions for SPAD more precisely in a standardized experimental set-up.
Methods: In the first experimental set up, albumin concentration was adjusted to 1%, 2%, 3%, or 4%, with a constant flow rate of dialysate of 700 ml/h. In the second experimental set up, we varied the flow rate of dialysate between 350, 500, 700, and 1000 ml/h, with a constant concentration of albumin (3%).
Results: We detected the optimal detoxification capacity of SPAD at a albumin concentration of 3%. A further increase of the albumin concentration to 4% did not significantly increase the efficiency. We observed a gradual increase of the detoxification efficiency for albumin bound substances, from 350 ml/h to 700 ml/h (for bilirubin) or 1000 ml/h (for bile acids) of dialysate flow. Water-soluble toxins (ammonia, creatinine, urea, uric acid) were already removed almost completely with minimal flow rates and albumin concentrations.
Conclusion: In conclusion, this study confirmed that SPAD is effective in eliminating albumin-bound as well as water-soluble toxins using a simulation of ALF. This findings could help to optimize bridging therapies for patients with ALF awaiting OLT.

P.1110

Bile as a diagnostic tool after liver transplantation: miRNA pattern and protein based biomarkers may predict acute cellular rejection

Rosa Schmuck[1], Anja Reutzel-Selke[1], Nathanael Raschzok[1], Mehmet HMorgul[2], Benjamin Struecker[1], Moritz Schmelzle[1], Robert Öllinger[1], Sabine Boas-Knoop[1], Marcus Bahra[1], Andreas Pascher[1], Johann Pratschke[1], Igor MSauer[1].
[1]General, Visceral and Transplantation Surgery, Experimental Surgery and Regenerative Medicine, Charité – Universitätsmedizin Berlin, Berlin, Germany; [2]Department of Visceral, Transplantation, Thoracic, and Vascular Surgery, Universitätsklinikum Leipzig, Leipzig, Germany.

Introduction: The ability to adjust immunotherapy to the individual risk of acute cellular rejection (ACR) would have great impact on the long-term outcome of orthotopic liver transplantation (OLT). Thus, diagnostic tools are required to assess a patient's individual risk and refine the diagnosis of ACR. Given that bile rather than blood depicts the local inflammation in the liver, this study aims to investigate alterations in bile composition during ACR.
Methods: Protein-based biomarkers and expression of miRNAs were analyzed during the first two weeks after transplantation and on clinical suspicion of ACR in the bile of 45 OLT recipients with external bile drainage systems (25 with ACR, 20 w/o ACR), and correlated to the clinical course after OLT.
Results: Levels of CD44, miR-122, miR-133a, miR-148a and miR-194 were significantly higher during the early postoperative period in the bile of patients who developed ACR within the first 6 months after OLT. miRNA 122 expression was most effective in discriminating both groups (PPV: 90%, sensitivity: 88%, specificity: 89%).
During ACR, levels of CD44, CXCL9, miR-148a and miR-194 were significantly elevated compared to the no rejection group. A PPV of 86% could be achieved through combining CD44 and CXCL9 cut-offs, (sensitivity: 75%, specificity: 89%).
Conclusion: Bile analysis could further our understanding of the local inflammatory process during rejection, by detecting specific responses against the transplanted liver. Secretome and miRNA examination in bile may lead to the identification of predictive biomarkers for ACR, and may improve noninvasive diagnosis in case of clinical suspected ACR.

P.1111

Successful transplantation after normothermic machine perfusion of a liver procured from a donor with cold agglutinin formation secondary to mycoplasma pneumoniae infection – a case report

Peri Kocabayoglu[1], Marianne breyer[2], Andreas Paul[1], Maren Schulze[1].
[1]Department of general Visceral and Transplant Surgery, University Hospital Essen, Essen, Germany; [2]Department of Transfusion Medicine, University Hospital Essen, Essen, Germany.

Objective: We report a successful liver transplantation performed following short-term normothermic machine perfusion (NMP) of the graft using the OrganOx metra. The liver was procured from a 17-year old brain dead donor with cold agglutinin formation in the serum secondary to Mycoplasma pneumoniae infection.
Methods: This is a case of an innovative method of organ preservation which was employed for reconditioning of a liver procured from a 17-year-old brain dead organ donor who died due to cerebral complications of an initial Mycoplasma pneumaniae infection. The serum of the donor showed cold agglutinin formation before organ procurement. At time of procurement, an initial normothermic in situ perfusion after aortic cross clamping was performed, followed by cold perfusion and static cold storage of the liver at the donor hospital. Micro-embolism of the graft vasculature could not be ruled out, and the organ was finally allocated through EuroTransplant as a competitive center offer. Upon arrival of the liver at the recipient hospital, the graft was perfused under normothermic conditions using three packed red blood cells as well as substrates on the OrganOx metra according to the manufacturer's protocol (OrganOx, Oxford, UK). This was performed in order to reduce the presence of cold agglutinins within the graft and to remove remaining donor blood from the vasculature. During machine perfusion, flow parameters as well as blood gases were constantly monitored. The liver was transplanted successfully to a 43-year-old recipient with alcoholic liver cirrhosis.
Results: Donor serum showed cold agglutinin formation as well as clotting at room temperature. Rewarming of the serum led to a solution of the clots. As for the graft, during organ perfusion at 37°C using the OrganOx metra, flow parameters were constantly measured and were within normal limits. Blood gas analyses showed a continuous drop in lactate as well as persistence of a pH within normal range throughout NMP. Peak transaminases showed AST 203 U/l and ALT 232 U/l within the first week following transplant. There was no need for substitution of coagulation factors both during and after transplantation. The patient was discharged in good clinical condition on day 14 post-transplant.
Conclusions: This is a rare case report of liver transplantation following organ resuscitation through NMP of a graft procured from a donor showing cold agglutinin formation secondary to Mycoplasma pneumoniae infection. Normothermic machine preservation proved to be a safe and effective method to restore and assess organ quality as well as function throughout the preservation period in a case of probable vascular damage due to cold agglutinin formation in a graft.

P.1112

Outcomes of living and deceased donor liver transplant recipients according to the MELD score

Jae Geun Lee[1], Juhan Lee[1], Jung Jun Lee[2], Seung Hwan Song[1], Jee Youn Lee[1], Su-kyung Kwon[1], Dong Jin Joo[1], Man Ki Ju[1], Gi Hong Choi[1], Jin Sub Choi[1], Soon Il Kim[1], Myoung Soo Kim[1].
[1]Department of Surgery, Yonsei University College of Medicine, Seoul, Korea; [2]Department of Surgery, CHA Bundang Medical Center, CHA University, Seoul, Korea.

Background: Living donor liver transplantation (LDLT) has developed as an alternative to decease donor liver transplantation (DDLT) to overcome the critical shortage of deceased organ donations. However, the evidence supporting a LDLT for high model for end stage liver disease (MELD) score recipient is weak. We compared the outcomes of LDLT and DDLT according to MELD scores.
Methods: The study included 498 adult patients who underwent liver transplantation between 2006 and 2014 at Severance Hospital. Patients with re-transplantation and fulminant liver failure were excluded from the study. Recipients were categorized according to their MELD score into low (MELD score ≤25) and high (MELD score >25) MELD group.
Results: About 76.5% of patients are male and median age is 53. Major origin of liver cirrhosis is Hepatitis B virus and 50% of patients had HCC. There were no significant difference gender, donor gender, age, HBV, HCV, HCC and DM. However, age of donor, CTP score and MELD score in DDLT were significantly higher than LDLT. In both LDLT and DDLT groups, patients with high meld score show significantly lower graft survival than patients with low MELD score. (p =0.019 in LDLT and p=0.009 in DDLT) However, in both high and low meld group, there were no significant difference of survival between LDLT and DDLT. Non-HBV, HCC, High MED are risk factor for graft mortality in overall patients. However, HCV and high MELD are risk factor in patients without HCC.
Conclusion: High MELD score is risk factor for Graft mortality in liver recipients regardless combined HCC. There was no significant difference between LDLT and DDLT, in patients with both high MELD and low MELD. When deceased donor organs are scare, LDLT with donor safety maybe good therapeutic option in patient with high MELD score.

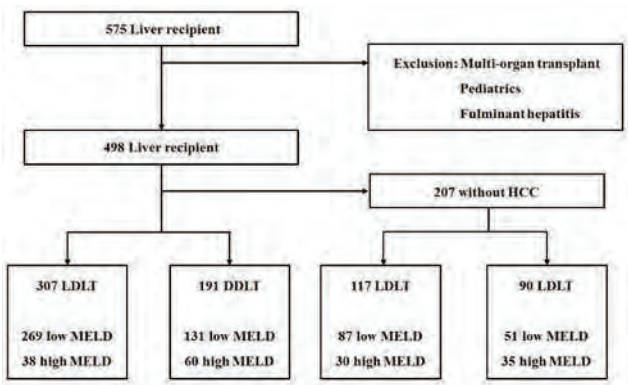
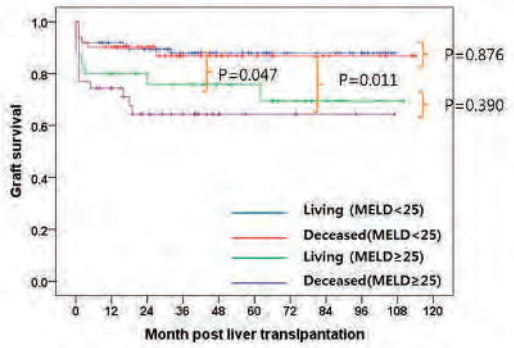


Figure1. Graft survival of patients without HCC according to MELD score and donor type



References:
[1] Al Sebayel M. *Living donor liver transplant versus cadaveric liver transplant survival in relation to model for end-stage liver disease score.*. 2015;47(4):1211-3

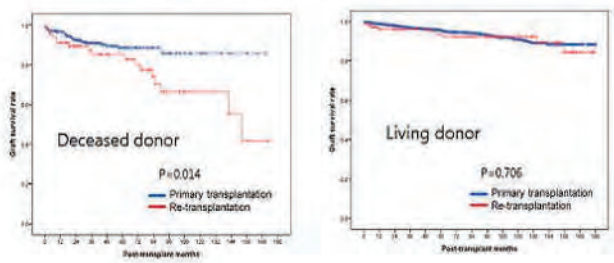
P.1113

Outcomes of second kidney transplantation compared primary transplantation

Jae Geun Lee, Kwanbum Lee, Juhan Lee, Seung Hwan Song, Jee Youn Lee, Su-kyung Kwon, Dong Jin Joo, Kyu Ha Huh, Myoung Soo Kim, Soon Il Kim, Yu Seun Kim.
Department of Surgery, Yonsei University College of Medicine, Seoul, Korea.

Background: Re-transplantation is associated with an improved overall survival of patients with a failed renal allograft. Some studies showed the outcomes of retransplantation are than those of primary procedures. But in living donor kidney transplantation (LDKT), the few study was reported.
Objectives: The aim of study was to know outcomes in re-transplantation and primary transplantation in kidney recipients.
Methods: Medical records of 1922 Kidney recipients between Jan. 2000 and Dec. 2014 was retrospectively reviewed. Pediatric recipients and recipients who underwent multi-organ transplantation were excluded.
Results: 1686 kidney recipients underwent primary transplantation (PT) and 179 kidney recipients underwent re-transplantation (RT). The portion of male was higher in RT than PT. (74.9% vs. 59.7%, p <0.001). The portion of deceased donor kidney transplantation (DDKT) was higher in PT than RT. (39.1% vs. 18.3%, p <0.001). The rate of delayed graft function was higher in PT than RT. (6.3% vs. 15.1%). In DDKT, Graft survivals of PT at 1 year, 3years, 5 years were 96.7%, 90.9% and 88.5%, respectively. Graft survivals of RT at 1 year, 3years, 5 years were 91.1%, 89.4% and 85.2%, respectively. However, in LDKT, there was no difference of graft survival between PT and RT group. In LDKT, acute rejection and recipient age were risk factor of graft survival by multivariant analysis. In DDKT, acute rejection, delayed graft function and recipient age were risk factor of graft survival by multivariant analysis.
Conclusion: In LDKT, re-transplantation is not risk factor of graft loss. Proper selection of living donor can be expected to good outcomes of

Fig 1. Graft survival according to primary transplantation and re-transplantation in deceased and living donor kidney transplantation



References:
[1] Barba Abad J. Impact of renal retransplantation on graft and recipient survival. *Arch Esp Urol.* 2011;64(4):363-70

P.1114

CoCl2 induced ER stress protein mediated cytotoxicity in FK506 treated hepatoma HepG2 cells

Soo Jin Na Choi.
Surgery, Chonnam National University Hospital, Gwangju, Korea.

Background: The effects of FK506 on the endoplasmic reticulum (ER) mediated stress pathway accelerates CoCl2 - induced cytotoxicity in human hepatoma HepG2 cell line were investigated.
Methods: We examined the effects of FK506 on CoCl2 - induced cytotoxicity by western blottings of poly ADP-ribose polymerase(PARP), CHOP, GRP78, Nrf2, ATF4, ATF6, XBP-1, Bak, Bax, and Bcl-2. And the catalytic acitivity of caspase-3 and -12 caspase in HepG2 cells was also measured.
Results: FK506 and CoCl2 significantly induces the synergistic effect of HepG2 cytotoxicity in dose dependent manner. Increased active-PARP expression occurred at 24 hours after FK506 treatment on cobalt chloride-induced HepG2 cytotoxicity and peak activation of cleaved caspasec-3 was also observed at 24 hours. FK506 aggravates cobalt chloride-induced HepG2 cytotoxicity. GRP78 expression was increased 24 hours after FK506 treatment on cobalt chloride-induced HepG2 cytotoxicity. CHOP and caspase-12 expressions were increased 24 hours after FK506 treatment on cobalt chloride-induced HepG2 cytotoxicity. Expressions of ATF4 and ATF6 were same manners. Expression of XBP-1 was decreased beginning at 6 hours. FK506 exasperate endoplasmic reticulum stress by cobalt chloride-induced cytotoxicity. Bcl-2 protein expression decreased, but FK506 induces expression of Bak and Bax by cobalt chloride-induced cytotoxicity. Nrf2 expression was also noted.
Conclusions: FK506 and CoCl2 significantly induces the synergistic effect of cytotoxicity in dose dependent manner. FK506 aggravates cobalt chloride-induced cytotoxicity. FK506 exasperate endoplasmic reticulum stress by cobalt chloride-induced cytotoxicity. FK506 accelerates expression of ER-stress related nuclear transcriptional factor.

P.1115

Changes in T cells in peripheral blood after adult liver transplantation

Jong Man Kim[1], Nuri Lee[1], Chanwoo Cho[1], Jisoo Lee[1], Gyu-Seong Choi[1], Jae Berm Park[1], Choon Hyuck D.Kwon[1], Eun-Suk Kang[2], Sung Joo Kim[1], Jae-Won Joh[1].
[1]Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; [2]Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea.

Backgrounds: T lymphocytes are an essential component of allograft rejection and tolerance. The aims of the present study are to analyze the characteristics of T cell subsets between deceased donor liver transplantation (DDLT) patients and living donor liver transplantation (LDLT) patients and to investigate the potential role of T cell subsets in cytomegalovirus (CMV) infection, acute rejection, and graft failure.
Methods: Between April 2013 and June 2014, 64 patients underwent adult LT. All patients received basiliximab as induction therapy and tacrolimus as maintenance therapy. The distribution of peripheral blood T lymphocyte subsets pretransplant and 4, 8, 12, and 24 weeks post-transplant were serially monitored.
Results: Patient characteristics did not vary between DDLT and LDLT groups except for Child-Pugh class, model for end-stage liver disease score, and cold ischemic time. However, the Vδ1/Vδ2 ratio in the DDLT group was higher than in the LDLT group (P=0.045). Comparison between LDLT and DDLT groups revealed that CD4+ T cells, CD8+ T cells, CD4/CD8, Vδ1 cells, Vδ2 cells, and γδ T cells did not change significantly over time. The Vδ1/Vδ2 ratio in patients with CMV infection was higher than in patients without CMV infection. The absolute CD3+ and CD8 T cell counts in patients with biopsy-proven acute rejection (BPAR) were higher than in patients without BPAR. The absolute lymphocyte counts, CD4+ T cell, γδ T cell, and Vδ2 γδ T cell counts in patients with graft failure were lower than in patients without graft failure.
Conclusion: CD3+ T cells are different between DDLT and LDLT groups. Patients with BPAR showed elevated CD3+ and CD8+ T cells. The present study suggests that LDLT patients receive high doses of immunosuppression compared with DDLT patients. Vδ2 γδ T cells are closely associated with CMV infection and graft failure.

P.1116

Differences in peripheral blood lymphocytes between brand-name and generic tacrolimus treated in stable liver transplant recipients

Jong Man Kim[1], Nuri Lee[1], Chanwoo Cho[1], Jisoo Lee[1], Gyu-Seong Choi[1], Jae Berm Park[1], Choon Hyuck D.Kwon[1], Eun-Suk Kang[2], Sung Joo Kim[1], Jae-Won Joh[1].
[1]Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; [2]Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea.

Backgrounds: Brand-name and generic tacrolimus have been used for prevention liver allograft rejection. However, no study has performed an analysis of peripheral lymphocytes in blood between brand-name and generic tacrolimus treated patients. Aims of present study compared peripheral blood lymphocytes between brand-name and generic tacrolimus group in stable liver transplant recipients.
Methods: Sixteen patients, who underwent ABO-compatible living donor liver transplantation between 2012 and 2013, have stable graft function. Ten patients received brand-name tacrolimus and six patients received generic tacrolimus. Lymphocytes were analyzed in peripheral blood obtained preoperatively and 4, 8, 12, and 24 weeks after liver transplantation.
Results: Regarding the baseline and perioperative characteristics, there were no statistically significant differences between the two groups. Immunosuppression also was not different. Subtype analysis of T cell populations carried out in parallel showed similar levels of CD3, CD4, CD8, and γδ T cells in brand-name tacrolimus group and generic tacrolimus group in stable liver transplant recipients. However, levels of CD4+Foxp3+ and CD3-CD56+ T cells were higher in the brand-name tacrolimus group than in the generic tacrolimus group after 8 weeks post-transplantation.
Conclusions: The present study suggests that brand-name tacrolimus might have more potential immunosuppressive activity than generic tacrolimus regarding CD4+Foxp3+ T cells contribute graft tolerance.

P.1117

Selection of immunosuppression in hepatitis C virus liver transplant recipients prevents biopsy-proven acute rejection

Jong Man Kim[1], Kwang-Woong Lee[2], Gi-Won Song[3], Bo-Hyun Jung[3], Hae-Won Lee[4], Nam-Joon Yi[2], Choon Hyuck DavidKwon[1], Shin Hwang[3], Kyung-Suk Suh[2], Jae-Won Joh[1], Suk-Koo Lee[1], Sung-Gyu Lee[3].
[1]Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; [2]Department of Surgery, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Korea; [3]Division of Hepatobiliary Surgery and Liver Transplantation, Department of Surgery, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; [4]Department of Surgery, Seoul National University Boramae Medical Center, Seoul, Korea.

Introduction: The relationship between patient survival and biopsy-proven acute rejection (BPAR) in HCV liver transplant (LT) recipients remains unclear. The aims of this study were to compare the characteristics of patients with and without BPAR and to identify risk factors for BPAR.
Methods: We retrospectively reviewed the records of 169 HCV RNA-positive patients who underwent LT at three centers.
Results: BPAR occurred in 39 patients (23.1%) among the HCV RNA positive recipients after LT. The 1-, 3-, and 5-year patient survival rates were 92.1%, 90.3%, and 88.5%, respectively, in patients without BPAR and 75.7%, 63.4%, and 58.9% in patients with BPAR ($P<0.001$). Multivariate analyses showed that BPAR was associated with lack of basiliximab induction and used cyclosporine in HCV RNA positive LT recipients
Conclusion: The results of the present study suggest that the selection of immunosuppression in HCV RNA-positive LT recipients should be carefully reviewed to prevent BPAR and to improve patient survival.

P.1118

Hepatitis C patients in a multicenter study who received living donor liver transplants are likely to have increased survival

Jong Man Kim[1], Kwang-Woong Lee[2], Gi-Won Song[3], Bo-Hyun Jung[3], Hae Won Lee[4], Nam-Joon Yi[2], Choon Hyuck D.Kwon[1], Shin Hwang[3], Kyung-Suk Suh[2], Jae-Won Joh[1], Suk-Koo Lee[1], Sung-Gyu Lee[3].
[1]Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; [2]Department of Surgery, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Korea; [3]Division of Hepatobiliary Surgery and Liver Transplantation, Department of Surgery, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; [4]Department of Surgery, Seoul National University Boramae Medical Center, Seoul, Korea.

Backgrounds: No consensus has been reached regarding comparisons of outcomes for patients with hepatitis C virus (HCV) who received living donor liver transplantation (LDLT) versus deceased donor liver transplantation (DDLT). The aims of this study were to compare characteristics between LDLT and DDLT groups and to identify risk factors for patient survival.
Methods: We retrospectively reviewed the multicenter records of 192 HCV RNA-positive patients who underwent LT.
Results: Thirty-five patients received a DDLT and 146 received a LDLT. The median donor age and model for end-stage liver disease (MELD) score in the DDLT group were younger and lower than in the LDLT group ($P<0.001$). No significant differences were observed between the two groups regarding their baseline characteristics. Also, there were no statistically significant differences regarding use of induction agent, use of cyclosporin or tacrolimus, use of mycophenolate mofetil, steroid use for at least six months, HCV recurrence, or biopsy-proven acute rejection after LT. The 1-year, 3-year, and 5-year patient survival rates were 66.7%, 63.0%, and 63.0% in the DDLT group and 86.1%, 82.3%, and 79.5% in the LDLT group ($P=0.024$), respectively. Recipient age ≥ 60 years, LDLT, and use of tacrolimus were factors that were positively associated with patient survival in multivariate analyses.
Conclusion: This study concludes that patient survival among LDLT patients is higher than among DDLT patients, which suggests that HCV patients should receive a LDLT if an appropriate donor is available.

P.1119

A modified heterotopic auxiliary liver transplantation for wilson's disease with the graft implanted in the splenic fossa

Kefeng Dou, Kaishan Tao, Zhaoxu Yang, Xiao Li, Zhuochao Zhang. Department of Hepatobiliary Surgery, Xijing Hospital, the Fourth Military Medical University, Xi'an, People's Republic of China.

Introduction: Auxiliary liver transplantation(ALT) was initially indicated for potentially reversible fulminant hepatic failure(FHF), and recently has been proven to one of the effective treatments for Wilson's disease(WD). Conventional auxiliary liver transplantation includes orthotopic liver transplantation and heterotopic liver transplantation. But the conventional procedure could not avoid the problem of space, functional competition and hemodynamic variation.
Methods: We modified the heterotopic auxiliary partial liver transplantation (HAPLT) to have a splenectomy, thus to have plenty of space implanting graft into the splenic fossa. The recipient's splenic artery and vein was anatomosed to the graft's hepatic artery and portal vein. Anastomosis of the graft's hepatic vein and the recepient's caval orifice of the left renal vein established the outflow.
Results and Discussion: We start our first modified HAPLT for WD in June 2007. The patient recovered well after the transplantation and has been symptom-free during a 8-year follow-up. Since then, 14 WD patients underwent modified HAPLT in our center: 9 received graft(left lateral lobe) from living donor, and the other 5 cases received a split left lateral lobe from DCD(donation after cardiac death) donors. Median follow-up was 57months(28days-104months). Thirteen of 14 patients(92.9) were alive. One patient died at the 28th postoperative day because of pneumonia.
Conclusion: The modified HAPLT is more safe and simple. It might be an effective treatment for WD patients with splenomegaly.

P.1120

Clinical Outcomes with Modified Release Tacrolimus in Liver Transplant Recipients: a single centre observational study

Elankumaran Krishnan, Anand Ramamoorthy, Mahesh Gopasetty, Manish C Varma, Anand K Khakhar.
Apollo Transplant Institues, Apollo hospitals, Chennai, India.

Modified release tacrolimus has been studied in several centres for both de novo and conversion in long-term stable recipients. The aim of this study is to analyse the clinical outcomes of 1:1 dose conversion from BD to OD tacrolimus in liver transplant recipients.
Methods: The total number of patients on modified release tacrolimus was 30, of which de novo usage in 4 patients and conversion in long-term stable recipients in 26 patients with a dose conversion of 1:1. A mean follow-up period in the studied group was 3 months. The compliance of these patients was evaluated with a questionnaire.
Results: The mean age of these patients was 50.2± 11.91 yrs. The reason for conversion being non-compliance in 57.7% and renal dysfunction in 42.3% of patients. The trough levels were maintained in comparable range both before and after conversion. There were no severe rejections or major infections in these patients were documented. There was a 100% perception of convenience in all these patients with improved compliance.
Conclusion: Switching from tacrolimus twice daily to modified release once daily regimen is safe and has the advantage of improving adherence.
References:
[1] Safety and efficacy of once-daily modified-release tacrolimus in liver transplant recipients: a multicenter postmarketing surveillance in Japan. Uemoto, Abe, Horike Transplant Proc. 2014 Apr;46(3):749-53.
[2] Advagraf de novo in liver transplantation: a single-center experience. Ortiz de Urbina J1, Valdivieso A, Matarranz A, Gastaca M, Fernandez JR, Bustamante J, Gonzalez J et al., Transplant Proc. 2011 Apr;43(3):724-5.

P.1121

Long term observation of children after liver transplantation across ABO blood groups. A single center experience

Małgorzata Markiewicz-Kijewska[1], Joanna Teisseyre[1], Piotr Kalicinski[1], Marek Szymczak[1], Hor Ismail[1], Dorota Broniszczak[1], Adam Kowalski[1], Marek Stefanowicz[1], Irena Jankowska[2].
[1]Pediatric Surgery and Organs Transplantation, Children's Memorial Health Institute, Warsaw, Poland; [2]Gastroenterology, Hepatology and Nutrition Disorder, Children's Memorial Health Institute, Warsaw, Poland.

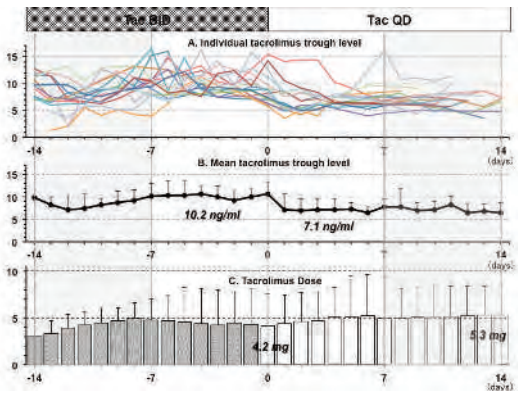
Introduction: Liver transplantation has become a routine treatment for children with liver failure. Organ shortage or urgency of transplantation is the cause for accepting suboptimal donors including donors with incompatible blood groups. The aim of our study was to assess long term post transplant course of children after ABO incompatible (ABOi) liver transplantations performed in our center. We performed retrospective analysis of all ABOi liver transplantation for: indications for transplantation, immunosuppression, history of acute or chronic rejection and long term graft function and graft and patient survival.
Material and Methods: Between 1997 and 2014 we transplanted 63 patients aged 0,2-20,5 years (mean 1,36 years) and body mass 4,43-77 kg (mean 9,9 kg). 2 patients which died during transplantation were excluded from further analysis., so finally 61 patients were evaluated. MELD/PELD score was 4-40 points at transplantation (median 34), 41 of patients were transplanted urgently (67,1%), 20 electively (32,9%). They received 64 ABOi grafts.
Results: 15/61 patients died (24,6%) after transplantation - including 9 pts which died 7-93 days within posttransplant hospitalization and 6 later, between 3-56,5 months after Tx. The actual follow up of living patients is 1,65-15,45 years (mean 7,19 years). The immunosuppression consisted of tacrolimus in 59 patients, MMF in 51 pts and steroids in 35 pts. Antilymphocytic globulins or antiIL2rec antibodies were administered to 54 patients. Early acute rejection (within first 3 months after Tx) was observed in 24 pts (including 3 pts with humoral rejection), late acute rejection episodes developed in 11 patients (including 4 pts after one year). All but three AR resolved after steroid boluses, two patients were treated additionally with ATG or Rituximab and 3 with plasma exchange due to AMR. Late AR were treated with methylprednisolon and intensification of immunosuppression. There were 2 children with ductopenic chronic rejection in this series (3,3%). Immunological problems were the cause of 3 graft losses (4,8 % grafts) and 2 of these patients died (3,3 %).
Conclusions: liver transplantation with ABOi grafts can be a life saving procedure in patients with ALF or acute decompensation of chronic liver disease (especially if suitable donor is not available). Morbidity and mortality which could be connected to ABOi transplantation are not significantly different when compared to patients with ABO compatible grafts.

P.1122

Early conversion from twice-daily tacrolimus to prolonged-release tacrolimus in liver transplantation: a single-center experience

Yasuhiro Ogura, Hisashi Imai, Hideya Kamei, Tomohide Hori, Nobuhiko Kurata, Yasuharu Onishi.
Transplantation Surgery, Nagoya University Hospital, Nagoya, Japan.

Background: Twice-daily tacrolimus (Tac BID) has been a mainstay of immunosuppression therapy for liver transplantation over the last 20 years. Prolonged-release tacrolimus (Tac QD) was developed to increase compliance, and widely used in organ transplantation. However, in Japan, Tac QD introduction to de novo liver transplant patients was initially tried, but it was not spread as a standard protocol due to unstable pharmacokinetics. Therefore, the conversion from Tac BID to Tac QD is usually done in stable patients months or years after liver transplantation in Japan. The aim of this study is to assess the early conversion of Tac QD during liver transplant hospital stay.
Methods: Eighteen liver transplants (excluding pediatric recipients) were performed during 2014-2015. All consecutive cases except 2 early-expired patients were enrolled. Our standard immunosuppression is oral Tac BID and steroid taper, and we add mycophenolate mofetil if indicated. Conversion criteria from Tac BID to Tac QD were, i) relatively stable liver function with stable trough level by oral Tac BID, and ii) good general condition (no or well-controlled complications). We did not fix the exact conversion date because each patient's recovery was different. Dose conversion rate from Tac BID to Tac QD was set at 1:1. Total follow-up period after Tac QD conversion in this study was 283-587 days.
Results: Median conversion days after liver transplant were 27 days. Sixty-two percent of patients were converted within 4 weeks after liver transplant, and 56% discharged hospital within 2 weeks after conversion. The comparison of the last week of Tac BID and the first week of Tac QD revealed that the mean tacrolimus trough level declined 30.4% from 10.2ng/ml to 7.1ng/ml, resulting in the 26.2% (from 4.2mg to 5.3mg per day) tacrolimus dose increase during the first 2 weeks after conversion. Adverse events after conversion were limited, and all patients currently showed normal liver function at the outpatient clinic.



Conclusions: The findings from this single center experience suggest that early conversion from Tac BID to Tac QD is safe and feasible in our conversion criteria. As dose adjustments after conversion were necessary, early conversion should be done in-hospital to monitor liver function and tacrolimus trough level closely. There were no serious adverse events in short term, but the benefit of early Tac QD conversion for a long term should be investigated.
References:
[1] Adam, R. et al. *Am J Transplant*, 2015; 15(5), 1267-1282.
[2] Uemoto, S. et al. *Transplant Proc*, 2014; 46(3), 749-753.
[3] Wu, Y. J. et al. *Ann Transplant*, 2016; 21, 30-34

P.1123

Liver transplantation in Germany – with specific focus on adult transplantation of extremely ill patients receiving „bottom-up" immunosuppression early after transplantation

Hans Schlitt[1], Andrea Proneth[1], Andreas ASchnitzbauer[1], Gabi Kirchner[2], Barbara Sinner[3], Martin Loss[1], Marcus NScherer[1], Edward KGeissler[1], Annette Pross[1], Ivan Gözce[1], Volker Benseler[1].
[1]Surgery, University of Regensburg, Regensburg, Germany; [2]Internal Medicine II, University of Regensburg, Regensburg, Germany; [3]Anaesthesiology, University of Regensburg, Regensburg, Germany.

Since the introduction of MELD-score based allocation in Germany by the end of 2006, the majority of waiting list patients receiving a liver graft, have had a MELD score of >30 at the time of transplantation; in fact, many patients had a labMELD score in that high range, much fewer a matchMELD score with markedly lower labMELD scores. Patients with labMELD scores above 30 are generally in very bad clinical condition with a high rate of decompensated cirrhosis, highly catabolic state, frequently on renal-replacement therapy, and sometimes even on artificial ventilation – some even in the state of ongoing (mostly bacterial) infection.
If such patients are transplanted – in many cases with suboptimal donor organs, which are the standard in Germany currently – they carry a very high risk of infections and wound healing problems, as well as a high rate of graft dysfunction, not related to acute rejection, in addition to a considerable rate of renal dysfunction around the time of transplantation – with ensuing high morbidity and mortality. In particular, their risks of infection, renal dysfunction, wound healing disturbances, and (infection-related) mortality are very much higher than that of "standard" liver transplant recipients, generally reported on in the broad literature. Therefore, in our center we aimed at a rather low immunosuppressive treatment early after transplantation, starting with very "mild" and calcineurin-inhibitor (CNI)-free treatment – and introducing CNIs not before posttransplant day 5, if at all.
In our study cohort we will present more than 150 adult patients transplanted over the last 10 years, who have received a CNI-free immunosuppression for at least 5 days (and frequently much longer) after transplantation. In spite of their bad condition at the time of transplantation, most of the patients recovered – although slowly -, renal function in many of the patients returned, and none of the patients had critical acute rejection episodes in spite of an initially very low immunosuppressive treatment without the use of CNIs for an average of more than 10 days. Detailed data will be presented at the meeting.
In conclusion, the data show that liver transplantation of critically ill end-stage cirrhotic patiens with high MELD scores, mostly with renal dysfunction, and frequently with ongoing infection requires aggressive adjustment of early postoperative immunosuppressive treatment in a way called "bottom-up" immunosuppression that is rather mild and is free of CNI for quite some time after transplantation.

P.1124

ABO-incompatible liver transplantation – a single center experience

Mariana Mihaila[1], Dana Tomescu[2], Laurentiu Micu[1], Florin Botea[3], Irinel Popescu[3].
[1]Department of Internal Medicine, Fundeni Clinical Institute, Bucharest, Romania; [2]Department of Anesthesia and Intensive Care, Fundeni Clinical Institute, Bucharest, Romania; [3]Center of General Surgery and Liver transplantation, Fundeni Clinical Institute, Bucharest, Romania.

Introduction: ABO-incompatible liver transplantation is not a routine intervention due to the high risk of hyperacute rejection. However, it represents a therapeutic option in emergency situations. We present our center's experience regarding ABO-incompatible liver transplantation between April 2000 – December 2015.
Patients and Methods: Between April 2000 – December 2015, 8 ABO-incompatible liver transplantations were performed in adults, as follows: 7 cases of whole liver transplantation and one case of living donor liver transplantation (LDLT). Of the 7 cases with whole liver, 3 were re-transplantations (in all these 3 patients, the first liver transplantation was ABO-compatible). All patients received induction therapy with basiliximab. The titre of isohemagglutinins was determined daily in the first 21 postoperative days, and plasmapheresis was performed in case of titres higher than 1:16.
Results: All patients with an initial ABO-incompatible liver transplantation are alive at the end of the follow-up period. Of the three re-transplanted patients, 2 died during the first month after re-transplantation, one through sepsis and the other through cardio-circulatory failure. We did not record any cases of hyperacute rejection.
Conclusion: ABO-incompatible liver transplantation is feasible and may represent an alternative in emergency situations, when an ABO-compatible donor is not available.

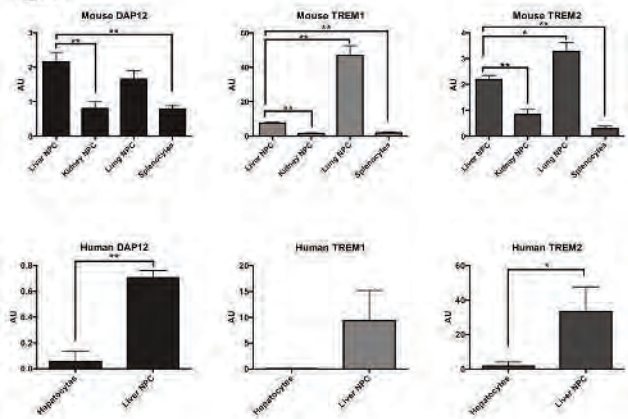
P.1125

Expression of DAP12 and its triggering receptor TREM2 by liver dendritic cells is associated with negative regulation of their allostimulatory function and transplant tolerance

Yoshihiro Ono, Osamu Yoshida, Shin Yokota, Daniel Fantus, Angelica Perez-Gutierrez, David A Geller, Angus WThomson.
Surgery, Starzl Transplant Institute, University of Pittsburgh, Pittsburgh, PA, United States.

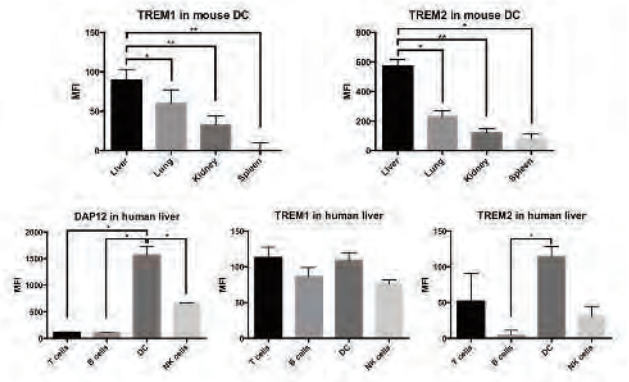
Background: DNAX-activating protein of 12kDa (DAP12) is a transmembrane adaptor protein that is highly expressed by myeloid cells and regulates their maturation/function. We have shown previously that DAP12 expression by liver allografts negatively regulates donor dendritic cell (DC) migration to host lymphoid tissue and alloreactive T cell responses. Moreover, liver allografts from DAP12-/- mice, unlike those from wild-type (WT) donors, are rejected acutely. By associating with triggering receptors expressed on myeloid cells (TREMs), DAP12 can either potentiate (TREM1) or inhibit (TREM2) leukocyte activation. We hypothesized that characterization of DAP12 in relation to TREM1/2 expression would provide important new insights into the role of these molecules in regulating DC function, alloimmunity and transplant tolerance.
Methods: Freshly-isolated liver, lung and kidney parenchymal (PC) and non-parenchymal cells (NPC) and bone marrow (BM) cells, blood and spleen mononuclear cells (MNC) from 8-12 week-old C57BL/6 mice were analyzed by RT-PCR for their expression of DAP12, TREM1 and TREM2. Human liver PC and NPC and blood MNC were also evaluated by RT-PCR. Flow cytometric analysis was performed in mouse splenocyte, liver, kidney and lung NPC and human liver NPC to confirm expression of these molecules.
Results and Discussion: Gene expression of DAP12 and TREM 1 and 2 by NPC was significantly higher than that by PC in mice and human. In mice, DAP12 gene expression by liver NPC was higher than that of lung, kidney, BM and blood MNC and significantly higher than that expressed by spleen cells. TREM1 and 2 genes were expressed at high levels by liver and lung NPC.

Figure 1



However, in flow analyses the intensity of TREM1 and 2 expression by mouse liver myeloid DC was significantly higher than that by lung, kidney and spleen DC. In human and mouse liver, TREM2 expression in DC was higher than that in T cells, B cells and natural killer cells. Human liver DC expressed DAP12 at significantly higher levels than other immune cells in the liver.

Figure 2



Since DAP12-/- mouse liver grafts are rejected acutely, DAP12 and its receptors play a crucial role in liver transplant tolerance. In this study, we evaluated the gene and protein expression of DAP12, TREM1 and 2 in different organs and cell subtypes. Better understanding of these roles could lead to improved approaches to inducing organ transplant tolerance or reducing/ eliminating immunosuppression after liver transplantation.

Conclusions: DAP12 and its associated triggering receptor TREM2 are expressed highly by liver myeloid DC with important implications for the role of those molecules in negative regulation of DC function and attenuation of the allloimmune responses.

References:

[1] Sumpter TL. DAP12 promotes IRAK-M expression and IL-10 production by liver myeloid dendritic cells and restrains their T cell allostimulatory ability. *J Immunol*. 2011; 186(4):1970-1980.

[2] Yoshida O. DAP12 deficiency in liver allografts results in enhanced donor DC migration, augmented effector T cell responses and abrogation of transplant tolerance. *American journal of transplantation*. 2014; 14(8):1791-1805.

P.1126

Single use of anti-platelet agent for prevention of early hepatic artery thrombosis after liver transplantation

Young Yeon Choi, Young Seok Han, Jae Min Chun, Heon Tak Ha, Yoon Jin Hwang.

Liver-Pancreas Transplantation, Kyungpook National University School of Medicine, Kyungpook National University Hospital, Daegu, Korea.

Hepatic artery thrombosis(HAT) after living donor liver transplantation(LDLT) is the most serious vascular complication that often results in irreversible damage to liver graft. Until now, it has been reported that the combination or sequential use of low molecular weight heparin(LMWH) and anti-platelet agent are effective in preventing HAT after liver transplantation(OLT). However, anti-coagulation therapy including LMWH has always had an unexpected bleeding risk. Hence, we have solely used anti-platelet agent from immediate postoperative period. However, the effect or safety for single use of anti-platelet agent in OLT is not well known. We performed 51 OLT (32 living donor liver transplantation and 19 deceased donor liver transplantation) between January 2015 and December 2015. Seven of 51 cases were ABO incompatible LDLT. Anti-platelet prophylaxis with aspirin (100 mg per day) was administered orally or through the levin tube, if there was no evidence of bleeding and serum platelet count was more than 30,000 per cubic millimeter. The patency of vascular structure was confirmed by follow-up imaging studies. Anti-platelet prophylaxis was lately started in 1 recipient because of immediate postoperative bleeding and it was re-administered after bleeding control. And anti-platelet agent has not been used in one patient whose platelet count was under 50,000 per cubic millimeter during 6 months after transplantation We have never experienced HAT.

Conclusively, single use of anti-platelet agent is safe and effective after liver transplantation and additional anti-coagulation therapy is not necessary for the prevention of HAT.

P.1127

Aggressive preoperative haemodialysis improves left ventricular ejection fraction in primary hyperoxaluria for combined kidney and liver transplantation

Ilankumaran Kaliamoorthy, Akila Rajakumar, Gomathy Narasimhan, Chandrasekar Venkatraman, Joy Varghese, Mettu Srinivas Reddy, Mohamed Rela.
Institute Liver Disease and Transplantation, Global Health City, Chennai, India.

Introduction: Primary hyperoxaluria (PH) is a rare autosomal recessive disease characterized by a deficiency of glyoxalate metabolism, overproduction and accumulation of oxalate in the body. Excess urinary oxalate excretion results in urolithiasis and ultimately renal failure. Oxalate deposition can occur in various organs including kidneys, heart, vascular and the skeletal system. Patients usually present with renal failure and they would require combined liver and kidney transplantation (CLKT) either as simultaneous or sequential procedures. Cardiomyopathy with low left ventricular ejection fraction (LVEF), which is common in this group of patients has shown to pose a major risk in the intraoperative period because of impaired ability to improve the contractile response of the heart at peak stress which might preclude the candidacy of these patients. Aggressive preoperative dialysis has shown to improve the ejection fraction by reducing oxalate burden and hence cardiac deposits making these patients fit enough to undergo liver and kidney transplantation.
Materials and Methods: We performed a retrospective study to analyze the cardiac status of our PH patients and the effects of preoperative aggressive dialysis in PH patients with low LVEF presenting for combined liver kidney transplantation between October 2009 and December 2015.
Results: A total of 9 patients with PH underwent CLKT during this period of which 5 were simultaneous LKT and 4 were sequential LKT. The study group included 5 children (<16 years of age) and 4 adults; 6 females and 3 males. 2 patients received DDLKT, 4 patients received simultaneous LDLKT, 1 patient received sequential LDLKT, 2 patients received live liver transplantation followed by cadaveric kidney transplantation.
Out of 9 recipients, 6 patients had low ejection fraction, at the time of presentation to our Institute, ranging from 33% to 42%. All six patients were then subjected to aggressive hemodialysis following which there was significant improvement of EF. We have found that there was 15-20% increase in EF in 3 months time in all patients. Our target was to improve EF to 45-50% before surgery. Intraoperative Tranesophageal Echocardiography and pulse contour cardiac output monitoring has been used in these patients as needed. There was no perioperative cardiac morbidity or mortality in these patients. One patient developed acute kidney graft rejection and required redo kidney transplant 8 months later. All these 6 patients recovered well and have 100% survival at one year.
Conclusion: Our data shows that in patients with PH requiring CKLT, aggressive hemodialysis for 3 months would significantly improve cardiac function and CLKT can be safely performed. Appropriate cardiac output monitoring in these patients will help in judicious fluid/inotrope management of these patients.

Patient ID	Age in years/Sex	Ejection Fraction in Echocardiogram at initial presentation	Intensive Dialysis duration	Ejection Fraction in Echocardiogram after aggressive hemodialysis
1	9/Female	36%	3 months	47%
2	11/Male	42%	2 months	50%
3	15/Female	40%	3 months	51%
4	24/Female	38%	7 months	54%
5	8/Female	40%	3 months	48%
6	19/Male	42%	4 months	50%

P.1128

A case of successful deceased donor liver transplantation of grand old age recipient

Pyoung-Jae Park, Sae Byeol Choi, Tae Wan Lim, Wan Bae Kim, Sang Yong Choi.
Surgery, Korea University Guro Hospital, Seoul, Korea.

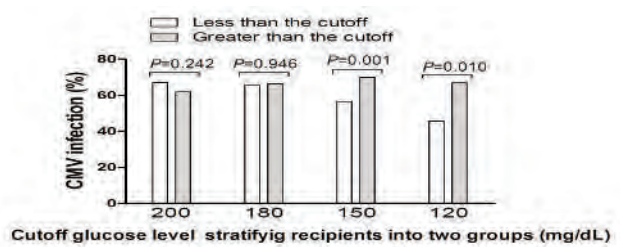
Patients older than 60 are undergoing transplantation with increasing frequency. Although reports that overall short-term patient survival rates in seniors undergoing liver transplantation are comparable to survival rates of younger adults, specific subgroups of older patients may not fare as well. Seniors with far-advanced end-stage liver disease are high-risk for liver transplantation and have poor survival rates.
We reported a case of successful deceased donor liver transplantation of 79 year-old recipient with 36 MELD score and in Korea, 2nd case of 79 years old recipient since KONOS was established.
A 79 year-old male patient having chronic hepatitis from hepatitis B virus with cirrhosis was admitted for altered mentality. He had arrhythmia and type 2 diabetes mellitus. He had suffered pancreas non-Hodgkin's lymphoma 11 years ago and totally recovered. Before admission, his performance status and occupation activity was good.
He had taken lamivudine and adefovir, but they were discontinued for 5 months. And hepatitis B was flared up and virus DNA titer increased to 9.89*106 copies/mL. Total bilirubin level was 17.43 mg/dL and INR prothrombin time was increased to 1.83. And then total bilirubin level was getting worse to 47.94 mg/dL and mentality was deteriolated to semicoma. MELD score was 24 at admission, but MELD score was increased to 36 just before liver transplantation. He was registered to status IIA on KONOS for deceased donor liver transplantation.
He was allocated from 40 year old male brain death donor. Size and condition of allograft were excellent and deceased donor liver transplantation was performed.
Postoperative course was good but mental recovery was improved steadily. Extubation at postoperative 9 days was done prudently, but aspiration pneumonia and multiple lung abscess were suffered. He was eventually improved through aggressive supportive care and antibiotics management. Moreover, acute cerebral infarction arising from atrial fibrillation was occured. But eventually he was improved undergoing rehabilitation and discharged at postoperative 60 days.
Triple combination immunosuppressant therapy (tacrolimus, mycophenolate mofetil and steroid) was selected and lower dose of immunosuppressant was adminstered during management of pneumonia and lung abscess.
For prevention of recurrence hepatitis B virus, combination of anti-hepatitis B immunoglobulin and tenofovir was used, but tenofovir administration was discontinued due to renal insufficiency.
He was recovered healthy and followed up 1 year after liver transplantation. Although seniors with far-advanced end-stage liver disease are high-risk for liver transplantation and have poorer survival rates, we suggested that liver transplantation for selected cases of a grand old age would be possible, and life expectancy, risk factor, and allocation policy will be investigated through large scale study.

P.1129

Postoperative blood glucose level lesser than 150 mg/dl is associated with decreased cytomegalovirus infection in seropositive liver transplant recipients

Hyo-Won Park, Sangbin Han, Jun Chul Shin, Jeongbo Park, Gaab Soo Kim.
Anesthesiology and Pain Medicine, Samsung Medical Center, Seoul, Korea.

Background: The maintenance of glycemic homeostasis is difficult early after liver transplantation due to the great extent of hepatic ischemia reperfusion injury, immunosuppression, and surgical stress in combination with chronically increased insulin resistance. Thus, hyperglycemia is frequently occurred during this critically ill period. Perioperative hyperglycemia is known to increase the risk of infectious complications in patients undergoing liver transplantation. However, the impact of postoperative hyperglycemia has not been evaluated. Thus, we hypothesized that lower blood glucose level during the early post-transplant period decreases the risk of cytomegalovirus (CMV) infection.
Methods: A total of 793 CMV-seropositive recipients (R+) who received living liver graft from CMV-seropositive donors (D+) were included in the retrospective cohort study. The association between mean postoperative glucose level and the incidence of CMV infection within 2 months after transplantation was evaluated by means of backward stepwise binary logistic. CMV infection was defined when a CMV pp65 antigenpositive is more than 1 in 400,000 white blood cells. The following factors were considered in multivariable analysis: age, gender, body mass index, graft macrosteatosis, graft-to-recipient weight ratio, ABO blood-type mismatch, diabetes, neutrophil-to-lymphocyte rate, high sensitivity C-reactive protein, ascites, graft ischemia time, etiology of liver disease, operative year, model for end-stage liver disease score, intraoperative blood loss, operative time, immunosuppression degree, and the number of transfused RBCs. Variables of P >0.10 were removed. The secondary outcomes were the maximum CMV titer and the incidence of CMV disease. CMV disease was defined as a positive antigenemia assay with more than 1 of the followings: unexplained fever, fatigue or general myalgia, leukopenia, thrombocytopenia, or biopsy-confirmed tissue-invasive CMV disease.
Results: Mean blood glucose level <200 or 180 mg/dL was not associated with decreased CMV infection. However, as shown in Fig. 1, mean blood glucose level <150 mg/dL was significantly associated with decreased CMV infection (69.2% versus 54.2%, P <0.001). Multivariable analysis showed that mean blood glucose <150 mg/dL is independently associated with CMV infection risk (odds ratio 0.50, 95% confidence interval 0.34-0.75, P <0.001). Fig. 2 shows that the maximum CMV titer was significantly higher in hyperglycemic recipients [6 (0-30) versus 2 (0-16), P <0.001]. CMV disease incidence was higher in hyperglycemic recipients with a marginal significance (16.9% versus 12.0%, P=0.080).
Conclusions: Strict glycemic management with the target blood glucose level <150 mg/dL might decrease the risk of early onset CMV infection in seropositive recipients after liver transplantation.



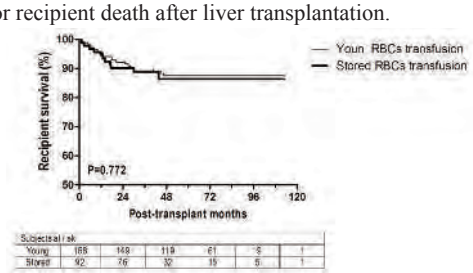
References:
[1] Kim JM, Kim SJ, Joh JW, et al. Is cytomegalovirus infection dangerous in cytomegalovirus-seropositive recipients after liver transplantation? Liver Transpl 2011;17:446-55.
[2] Wadhawan M, Gupta S, Goyal N, et al. Cytomegalovirus infection: its incidence and management in cytomegalovirus-seropositive living related liver transplant recipients: a single-center experience. Liver Transpl 2012;18:1448-55.
[3] Park CS. Predictive roles of intraoperative blood glucose for post-transplant outcomes in liver transplantation. World J Gastroenterol 2015;21:6835-41.

P.1130

Transfusion of old red blood cells stored during greater than 2 weeks is not associated with liver transplant death

Sangbin Han, Jeongbo Park, Jun Chul Shin, Ji Hye Kwon, Gaab Soo Kim.
Anesthesiology and Pain Medicine, Samsung Medical Center, Seoul, Korea.

Background: Red blood cells (RBCs) transfusion is essential for some recipients during and after surgery to maintain sufficient tissue oxygen supply. The quality of RBCs product decreased significantly after 2 weeks of cold storage, which is so-called RBCs storage lesion. It is unclear whether RBCs storage lesion increase post-transplant death risk while previous studies showed conflicting results. Thus, we aimed to evaluate the impact of transfusion of RBCs stored during ≥2 weeks on post-transplant death.
Methods: A total of 1721 units of RBCs transfused to 260 recipients during 2 weeks of adult-to-adult living donor liver transplantation were included in the study while recipients with mean RBCs storage duration of <2 weeks and, however, with at least 1 unit of RBCs stored during ≥2 weeks were excluded. The association between stored RBCs transfusion, which was defined the mean duration of transfused RBCs was ≥2 weeks, and death risk was evaluated by means of the backward stepwise Cox regression. The following factors were considered in the multivariable model: donor/recipient age, donor/recipient gender, body mass index, graft macrosteatosis, graft-to-recipient weight ratio, ABO blood-type mismatch, intermittent hepatic inflow occlusion during graft procurement, graft ischemia time, etiology of liver disease, hepatocellular carcinoma presence and biology, operative year, model for end-stage liver disease score, intraoperative blood loss, immunosuppression degree, and the number of transfused units of RBCs, fresh frozen plasma, platelet concentrates, and cryoprecipitate. Variables of P >0.10 were removed.
Results: All allogeneic RBCs were transfused after leukoreduction and irradiation. The mean storage duration of stored RBCs group and young RBCs group was 17 (15-19 days) and 8 (6-10 days), respectively. The maximum storage duration of two groups were 20 (18-23 days) and 10 (8-12 days), respectively. Fig. 1 show the insignificance of stored RBCs transfusion on recipient death (hazard ratio 0.90, 95% confidence interval 0.42-1.90, P=0.772). Multivariable analysis confirmed that stored RBCs transfusion was not significantly associated with death risk (odds ratio 0.91, 95% confidence interval 0.43-1.93, P=0.803). There were no recipients who died within 1 month of transplantation. There were no significant differences in mortality within 12months between stored RBCs group and young RBCs group (3 months, 2.2% versus 1.2%, P=0.616; 6 months, 3.3% versus 2.4%, P=0.701; 12 months, 5.4% versus 4.8%, P=0.775).
Conclusions: We found no evidence of stored RBCs as a contributing factor for recipient death after liver transplantation.



References:
[1] Dunn LK, Thiele RH, Ma JZ, Sawyer RG, Nemergut EC. Duration of red blood cell storage and outcomes following orthotopic liver transplantation. Liver Transpl 2012;18:475-81.
[2] Cywinski JB, You J, Argalious M, et al. Transfusion of older red blood cells is associated with decreased graft survival after orthotopic liver transplantation. Liver Transpl 2013;19:1181-8.

P.1131

The immunoexpression of HSP70, Glipican3, glutamin synthetase and beta-catenin in HCC post-liver transplantation. The association between Glipican3 andbeta-catenin with the presence of larger nodules

Elaine Ataide, Simone RPerales, Raquel BStucchi, Cecilia AFEscanhoela, Larissa BECosta, Ilka FSFBoin.
Unit Of Liver Transplantation, State University of Campinas, Campinas, Brazil.

Introduction: Hepatocellular carcinoma (HCC) is the sixth leading cause of cancer worldwide. Its recurrence has indices in literature ranging between 6% and 26%.
In the literature, many factors are associated with the higher risk of recurrence, without a clear definition of the best method that could possibly predict this highly lethal event.[1][2][3]
Objective: The aim of this study was to evaluate the immunoexpression of immunohistochemical markers: HSP70, Glipican3, Glutamin synthetase and Beta-Catenin, as long as studying their association with tumor characteristics and prognosis of patients undergoing liver transplantation for HCC.
Methods: We studied 90 patients who underwent liver transplantation between 1998 and 2012. Afterward we evaluated factors related to survival, tumor recurrence and the correlation of expression of the immunohistochemical markers.
Results: Immunohistochemical markers glutamin synthetase showed a positive trend toward a better survival. Positive HSP70 patients had a higher prevalence of histologic grade III. Patients with positive Glipican3 showed larger lesions and there was a higher number of patients with AFP greater than 200 ng / ml. Patients with positive Beta-Catenin showed larger nodules and there were more patients with histologic grade III. The association between Beta-catenin and Glipican3 presented positive association with larger nodules.[4][5][6][7]
Conclusion: Most of the markers studied presented correlation with at least one of the variables studied, confirming our hypothesis that these markers can indeed assist in assessing the prognosis of patients undergoing liver transplantation for HCC.
References:
[1] Chuma M, Sakamoto M, Yamazaki K, Ohta T, Ohki M, et al. Expression profiling in multistage hepatocarcinogenesis: identification of HSP70 as a molecular marker of early hepatocellular carcinoma. Hepatology. 2003 Jan; 37(1):198-207
[2] 1. Bioulac-Sage P, Balabaud C. Marker panels for the diagnosis of small hepatocellular carcinoma, a never-ending story. Clin Res Hepatol Gastroenterol. 2011 Sep; 35(8-9):513-5.
[3] 1. Kandil DH, Cooper K. Glypican-3: a novel diagnostic marker for hepatocellular carcinoma and more. Adv Anat Pathol. 2009 Mar; 16(2):125-9.
[4] 1. Ning S, Bin C, Na H, Peng S, Yi D, Xiang-hua Y, et al. Glypican-3, a novel prognostic marker of hepatocellular cancer, is related with postoperative metastasis and recurrence in hepatocellular cancerpatients. Mol Biol Rep. 2012 Jan; 39(1):351-7.
[5] 1. Osada T, Sakamoto M, Nagawa H, Yamamoto J, Matsuno Y, et al. Acquisition of glutamine synthetase expression in human hepatocarcinogenesis: relation to disease recurrence and ossible regulation by ubiquitin-dependent proteolysis. Cancer. 1999 Feb 15; 85(4):819-31.
[6] 1. Nejak-Bowen KN, Monga SP. Beta-catenin signaling, liver regeneration and hepatocellular cancer: sorting the good from the bad. Semin Cancer Biol. 2011Feb; 21(1):44-58.
[7] 32. Monga SP. Role of Wnt/β-catenin signaling in liver metabolism and cancer. Int J Biochem Cell Biol. 2011 Jul;43(7):1021-9.

P.1132

The possibility of radiotherapy for downstaging before living donor liver transplantation for hepatocellular carcinoma with portal vein tumor thrombus

Jin yong Choi[1], Jong Man Kim[2], Choon Hyuck Kwon[2], Jae-Won Joh[2], Gyu-Seong Choi[2], Jae Berm Park[2], Sung Joo Kim[2].
[1]Department of Surgery, Seoul National University College of Medicine, Seoul National University Hospital, Seoul, Korea; [2]Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea.

Background and Aims: Advanced HCC with portal vein tumor thrombus (PVTT) patients are excluded for liver transplantation (LT), according to the Milan criteria. But after the development of conformal RT, there have been several trials on RT as a bridge or downstaging management approach to LT. The purpose of our study is to evaluate the value of living donor liver transplantation (LDLT) following RT in PVTT patients.
Methods: This study took place between May 1996 and March 2013; a, total of 1360 patients were treated by LT in our institution, and 5 of those recipients had RT due to PVTT. To confirm the value of LDLT following RT in PVTT, we did a propensity-matched study retrospectively.
Results: There were no statistically significant differences in the clinical characteristics of the two groups. All LT was done by LDLT with duct to duct anastomosis and the mean operation time was 588 minutes. During the follow-up periods, in the LDLT following RT group, two recipients exhibited disease progression, but in the RT alone group, all patients had tumor ingrowths or either intra- or extra-hepatic metastasis. The OS for the LDLT following RT group was 1055 days and that of the RT alone group was 367 days, and there was a statistically significant difference.
Conclusions: LDLT following RT can be the treatment of choice for PVTT in select patients and when bile duct anastomosis was performed in RT recipients, a hepaticojejunostomy was recommended to prevent biliary complications.

P.1133

Impact of autologous and homologous blood transfusion in liver transplant on outcome of patients with hepatocellular carcinoma

Walid Elmoghazy[1,2], Glenda Meeberg[1], Norman Kneteman[1].
[1]Department of Surgery, Hepatobiliary, Pancreatic Surgery, and Transplantation, University of Alberta Hospital, Edmonton, AB, Canada; [2]Department of Surgery, Sohag University, Sohag, Egypt.

Aim: To evaluate the effect of transfusing packed red cells during and after transplantation on the outcome of patients with hepatocellular carcinoma (HCC).
Patients and Methods: Our study included HCC patients who underwent liver transplantation at University of Alberta Hospital between October 1990 and April 2013 and had a minimum 1 year of follow-up. We studied the impact of transfusing autologous blood during transplant, homologous blood during and after transplant on the outcome. Endpoints included HCC recurrence, 1- and 5-years graft loss or patient death. Cox's proportional hazard regression model was used to check the risk factors and area under curve was use to determine the cut-off value.
Results: A total of 150 patients were transplanted during this period with a mean follow-up of 4.8 ± 3.8 years. The mean age for patients was 55.9 ± 6.8 years, and 130 patients (86.7%) were males. Forty four patients (29.3%) had autologous blood during transplant, 90 patients (60%) received homologous blood transfusion during surgery, and 97 patients (64.7%) required blood transfusion at some point in the early post-transplant period (during hospital admission). The overall survival was 65.3% and 20 patients had HCC recurrence (13.3%). Using autologous or homologous blood transfusion intra-operatively had no significant effect on HCC recurrence, graft loss or patient death. Using blood transfusion in the early post-transplant period was associated with significantly higher graft loss and patient death (P=0.016) (HR=5.96, CI= 1.392-25.485). 1- and 5-year survivals were 96.2%, and 85.2%, respectively in patients who did not receive post-transplant transfusion and 79.3% and 62.4%, respectively in patients who had been transfused however; it had no impact on HCC recurrence. ROC analysis revealed that post-transplant transfusion is a predictor of graft loss or patient death (P=0.014) and the area under curve was 0.66, we choose post-transplant transfusion of 8 units as a cutoff value and it had sensitivity of 27% and specificity of 85%.
Conclusions: Autologous blood transfusion during liver transplant is not associated with increased risk of HCC recurrence. Blood transfusion in the early post-transplant period is a potential risk factor for graft loss and patient death

P.1134

Predictors of intra-operative red blood cell transfusion during liver transplant: A single center experience over 20 years

Walid Elmoghazy[1,2], Glenda Meeberg[1], James Shapiro[1], David Bigam[1], Norman Kneteman[1].
[1]Department of Surgery, Hepatobiliary, Pancreatic Surgery, and Transplantation, University of Alberta Hospital, Edmonton, AB, Canada; [2]Department of Surgery, Sohag University, Sohag, Egypt.

Background: Liver transplant has been associated with major blood loss and massive transfusion due to several factors. Despite improvement over the last decade, blood and blood products transfusion is still an issue during the transplant. In this study, we aimed to assess predictors for the need of intra-operative red cell transfusion during liver transplant.
Patients and Methods: This study includes adult patients who had liver transplant only between 1990 and 2013 due to chronic end-stage liver failure with a follow-up of at least one year post-transplant. Patients who had fulminant liver failure, combined liver-kidney transplants and children were excluded.
Several variables related to donor, recipient, graft, and operation were analyzed.
Data was summarized as mean ± standard deviation for continuous variables and number (%) for categorical variables. Correlation between blood transfusion and other factors was done using Person's and Sperman's methods, and regression analysis was used to determine the independent predictors for the need of blood transfusion. Survival of patients was calculated using Kaplan-Meir method.
Results: A total of 963 liver transplants met criteria and were included in this study. Mean age of patients at time of transplant was 51.5 ± 10.1 years, and 623 (65%) patients were males. Mean follow-up was 7.2 ± 5.5 years. The main indication for transplant was HCV in 211 (22%) patients followed by alcoholic cirrhosis in 144 (15%) patients. Mean MELD score at transplant was 18.1 ± 9.5 and 485 (50.8%) were Child-Pugh class C. During Transplant, 284 (30%) patients did not receive blood transfusion, 679 (70%) patients had a mean transfusion of 5.6 ± 8.8 units of packed RBCs during liver transplant procedure. Univariate analysis showed that pre-transplant MELD, INR, bilirubin, creatinine, albumin, Child-Pugh score, and clinical status, intra-operative renal replacement therapy, transfusion of platelets and plasma, liver graft type and transplant year correlated with the need for transfusion of packed RBCs ($P<0.05$). Multivariate regression analysis showed that intra-operative red blood cell transfusion is related to Child-Pugh score and clinical status at time of transplant, intra-operative continuous replacement therapy, and transplant year ($P<0.05$).
Survival of patients at 1, 5, and 10-years was 94.6%, 81.7%, and 64%, respectively. Blood transfusion was associated with lower patient survival at 1 year post-transplant.
Conclusions: Need for intraoperative red cell transfusion has improved over time, and it is related to liver reserve, clinical status and use of intra-operative renal replacement therapy, and it is associated with lower one year survival post liver transplant.

P.1135

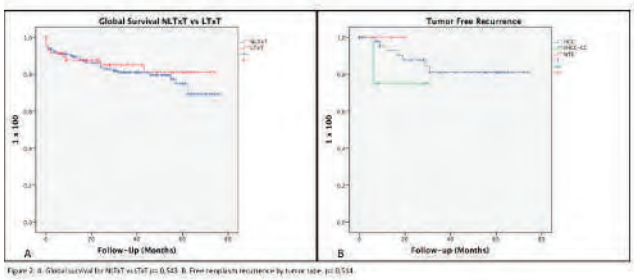
Long term results of liver transplantation for malignances

Nestor Pedraza Alonso, Andres Fraile, Victorio Cervera, Eugenia Serio, Silvina Yantorno, Pablo Barros Schelotto, Valeria Descalzi, Gabriel Gondolesi.
Instituto de Trasplante Multiorganico, Hospital Universitario Fundación Favaloro, Buenos Aires, Argentina.

Background: Transplantation has become one of the main accepted therapy for patients with liver malignances. Although inclusion criteria have been well established for hepatocellular carcinoma (HCC) recipients, not consensus has been yet established to transplant neuroendocrine tumors (NET) and combined hepatocellular cholangiocarcinoma (cHCC-CC) patients. The aim of the present study is to review the outcome of cancer patients receiving liver transplant in our center.
Methods: From 9/2009 to 12/2015, 295 adult liver transplants were performed. Patients with HCC, cHCC-CC and NET were grouped as Liver Transplant for tumor (LTxT) and were compared to no tumoral LTx (NLTxT). Demographics, transplant, and pathological variables, recurrence rate and survival were analyzed from a prospective database. Living donor, splits, combined and domino liver transplants were excluded from the analysis. All statistical analyses were performed by SPSS v 22.0
Results: 216 patients were included, from them, 59 were LTxT (27.3%): 52 corresponded to HCC, 4 to cHCC-CC and 3 to NET. Analyzed variables are included in table 1. Five years patient survival was 81 % for the LTxT group and 75% for NLTxT group (p:0.543) Fig1A. The global recurrence rate was 13.6%. In the LTxT group, at 74 months of follow-up, 79% of the HCC recipients are alive and 80.4% are recurrence free; cHCC-CC pts had 100% survival, with only one recurrence (25%) after 28 months of follow-up and NET pts had 100% survival and free of recurrence after 20 months of follow'up (p=0.515)Fig2B.;. When the HCC sub-group was analyzed, 22 pts were outside Milan and 30 within Milan criteria, 5-year survival and recurrence free patient was 78%/70% and 82%/91% respectively (p=0.925).
Conclusions: Our results support that LTxT indication have similar survival rates that those found at NLTxT. Strict criteria's for HCC needs to be followed, even for those exceeding Milan criteria. cHCC-CC found in the explant had comparable long term results to HCC recipients in our center. LTx for NET under selective criteria become a valid alternative.

		N	Media	SD	p
Age	NLTxT	156	51,8	13,1	0,04
	LTxT	59	58,9	9,2	
Gender	NLTxT	156	1,4	,5	0,07
	LTxT	59	1,2	,4	
Real Meld	NLTxT	148	23,1	6,8	0,173
	LTxT	59	12,8	6,2	
Additional Meld	NLTxT	145	19,7	6,7	0,000
	LTxT	59	21,5	5,3	
DRI	NLTxT	153	1,4	,2	0,004
	LTxT	59	1,6	,3	
Time in Waiting List	NLTxT	148	6,1	11,5	0,608
	LTxT	58	7,7	11,2	
Surgical Complication	NLTxT	156	,4	,4	0,616
	LTxT	59	,4	,4	

Figure 1: Demographic and variables analyzed



References:
[1] Song S, Moon HH, Lee S, Kim T, Shin M, Kim JM, et al. Comparison Between Resection and Transplantation in Combined Hepatocellular and Cholangiocarcinoma. Transplant Proc [Internet]. Elsevier Inc; 2013;45(8):3041–6. Available from: http://dx.doi.org/10.1016/j.transproceed.2013.08.064
[2] Frilling A, Giele H, Vrakas G, Reddy S, Macedo R, Wasan H, et al. Modified Liver-free Multivisceral Transplantation for a Metastatic Small Bowel Neuroendocrine Tumor: A Case Report. Transplant Proc [Internet]. Elsevier Inc.; 2015;47(3):858–62. Available from: http://dx.doi.org/10.1016/j.transproceed.2015.01.007
[3] Garancini M, Goffredo P, Pagni F, Romano F, Roman S, Sosa JA, et al. Combined Hepatocellular-Cholangiocarcinoma?: A Population-Level Analysis of an Uncommon Primary Liver Tumor. 2014;952–9

P.1136

Hepatocellular carcinoma is an independent predictor of recurrent hepatitis C infection following donation after cardiac death liver transplantation

Shiva Kumar.
Center for Liver Disease & Transplantation, Aurora St. Luke's Medical Center, Milwaukee, WI, USA &, Digestive Disease Institute, Cleveland Clinic Abu Dhabi, Abu Dhabi, United Arab Emirates.

Extended criteria donors, including donation after cardiac death (DCD) allografts, offer the potential of increasing the available donor pool in liver transplantation (LT). The impact of recipient hepatitis C (HCV) infection on outcome following DCD LT and donor and recipient variables that impact risk of recurrent HCV in this setting are not clear. We aimed to study the outcomes of DCD liver grafts used in HCV recipients and determine predictors of outcome.
We performed a retrospective analysis of all transplants performed over a 7 year period at a single center (July 2007 - Feb 2014). All DCD procurements were done by the same team of surgeons using standard protocol. HCV RNA positive recipients of solitary primary LT utilizing DCD allografts (Group 1) were compared to donation after brain death (DBD) HCV recipients (Group 2) and DCD non-HCV recipients (Group 3) during the same study period. The following variables were analyzed: donor age, warm and cold ischemic time, recipient age, MELD score, presence of hepatocellular carcinoma (HCC). HCV recurrence was defined as biochemical graft dysfunction with detectable HCV RNA by PCR, confirmed histologically. Severe recurrence was defined as presence of > stage 2 fibrosis within a year of LT or development of cirrhosis secondary to recurrent HCV. Primary outcome measures were incidence and severity of HCV recurrence and graft survival. Variables were compared using chi-square test for categorical variables and student's t-test for continuous variables. Competing risk analysis was used to determine incidence of HCV recurrence post LT.
196 LT were performed during the study period, of which, 159 were primary single organ LT. Among these, 64 patients (33%) were HCV RNA positive at the time of LT. Among HCV patients, 22 (36%) received a DCD allograft and 42 (64%) did not. There were 22, 42 and 27 patients respectively in Groups 1, 2 & 3. The 3 groups were similar with respect to all baseline variables, except MELD score at transplant, which was lower in Group 1 than Group 2 (21.3 vs. 27.1; $p < 0.01$).
HCV recurrence at 1 and 3 years in Group 1 vs. Group 2 were respectively 21.9% vs. 8.6% and 58.7% vs. 44.5% (p=0.10). Even though there was a trend toward greater HCV recurrence post DCD LT, this did not reach statistical significance. Despite this trend, there was no difference in graft survival among the 3 groups. Severe HCV recurrence, however, was more likely in DCD recipients: it was noted at 1 and 3 years in 11.4% and 29.7% of patients in Group 1 and only 2.9% and 13.8% respectively in Group 2 (p=0.05). In univariate analysis, the only predictor of HCV recurrence post DCD LT was the presence of HCC (HR (95% CI); 0.41(0.2-0.84); $p = 0.015$).
There was a trend towards greater risk of recurrent HCV in recipients of DCD LT. Longer duration of follow up is needed to determine impact of this observation on graft survival. Whether the presence of HCC plays a pathogenic role in the recurrence of HCV or whether it is just a surrogate marker for more aggressive HCV infection at higher risk of post-transplant recurrence is unclear. The presence of HCC may enable identification of a subset of patients with HCV at higher likelihood of recurrence following DCD LT.

P.1137

Immunohistochemical markers for prognosis of hepatocellular carcinoma after liver resection and liver transplantation

Soo Ho Lee[1], Jun Suh Lee[2], Gun Hyung Na[2], Young Kyoung You[2], Dong Goo Kim[2].
[1]Surgery, Uijeongbu St.Mary's Hospital, Uijeongbu, Korea; [2]Surgery, Seoul St.Mary's Hospital, Seoul, Korea.

Background: There are many treatment methods for HCC, but the most reliable treatments are surgical treatments such as liver resection (LR) and liver transplantation (LT)[1]. There were differences in progression and prognosis of hepatocellular carcinoma (HCC) after surgery between liver resection (LR) and liver transplantation (LT). In this study, immunohistochemical (IHC) markers associated with the prognosis of HCC were assessed.
Methods: Data were collected from 167 patients who underwent LT (n = 41) or LR (n = 126) for HCC. IHC markers including alpha fetoprotein (AFP), p53, Ki-67, cytokeratin 7 (CK7), and cytokeratin 19 (CK19) were compared between the treatment methods in tumor tissue.
Results: AFP- and p53-negative patients had a significantly higher survival rate than AFP- and p53-positive patients (AFP: disease free survival [DFS] p=0.006, overall survival [OS] p=0.016; p53: DFS p=0.005, OS p=0.038) in the LR group. CK19 was related to DFS (p=0.005), while CK7 (p=0.014) and CK19 (p=0.06) were related to OS in the LT group. When we combined factors that were significant in both groups (LR: AFP and p53, LT: CK7 and CK19), all negative patients had a higher survival rate (LR: DFS p=0.025, OS p=0.043, LT: DFS p=0.034, OS p=0.008).
Conclusions: p53 and AFP were predictors for poor prognosis of HCC after LR; CK7 and CK19 could be predictors for poor prognosis of HCC patients after LT.

References:

[1] Pang TC, Lam VW.: Surgical management of hepatocellular carcinoma. *World J Hepatol.* 2015;7(2):245-52.
[2] Zhou L, Rui JA, Wang SB, et al.: Early recurrence in large hepatocellular carcinoma after curative hepatic resection: prognostic significance and risk factors. *Hepatogastroenterology.* 2014;61(135):2035-41.
[3] Baccarani U, Piselli P, Serraino D, et al.: Comparison of de novo tumors after liver transplantation with incidence rates from Italian cancer registries. *Dig Liver Dis.* 2010;42(1):55-60.
[4] Chaiteerakij R, Addissie BD, Roberts LR, et al.: Update on biomarkers of hepatocellular carcinoma. Clin Gastroenterol Hepatol. 2015;13(2):237-45.
[5] Guzman G, Alagiozian-Angelova V, Layden-Almer JE, et al.: p53, Ki-67, and serum alpha feto-protein as predictors of hepatocellular carcinoma recurrence in liver transplant patients. *Mod Pathol.* 2005;18(11):1498-503.
[6] Durnez A, Verslype C, Nevens F, et al.: The clinicopathological and prognostic relevance of cytokeratin 7 and 19 expression in hepatocellular carcinoma. A possible progenitor cell origin. *Histopathology.* 2006;49(2):138-51.
[7] Morrison C, Marsh W, Jr., Frankel WL. A comparison of CD10 to pCEA, MOC-31, and hepatocyte for the distinction of malignant tumors in the liver. *Mod Pathol.* 2002;15(12):1279-87.
[8] Minervini MI, Demetris AJ, Lee RG, et al.: Utilization of hepatocyte-specific antibody in the immunocytochemical evaluation of liver tumors. *Mod Pathol.* 1997;10(7):686-92.
[9] Chan JK, Ip YT, Cheuk W. The utility of immunohistochemistry for providing genetic information on tumors. *Int J Surg Pathol.* 2013;21(5):455-75.
[10] Dodson RM, He J, Pawlik TM.: Resection and transplantation for hepatocellular carcinoma: factors influencing surgical options. *Future Oncol.* 2014;10(4):587-607.
[11] Poon RT-P, Fan S-T, Lo C-M, et al.: Intrahepatic recurrence after curative resection of hepatocellular carcinoma. *Ann Surg.* 1999;229(2):216-22.
[12] Gunay Y, Guler N, Akyildiz M, et al.: Management of patients with recurrent hepatocellular carcinoma following living donor liver transplantation: a single center experience. *Gulf J Oncol.* 2014;1(15):12-8.
[13] Yang Y, Nagano H, Ota H, et al.: Patterns and clinicopathologic features of extrahepatic recurrence of hepatocellular carcinoma after curative resection. *Surgery.* 2007;141(2):196-202.
[14] Lee KK, Kim DG, Moon IS, et al.: Liver transplantation versus liver resection for the treatment of hepatocellular carcinoma. *J Surg Oncol.* 2010;101(1):47-53.
[15] Kirstein MM, Vogel A.: The pathogenesis of hepatocellular carcinoma. *Dig Dis.* 2014;32(5):545-53.
[16] Moudgil V, Redhu D, Dhanda S, et al.: A review of molecular mechanisms in the development of hepatocellular carcinoma by aflatoxin and hepatitis B and C viruses. *J Environ Pathol Toxicol Oncol.* 2013;32(2):165-75.
[17] Lee C-W, Kuo W-L, Yu M-C, et al.: The expression of cytokeratin 19 in lymph nodes was a poor prognostic factor for hepatocellular carcinoma after hepatic resection. *World J Surg Oncol.* 2013;11(136).
[18] Liu G, Wang K, Li J, et al.: Changes in serum alpha fetoprotein in patients with recurrent hepatocellular carcinoma following hepatectomy. *J Gastroenterol Hepatol.* 2015;30(9):1405-11.
[19] Hsu CY, Liu PH, Lee YH, et al.: Using serum alpha-fetoprotein for prognostic prediction in patients with hepatocellular carcinoma: what is the most optimal cutoff? *PLoS One.* 2015;10(3):e0118825.
[20] Komuta M, Spee B, Vander Borght S, et al.: Clinicopathological study on cholangiolocellular carcinoma suggesting hepatic progenitor cell origin. *Hepatology.* 2008;47(5):1544-56.

P.1138

Should hepatocellular carcinoma with portal vein tumor thrombus be contraindicated for living donor liver transplantation?

Deok-Bog Moon, Sung-Gyu Lee, Shin Hwang, Ki-Hun Kim, Tae-Yong Ha, Gi-Won Song, Dong-Hwan Jung, Gil-Chun Park. Hepato-Biliary Surgery and Liver Transplantation, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea.

Purpose: Even though liver transplantation(LT) for HCC with portal vein(PV) invasion has been contraindicated because of poor prognosis after LT, living-donor liver transplantation(LDLT) for those patients is performed infrequently after informed consent because of strong request of donor & recipient. Our experiences of LT for HCC with PV invasion hinted that some patients could survived unexpectedly long. Here, we aim to find their outcomes and favorable prognostic factors by reviewing LT patients for HCC with PV invasion.
Methods: From October 1993 to December 2013, LT for HCC with PV invasion was performed in 54 patients(3.8%) among total 1473 LT patients for HCC. LDLT was 90.2% and deceased donor LT was 9.8%. Variables related to HCC were evaluated how much affected on survivals.
Results: The overall and disease free 5-year survival rate after LDLT for HCC with PV invasion were 37.9% and 35.0%, respectively. On pre-LT CT scan, portal vein tumor thrombus(PVTT) level were main PV 1.9%, 1st order brahcnes 29.6%, 2nd order branches 29.6%, 3rd order branches 38.9%. Among PVTT level, there were no overall and disease free survival differences. Pretransplant treatment for HCC was performed for 46 patients(85.2%) and the viability of PVTT on pre-LT CT scan disappeared in 13 patients(24.1%). The mean survival of the patients with history of pre-transplant treatment for HCC was better than the patient without them. The patients without viability of PVTT on pre-LT CT scan had significant better 5-year overall survival (100%) than the others (13.4%).
Conclusions: Among HCC patients with PV invasion, selected patients without viability of PVTT might be a good candidate for LT minimizing the previously reported harmful effect related to PVTT.

P.1139

Impact of endoscopic ultrasound guided fine needle aspiration on prospective liver transplant patients with cirrhosis and hepatocellular carcinoma or pyrexia of unknown origin

NarendraS. Choudhary[1], Rajesh Puri[2], Saigal Sanjiv[1], Neeraj Saraf[1], Rajiv R.Singh[2], Rinkesh Bansal[2], Vinit Shah[2], Haimanti Sarin[3], Mridula Guleria[3], Randhir Sud[2], Prashant Bhangui[1], Arvinder S.Soin[1]. [1]Institute of Liver Transplantation and Regenerative Medicine, Medanta-The Medicity, Gurgaon, India; [2]Digestive and Hepatobiliary Sciences, Medanta-The Medicity, Gurgaon, India; [3]Department of Cytopathology, Medanta-The Medicity, Gurgaon, India.

Background: Diagnosis of metastatic hepatocellular carcinoma (HCC) or etiology of pyrexia of unknown origin (PUO) is important in patients with cirrhosis for optimal management and to prevent flare up of malignancy or infectious disease after liver transplantation. However, there is very limited literature available in patients with HCC and there is no data on PUO in patients with cirrhosis.
Methods: the study was conducted from January 2013 to January 2016 at a tertiary care centre. A total of 76 (73 lymph nodes, 3 adrenal) endoscopic ultrasound (EUS) guided fine needle aspirations (FNA) were done in 72 patients. Indication of FNA was HCC in 47 and PUO in rest. Data is shown as median (25-75 IQR). Three patients received platelet transfusion and 17 patients received fresh frozen plasma before EUS FNA procedure.
Results: The study group aged mean 53.4±9.3 (SD) years, 60 were males. Parameters of severity of liver disease were CTP 7(6-10), MELD 12(8-18), 36 had ascites and 25 had ≥2 grade of esophageal varices. Indication of FNA was HCC in 47 and PUO in 25 patients. The cytopathological diagnosis were metastatic disease in 16 (including 1 adrenal), granulomatous change in 5 (2 AFB stain positive), histoplasmosis in 3 (including 2 adrenals), 46 nodes were reactive and 6 FNAs showed inadequate cellularity. The pathologic nodes were significantly larger at small axis when compared to reactive nodes [12(8.5-17) mm versus 8(6-12) mm respectively, p=0.001]. Complications included mild hepatic encephalopathy related to sedation in 2 and mild mucosal bleed that stopped on conservative management in 1, all these patients had Child's C status.
Conclusion: EUS guided FNA changed the management in 24/72 (33.3%) patients and precluded liver transplant in 16/47 (34%) patients with HCC.
Yogesh Saini; Payal Arora; Vipin Thomas

P.1140

Liver transplantation for unresectable intrahepatic cholangiocarcinoma originated from intraductal papillary neoplasm - case report

Sung-Pil Yun, Hyung-Il Seo.
Department of Surgery, Pusan National University Hospital, Busan, Korea.

Intrahepatic cholangiocarcinoma represents a controversial indication for liver transplantation, due to lower disease-free and overall survival rate achieved by liver transplantation for intrahepatic cholangiocarcinoma. Therefore, few centers reported satisfactory result after liver transplantation for intrahepatic cholangiocarcinoma, in highly selected patients. Also, there is few report of liver transplantation for unresectable intrahepatic cholangiocarcinoma. Here, we describe the successful performance of total hepatectomy with left side diaphragm resection and re-construction followed by living donor liver transplantation for unresectable intrahepatic cholangiocarcinoma. A 66-year-old female patient present with hepatic encephalopathy, biliary cirrhosis and malignant bile duct IPMT. She underwent cholecystectomy 30 years ago and repeatedly admitted due to recurrent hepatic encephalopathy. Initially, her CEA and CA 19-9 concentration were 94.6 ng/mL and 657.4 U/mL, respectively. CT scan and MRI revealed that left intrahepatic duct dilatation with solid and cystic mass at left lobe of liver suggesting malignant bile duct IPMT and ascites. PET CT showed no distant metastases. We planned living donor liver transplantation for curative resection and to treat hepatic encephalopathy. The donor was the patient's 30-years-old daughter. She was acceptable as a right lobe donor. During total hepatectomy, cholangiocarcinoma at left lobe invasion to left diaphragm was observed, and then we perform resection of diaphragm with pericardium and reconstruction with 2mm Gore Tex membrane. The pathological examination of resected liver revealed a well differentiated cholangiocarcinoma with periductal invasion (pT4) from intraductal papillary neoplasm and hepatoduodenal LN metastases (pN1), and metastatic cholangiocarcinoma was found on resected diaphragm. The postoperative course was uneventful, and in present, 18 months after transplantation, the patient is alive, without recurrence.

P.1141

Technical refinement to prevent hepatic outflow disturbance in right lobe living donor liver transplantation: New simplified one-orifice venoplasty

Joo Dong Kim, Dong Lak Choi.
Division of Hepatobiliary Pancreas Surgery and Abdominal Organ Transplantation, Department of Surger, Catholic University of Daegu College of Medicine, Daegu, Korea.

Middle hepatic vein (MHV) reconstruction is often essential to avoid hepatic congestion and serious graft dysfunction in living donor liver transplantation (LDLT). The aim of this report was to introduce evolution of our MHV reconstruction technique and the excellent outcomes of simplified one-orifice venoplasty.

We compared clinical outcomes with three types of our one-orifice technique through retrospective review of recipients who underwent LDLT using right lobe graft at our institution from January 2010 to June 2015; group I (n = 60) received one-orifice technique that create the wider single outflow with patchwork method including central patch, group II (n = 106) received one-orifice technique that create single outflow using patch venoplasty without central patch, and group III (n = 34) received more simplified one-orifice technique that invaginate reconstructed MHV into right hepatic vein without patch venoplasty.

Patient demographics and the overall complication rates did not differ significantly between the three groups, but cold ischemic time and operation time in group II and III were significantly lower than those in group I (123.0 min versus 104.7 min versus 109.6, P = 0.000 and 530.3 min versus 398.6 min versus 341.9 min, P=0.000, respectively). No significant difference between two groups in in-hospital mortality and overall survival was observed. The early patency rates of MHV were similar in the three groups; 96.7%, 98.1% and 100% on postoperative day 7 and 95.0%, 94.3% and 94.1% on postoperative day 14, respectively ($p > 0.05$). Peak values in liver functional index within one month after LDLT was not different between three groups and especially, MHV stent insertion during the early post-transplant period did not occur in group III during follow-up period.

This technique is easy to perform without a learning curve or vein patch such as autologous vein or cryopreserved cadaveric vessels. Furthermore, our new simplified one-orifice technique could be an effective method of overcoming technical difficulties and the outflow disturbance in right lobe LDLT without complex bench work to create large outflow.

References:

[1] Lee SG. Techniques of reconstruction of hepatic veins in living-donor liver transplantation, especially for right hepatic vein and major short hepatic veins of right-lobe graft. *J Hepatobiliary Pancreat Surg*. 2006: 13: 131.

[2] Yi NJ, Suh KS, Lee HW et al. An artificial vascular graft is a useful interpositional material for drainage of the right anterior section in living donor liver transplantation. *Liver Transpl*. 2007: 13: 1159.

[3] Kishi Y, Sugawara Y, Matsui Y et al. Alternatives to the double vena cava method in partial liver transplantation. *Liver Transpl*. 2005: 11: 101.

[4] Pomposelli JJ, Akoad M, Khwaja K et al. Evolution of anterior segment reconstruction after live donor adult liver transplantation: A single-center experience. *Clin Transplant* .2012: 26: 470.

P.1142

Is systemic heparinization necessary during living donor hepatectomy?

Joo Dong Kim, Dong Lak Choi.
Division of Hepatobiliary Pancreas Surgery and Abdominal Organ Transplantation, Department of Surger, Catholic University of Daegu College of Medicine, Daegu, Korea.

Systemic heparinization has traditionally been performed in most transplant centers during living donor hepatectomy (LDH) due to the possibility of graft vascular thrombosis during the warm ischemic period, which could impair graft function or cause graft loss. However, no consensus on the use of systemic heparinization during LDH has yet emerged. The aim of the present study was to compare donor and recipient outcomes with reference to systemic heparinization and to determine if systemic heparin need not be administered to living donors. We analyzed the outcomes via retrospective review of 206 LDHs performed in our institution from January 2011 to June 2015; 79 donors received systemic heparinization (group I) whereas 127 did not, but the liver graft was flushed with a heparinized perfusate (group II). Patient demographics, intraoperative parameters, laboratory data, postoperative complications, and survival rates were compared between the two groups. The overall complication rates did not differ significantly between the two groups but postoperative bleeding occurred more frequently in group I than group II (8.9% versus 2.4%, P = 0.035). Moreover, the incidence of significant postoperative bleeding (requiring red blood cell transfusion) in group II was significantly lower than in group I (1.6% versus 7.6%, P=0.03) The incidence of graft vascular thrombosis was similar in both groups and no graft loss caused by vascular thrombosis was evident during the early postoperative period. Moreover, no difference in either posttransplant graft function or survival rate was apparent between the two groups. The rates of decrease in donor hemoglobin, hematocrit, and platelet count levels during the early postoperative period were significantly higher in group I compared to group II.
In conclusion, omission of systemic heparinization during LDH is both feasible and safe, with no adverse effect on donor or recipient outcomes.
References:
[1] Yoo T, Kim SH, Kim YK, Cho SY, Park SJ. Low-dose heparin therapy during living donor right hepatectomy is associated with few side effects and does not increase vascular thrombosis in liver transplantation. *Transplant Proc*. 2013;45:222-224.
[2] Crotty C, Tabbakh Y, Hosgood SA, Nicholson ML. Systemic heparinisation in laparoscopic live donor nephrectomy. *J Transplant*. 2013;2013:138926.
[3] Lo CM. Complications and long-term outcome of living liver donors: A survey of 1,508 cases in five asian centers. *Transplantation*. 2003;75:S12-5.
[4] Goldfarb DA. Use of heparin and protamine sulfate during laparoscopic donor nephrectomy. *J Urol*. 2003;169:2433-2434

P.1143

The best level of recipient's biliary duct for anastomosis to prevent postoperative anastomosis stricture

Keisuke Arai, Kaori Kuramitsu, Takumi Fukumoto, Masahiro Kido, Motofumi Tanaka, Hisoka Kinoshita, Tetsuo Ajiki, Hirochika Toyama, Sadaki Asari, Tadahiro Goto, Ku Yonson.
Division of Hepato-Biliary-Pancreatic Surgery, Department of Surgery, Graduate School of Medicine, Kobe University, Hyogo, Japan.

Introduction: Currently, end-to-end ductal anastomosis is the standard biliary reconstruction technique because of the advantages for the treatment of postoperative anastomosis strictures. However, it is still unclear which level of recipient's biliary duct is best for anastomosis. The aim of this study is to compare the two different level of biliary duct anastomosis cite from the aspect of postoperative biliary complications.
Patients and Methods: All the recipients who underwent LDLT from 2006 to 2015 at our facility and performed postoperative magnetic resonance cholangiopancreatography (MRCP) are included in the study. Based on the level of anastomosis, recipients were divided into two groups; those who were anastomosed with hepatic duct (HD group), and those with common bile duct (CBD group). Patients were performed MRCP postoperatively after the removal of external biliary drainage tubes to detect the occurrence of biliary complication. A 3-dimensional (3D) reconstruction program (ziostationa2 ver.2.4; Ziosoft, Tokyo, Japan) was used to detect the angle of anastomosis from the MRCP images. Angle of anastomosis was defined as the angle between the vectors of graft bile duct and the recipient bile duct detected from the MRCP images (figure). The occurrence of postoperative biliary complications and the angle of anastomosis were compared between the two groups.
Results: Of all, 23 recipients were included in the study; 17 recipients (73.9%) were categorized in the HD group and 6 recipients (26.1%) were in the CBD group. There were no statistical differences for the characteristics of the recipients between the two groups. Biliary stricture occurred in 9 recipients (39.1%), and all the recipients were from the HD group (52.9%). There were no biliary leakage, and all the biliary strictures were treated endoscopically. The mean angle of anastomosis was greater in the CBD group compared to the HD group (114.3° vs. 89.9°, p = 0.0152).
Conclusion: From the study, it was suggested that anastomosis with recipient CBD resulted in less incidence of biliary strictures compared to HD anastomosis. Together with the fact that CBD anastomosis has greater angle compared with HD anastomosis, CBD anastomosis is favorable considering the approach for postoperative endoscopical treatment.

P.1144

Causes of mortality in patients transplanted for NASH or cryptogenic cirrhosis: A single center experience over 23 years!

Chandra S Bhati, Maria Rivera, Amit Sharma, Connie Jackson, Michael Idowu, Adrian Cotterell, Mohammed Siddiqui, Levy FMarlon.
Division of Transplant Surgery, Virginia Commonwealth University, Richmond, VA, United States.

Background: Nonalcoholic steatohepatitis (NASH) is growing indication for liver transplantation (LT) in the western world. Epidemiological data suggests that long-term mortality in patients transplanted for NASH or cryptogenic cirrhosis is similar or increased to other causes of chronic liver disease. it is unclear what constitutes long-term mortality in these patients after LT.
Aim: To evaluate causes of mortality in patients transplanted for NASH (or cryptogenic cirrhosis).
METHOD: This was a retrospective review of all patients transplanted between years 1990-2013 at Hume Lee transplant Center. Patients transplanted for cryptogenic cirrhosis who met criteria for metabolic syndrome prior to liver transplant were considered to have NASH-related cirrhosis in the absence of other identifiable etiology.
Results: Over the span of 23 years, 108 patients were transplanted for NASH or cryptogenic cirrhosis. Of the 108 patients, 34 patients died over the study period. Thirty-one patients received disease donor liver transplantation while three patients received right lobe living donor transplantation. The overall all-non-adjusted mortality rate during 23 years was 31%. The median age at the time of transplant was 59.5 (26-67 years). The average time from listing to transplant was 237 days. Mean patient survival was 8.9 years. Average recipient BMI at the time of transplant was 31.6 kg/m2 and average MELD score was 21.26. Average warm ischemia time was 41 min and cold ischemia time was 409 min. The leading causes of mortality in these patients were cancer (primary as well as distant, 23%) and infection (23%) followed by cardiac (11.8%). About 26% had history of smoking tobacco. Cardiac history was noted in 41 % recipients and 35 % had preexisting diabetes. Preexisting DM (p=0.001) and age over 60 years (p=0.007) were the only significant risk factors for mortality in this study. About one third of the patients (11) died during first 5 years and two third patients (20) died in 10 years period.
Conclusion: Pre-transplant NASH is associated with increased risk of cardiovascular disease and non-HCC malignancy; however, leading cause of long-term mortality in patients transplanted for NASH is infection and cancer. Preexisting DM and age over 60 years were the significant risk factors in recipients for mortality among NASH patients receiving liver transplantation.
References:
[1] Siddiqui MS, Sterling RK.Posttransplant metabolic syndrome.Int J Hepatol. 2012;2012:891516. doi: 10.1155/2012/891516. Epub 2012 Nov 27
[2] Chhatrala R, Siddiqui MB, Stravitz RT, Driscoll C, Sanyal A, Sargeant C, Luketic V, Sharma A, Sterling R, Matherly S, Puri P, Siddiqui MS,Evolution of serum atherogenic risk in liver transplant recipients: Role of lipoproteins and metabolic and inflammatory markers.Liver Transpl. 2015 May;21(5):623-30. doi: 10.1002/lt.24100. Epub 2015 Apr 15

P.1145

The correlation between platelet counts and spleen size after successful liver transplantation

Fatih Ensaroğlu[1], Serkan Öcal[1], Ahmet Sedat Boyacıoğlu[1], Haldun Selçuk[1], Fatih Hilmioğluu[1], Gökhan Moray[2], Mehmet Haberal[2].
[1]Gastroenterology, Başkent University, Ankara, Turkey; [2]Surgery, Başkent University, Ankara, Turkey.

Introduction: Thrombocytopenia secondary to splenomegaly is a frequent problem in cirhhosis related portal hypertension. Thrombocytopenia can negatively impact the survival of these patients. Multiple factors can contribute to the development of thrombocytopenia including splenic platelet sequestration, immunological processes, bone marrow suppression by chronic viral infection, and reduced levels or activity of the hematopoietic growth factor thrombopoietin. In this study we retrospectively investigated the patterns of platelet counts and changes of liver size in patients with successful liver transplantation.
Materials and Methods: The study included 45 patients who had successful liver transplantions (30 living related and 15 cadaveric) between January 1st, 2012 and December 31st, 2015 at Baskent University Ankara Hospital. Of these 45 patients 30 were males and 15 were females with the mean age of 44,9 years. The platelet counts before transplantation, the day of transplantation and 3, 7, 14 and 28 days after transplantation were compared. Similarly, ultrasonographic liver sizes before transplantation, the day of transplantation and 7 and 28 days after transplantation were compared.
Results and Discussion: The average platelet counts in 0, 3, 7, 14 and 28 days after transplantation were 89.6 ± 69.7 x 109/L, 51.8 ± 33.7 x 109/L, 67.8 ± 51.5 x 109/L, 130.5 ± 138.4 x 109/L and 178.1 ± 99.6 x 109/L. There was no difference between living related and cadaveric transplantations ($p>0.05$). The average spleen sizes 0, 7 and 28 days after transplantation were 168.4 ± 38.7 mm, 162.1 ± 42,0 mm and 146.1 ± 40.7 mm. The difference in spleen size was significant between day 0 and day 28 ($p<0.05$). There were moderate but significant negative correlations between platelet counts and spleen size in 0, 7 and 28 days after transplantation ($r= -457$ $p< 0.05$, $r= -343.4$ $p< 0.05$, $r= -359$ $p< 0.05$).
Conclusions: A steady decrease was observed in the platelet counts in the first three days after liver transplantation regardless of the type of donor. Improvement in thrombocytopenia was observed at 14. day of transplantation and normal platelet counts were reached at 28. day. Platelet counts improvement was paralleled with the reduction of spleen size.

P.1146

Long-term outcomes in pediatric live-donor liver transplantation

Neslihan Celik[1], Armando Ganoza[2], Frederick Vyas[1], James Squires[3], Veena Venkat[3], Geoffrey J.Bond[1], Kyle A.Soltys[1], Rakesh Sindhi[1], Robert H.Squires[3], Abhinav Humar[2], George V.Mazariegos[1].
[1]Hillman Center for Pediatric Transplantation, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA, United States; [2]Thomas E. Starzl Transplantation Institute, Division of Transplantation in the Department of Surgery, UPMC, Pittsburgh, PA, United States; [3]Division of Pediatric Gastroenterology, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA, United States.

Objectives: To determine the incidence of allograft and medical factors characterizing optimal patient outcomes 3 year or more after pediatric live-donor liver transplantation (LDLT).
Aim: To determine the incidence of allograft and medical factors characterizing optimal patient outcomes 3 year or more after pediatric live-donor liver transplantation (LDLT).
Methods: Children with a minimum of 3 year follow-up period after LDLT were reviewed in terms of ideal graft features and clinical outcomes including immunologic events and technical interventions.
Results: Between January 1997 and December 2015, 102 children received live-donor allografts. Overall Kaplan Meier patient and graft survivals are 92% and 89% respectively. Main indications were biliary atresia (n=49, 48%), acute hepatic necrosis (n=16, 15.7%), metabolic diseases (n=10, 9.8%) and malignant neoplasms (n=9, 8.8%). Median recipient age was 1.12 years (range 0.03-24.9 years). The allograft types were left lateral segment (n=90, 88.24%), left lobe (n=10, 9.8%) and right lobe (n=2, 1.96%). We further analyzed 80 patients with a minimum of 3 year follow-up period. Five patients (4.9%) required re-transplantation. The post-operative surgical complications were: intra-abdominal bleeding (n=15, 18.75%), biliary stricture (n=19, 23.75%), hepatic artery thrombosis (n=2, 2.5%) and portal vein thrombosis (n=6, 7.5%). Ventral hernia repair was performed in 31 patients (38.75%). Biopsy-proven acute cellular rejection was documented in 43 patients (53.75%) within the first 6 months after transplantation. PTLD was present in 4 patients (5%) but was not associated with graft loss or mortality. Immunosuppressive monotherapy and immunosuppressive-free patients were achieved in 48 (60%) and 6 (7.5%) patients respectively. Graft and extra-hepatic outcomes for 3 or more year follow-up period are shown in Table-1.
Conclusions: LDLT is a life-saving procedure with an excellent survival rate and outcomes for children. Intra-abdominal bleeding and biliary complications were the most graft related surgical complications and PTLD was the most common extra-hepatic morbidity but were not associated with graft or patient loss. Live-donor transplantation may also allow for less immunosuppression requirements and morbidity over the long term.

Table 1. Live-donor liver recipients with ≥3 year follow-up.

Category	Patient eligible for analysis	n (%) meeting criteria
Graft related		
No re-transplantation	80	75 (93.7%)
Lack of chronic rejection	52	52 (100%)
No biliary intervention	80	57 (71.2%)
No vascular intervention	80	71 (88.7%)
ALT, <60 IU/L	74	63 (85.1%)
Total bilirubin, ≤1 mg/dL	74	57 (71.2%)
Albumin, ≥3 g/dL	74	63 (85.1%)
GGT, <75 IU/L	74	58 (78.4%)
Extra-hepatic variables		
No PTLD	80	76 (95%)
Monotherapy or immmosuppressive-free	74	54 (73%)
No anti-diabetic medication	74	66 (89.2%)
No anti-hypertensive medication	74	68 (91.9%)
No anti-seizure medication	74	68 (91.9%)

P.1147

Durability of initial successful treatment for biliary anastomotic strictures in right lobe living donor liver transplantation

Kenneth Siu Ho Chok[1], Albert Chan[1], Jeff Dai[1], SuiLing Sin[1], Tiffany Wong[1], William Sharr[1], James Fung[2], TanTo Cheung[1], Brian She[1], KaWing Ma[1], See Ching Chan[1], ChungMau Lo[1].
[1]Department of Surgery, The University of Hong Kong, Hong Kong, Hong Kong; [2]Department of Medicine, The University of Hong Kong, Hong Kong, Hong Kong.

Background and Objective: Biliary anastomotic stricture (BAS) is a major complication after right-lobe living donor liver transplantation (RLDLT) and can result in significant morbidity and occasional mortality. There is little data on re-stricture after initial successful management of BAS. This study is to determine the risk factors for re-stricture after BAS as a complication of RLDLT.
Method: A retrospective study was performed on all adult patients who received RLDLT at a high-volume center during the period from August 2002 to December 2013.
Results: There were 387 RLDLTs in the study period, and 77 patients (19.9%) developed BAS. After endoscopic, radiological or surgical treatment, 72 patients were free of stricture for more than 1 year, whereas 5 patients (6.5%) developed re-stricture and required re-intervention with a median follow-up period of 80.8 months. On Multivariate analysis, a history of acute cellular rejection was associated with a significantly higher rate of re-stricture (p=0.022; 95% confidence interval 1.377-62.79). No patient required re-transplantation.
Conclusion: BAS patients with a history of acute cellular rejection should be monitored more closely even if their BAS has been successfully treated since they are more likely to develop re-stricture.

P.1148

Intrahepatic artery pseudoaneurysm-induced hemobilia caused by a plastic biliary stent after ABO-incompatible living donor liver transplantation: A Case Report

Jae Min Chun, Young Seok Han, Heon Tak Ha, Young Yeon Choi, Yoon Jin Hwang.
Liver-Pancreas Transplantation, Kyungpook National University School of Medicine, Kyungpook National University Hospital, Daegu, Korea.

Bile leakage after duct-to-duct anastomosis in living donor liver transplantation (LDLT) can mostly be managed by therapeutic endoscopic retrograde cholangiopancreatography (ERCP). Following this, various complications such as biliary infection, pancreatitis, perforation and bleeding can occur, and endoscopic sphincterotomy is primarily associated with post-ERCP bleeding; other causes have been published in case reports. In the present case, a plastic biliary stent used for treating liver abscesses and leakage at the bile duct anastomosis site after ABO-incompatible LDLT resulted in an intrahepatic artery pseudoaneurysm (PA) and hemobilia, which were managed by angiography and coil embolization. Although the complex postoperative course after LDLT can obscure the prompt diagnosis of an intrahepatic artery PA and haemobilia, biliary stenting should be considered as a possible cause.

P.1149

Late hepatic venous outflow obstruction following inferior vena cava stenting in the patient with deceased donor liver transplantation using modified piggyback technique: A case report

Jae Min Chun, Young Seok Han, Heon Tak Ha, Young Yeon Choi, Yoon Jin Hwang.
Liver-Pancreas Transplantation, Kyungpook National University School of Medicine, Kyungpook National University Hospital, Daegu, Korea.

Following liver transplantation, a few reports have documented hepatic venous outflow obstruction (HVOO) after inferior vena cava (IVC) stenting for the treatment of IVC stenosis. However, HVOO occurred early after IVC stenting and was mostly associated with living donor liver transplantation. We report a case of HVOO that occurred 31 months after IVC stenting in a man who received deceased donor liver transplantation (DDLT) using a modified piggyback (PB) technique. The cause of HVOO was unclear, but one possible explanation is that the balloon-expandable IVC stent might have compressed the IVC chamber on the donor liver side, which would have changed the outflow hemodynamics, resulting in intimal hyperplasia. Therefore, simultaneous hepatic venous stenting with IVC stent placement could help prevent HVOO in patients receiving DDLT with the modified PB technique.

P.1150

Does matching donor–recipient age impact long-term survival in liver transplantation?

Cristina Dopazo[1], Itxarone Bilbao[1], Lluis Castells[2], Mireia Caralt[1], Elena Fdez De Sevilla[1], Jose Luis Lazaro[1], Ramón Charco[1].
[1]Department of HPB Surgery and Transplants, Hospital Universitario Vall D'Hebron, Barcelona, Spain; [2]Department of Internal Medicine, Liver Unit, Hospital Universitario Vall D'Hebron, Barcelona, Spain.

Introduction: The characteristics of liver donor are changing in the last decade due to the shortage of organs and the high mortality in waiting list, leading to a more often use of extended criteria donors, including older donors.
Aim: To evaluate the matching donor-recipient age effect in the morbidity at one year post-transplant and in the long-term patient and graft survival.
Patients and Methods: Retrospective study from a prospectively obtained database which includes adult patients who underwent primary liver transplant (LT) from whole graft of brain dead donors. The recipients were divided in two groups: less than 60 years and 60 years or older. Both groups were divided based on donor age received (younger than 60 years and 60 years or older). To further adjust for baseline differences between recipients and donors, a propensity score analyses was performed. Median follow-up was: 68 (0-178) months.
Results: We analyzed 642 LT patients performed between January 2000 and December 2013. There were no differences regarding 1st year morbidity (hospital stay, rejection, surgical complications, retransplant) between groups *(see Table 1)*. Despite patient and graft survival was significant impaired in the group older donor/older recipient in the Kaplan-Meier analysis (p=0.004), the propensity score analysis showed donor aged ≥60 years did not increase the risk of death for recipients aged ≥ 60 (HR1.40, p 0.074) and <60 years (HR 1.47, p0.070).
Conclusions: Older donors did not negatively affect survival regardless of recipient age and comparable outcomes were achieved without increased rate of complications.

Table 1. Post-transplant morbidity and mortality during 1st year post-transplant

	D<60/R< (n=251, 39%)	D<60/R≥ (n=157, 25%)	D≥60/R< (n=144, 22%)	D≥60/R≥ (n=90, 14%)	p-value
Hospital stay (days)	17 (3-138)	15 (0-350)	17 (0-75)	17 (3-200)	0.210
Primary Non Function (n,%)	4 (1.21%)	0 (0%)	1 (0.69%)	1 (1.11%)	0.122
Acute rejection (n, %)	51 (22%)	37 (24%)	19 (13%)	12 (13%)	0.069
Arterial thrombosis (n,%)	5 (2.4%)	2 (1.6%)	5 (3.9%)	2 (2.5%)	0.753
Biliary complications (n,%)	20 (10%)	7 (6%)	7 (5%)	3 (4%)	0.107
Urgent retransplant (<7 days) (n,%)	2 (0.8%)	0 (0%)	2 (1.4%)	1 (1.1%)	0.457
Retransplant (>7 days 1st year) (n,%)	4 (1.6%)	1 (0.6%)	2 (1.4%)	0 (0%)	0.822
Mortality	34 (13.4%)	33 (21%)	13 (9%)	20 (22%)	0.007

P.1151

Low dose of anti-human T-lymphocyte globulin (ATG) warrant a good glomerular filtration rate after liver transplant on recipients with pre-transplant renal dysfunction

Cristina Dopazo[1], Ramón Charco[1], Mireia Caralt[1], Elizabeth Pando[1], Jose Luis Lazaro[1], Lluis Castells[2], Itxarone Bilbao[1].
[1]Department of HPB Surgery and Transplants, Hospital Universitario Vall D'Hebron, Barcelona, Spain; [2]Department of Internal Medicine, Liver Unit, Hospital Universitario Vall D'Hebron, Barcelona, Spain.

Introduction: Anti-T-lymphocyte Globulin (ATG)-based immunosuppression induction has gained increasing use in liver transplantation (LT) in patients with pre-LT renal dysfunction to delay initiation of calcineurin inhibitors. However, it has been historically associated with higher rate of infections.
Aim: The aim of this study was to evaluate the efficacy (acute rejection rate and renal function) and safety (post-LT mortality and adverse events) of induction immunosuppression based on reduced dose of ATG compared to basiliximab during the 1st year post-LT.
Methods: A single-center cohort study which analyze prospectively those adult patients who received a primary LT between 2012 and 2015 from brain-dead donors and pre-LT renal dysfunction (eGFR < 60 mL/min/1.73m2) and received ATG administered on day 1, 3 and 5 at 1mg/kg iv with dose adjustment according to CD2/CD3 levels (>20cel/μL) [ATG group]. This group was compared with a similar retrospective cohort with pre-LT renal dysfunction treated with basiliximab 20mg iv administered at day 0 and 4 post-LT [BAS group] between 2005-2011. In both groups, low tacrolimus (TAC) dose < 0.05mg/Kg twice daily was introduced according to urine output. Steroids were administered as maintenance during the first three months post-LT.
Results: Fifteen patients were included in each group with no differences in age, gender, indication for LT, pre-LT Diabetes Mellitus or MELD, however differences were observed regarding pre-LT renal function (ATG group 47±10 mL/min/1.73m2 vs BAS group 32±12 mL/min/1.73m2, p=0.003). The mean dose of ATG was 78±10 mg at day-one and day-three post-LT and only three patients (20%) required a third dose. Whole blood trough levels of TAC were kept in the low level during the first week achieving values of 2 (1-8)ng/dL in ATG group vs 5 (1-9)ng/dL (p=0.27) in BAS group. Significant improvement in renal function was observed at day 7 post-LT compared to pre-LT in both groups (59±19 vs 47±10 mL/min/1.73m2 in ATG group and 51±14 vs 32±12 mL/min/1.73m2 in BAS group, p=0.001).
No significant differences were found between groups in the incidence of acute rejection (33% in ATG group vs 20%, p=0.68), 1-year renal function (59±18 mL/min/1.73m2 in ATG group vs 61±16 mL/min/1.73m2, p=1) and 1-year patient survival (93% in ATG group vs 93%, p=1). No severe adverse events were observed in those patients receiving ATG with an incidence of overall infections of 33% (vs 47% in BAS group, p=0.71) and CMV infection of 40% (vs 40% in BAS group, p=1).
Conclusions: Low doses of Anti-T-lymphocyte Globulin can be safely and effectively used as induction immunosuppressive agent showing similar 1-year outcomes compared to induction with basiliximab and no increase of overall infections were observed.

P.1152

Evaluation of protocol biopsies after liver transplantation

Ebba Kroksmark[1], Annika Wernersson[2], Bo-Göran Ericzon[1,2], Henrik Gjertsen[1,2].
[1]Department of Transplantation surgery, Karolinska University Hospital, Stockholm, Sweden; [2]CLINTEC, Karolinska Institute, Stockholm, Sweden.

The utility of protocol liver allograft biopsies remain controversial, with exception of patients transplanted due to Hepatitis C cirrhosis who remain virus positive after transplantation. Still, biopsy and histology allows evaluation of changes in grafts with normal clinical and biochemical function. In our institution we have regularly performed protocol biopsies since the start of the liver transplant program in 1984. We here report risks and benefits of protocol biopsies in patient with normal liver function tests.
Protocol biopsies from all adult, HCV negative patients transplanted in our institution during 1998 and 2008 were evaluated and correlated to blood samples and medical event from the medical records.
There were four minor and one major complication related to the biopsy procedure. One patient got an infection which provoked an earlier retransplantation.
31% of the biopsies showed normal or minimal histological changes, whereas 69% biopsies where considered as pathological, where 13% had changes correlating to immunological events and rejection and. 13% of the biopsies showed signs of recurrent disease.
In 29% the biopsy findings led to changes in the pharmacological treatment, which included increase, decrease and switch of immunosuppression.
Over a five year period 67% of the biopsies remained unchanged, 11% got worse and 22% improved their histological picture.
Liver biopsy is an invasive procedure with potential risks, and should be avoided if the risks exceed the benefits. We had one major complication in 85 biopsies in 53 patients. A majority of patients experienced histological changes in their biopsies despite of normal clinic and biochemical status. In 29 % of the cases changes was made in the medical treatment that in 22% of the cases resulted in improved histological picture in later biopsies. In conclusion; Liver allograft biopsies is an invasive procedure with potential risk for severe complications, but still gives valuable information which might improve graft and patient survival.

P.1153

Liver transplantation in the presence of diffuse portal vein thrombosis: A 16-year experience with cavoportal hemitransposition

Sofia Gyllensward, John Softeland, Michael Olausson.
The Transplant Institute, Sahlgrenska University Hospital, Gothenburg, Sweden.

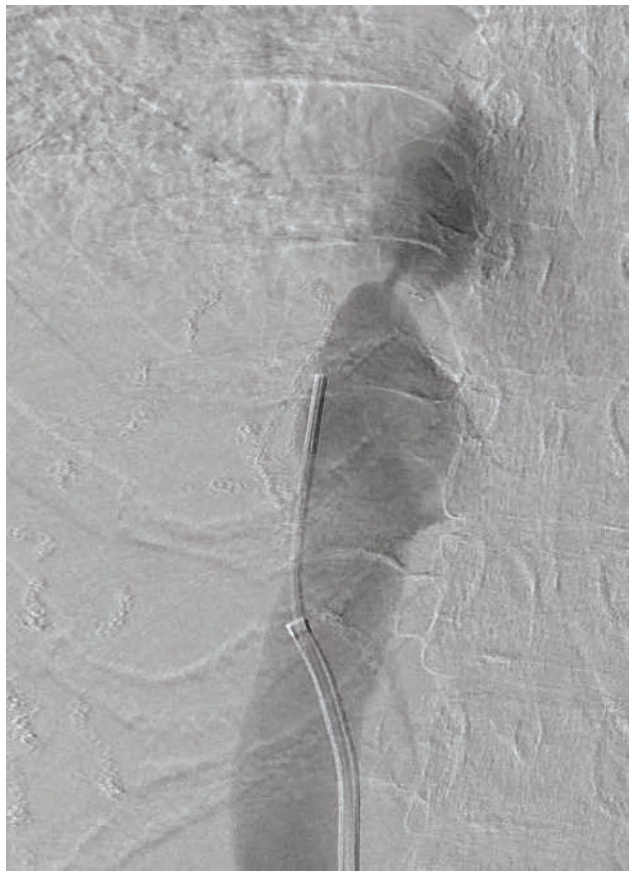
Background: During the last two decades surgical strategies for handling patients with thrombosis of the porto-mesenteric axis have evolved. Without cavoportal hemitransposition (CPHT), liver transplantation would not be possible for this patient group and long-term survival would be negligible. Here we review our experience with liver transplantation in patients with diffuse portomesenteric thrombosis, where surgical restoration of hepatopetal blood flow is not possible with standard methods, such as portal vein thrombectomy or venous interposition graft.
Due to the increase in infrahepatic venous pressure that this procedure entails and the persistence of portal vein obstruction, concerns about potential long-term problems have been voiced. These concerns have included persistent variceal bleeding, intractable ascites, lower extremity edema, hypersplenism and kidney failure.
Patients and Methods: From June 1999 to October 2008, 14 LTX on 12 patients were performed with CPHT at Sahlgrenska University Hospital. Follow up data was collected and analyzed retrospectively for the period 1999-2015. No patients were lost to follow-up.
Results: Patient survival at 1 and 5 years was 67 % and 58 % respectively. After first hospital discharge no patient required retransplantation and no patients experienced chronic liver failure in their grafts. Postoperatively, seven patients received continuous renal replacement therapy (CRRT) but two years after transplantation none of the patients were in need of dialysis and no patient is currently on hemodialysis. No patient underwent renal transplantation during follow-up. Of the eight patients discharged from hospital only one patient had any signs of ascites production but five patients were reported to have lower extremity edema during follow-up. Furthermore five patients had signs of gastrointestinal bleeding during follow-up after hospital discharge but none have needed any interventions.
Conclusions: For LTX recipients with diffuse PVT, where other techniques are not feasible, CPHT does not result in unacceptable long-term complications and the long-term results are satisfactory.

P.1154

Technical solution to upper caval stricture after liver transplantation

Sofia Gyllenswärd[1], John Softeland[1,2], Niclas Kvarnström[1,2], Bengt Gustafsson[1], William Bennett[1], Michael Olausson[1,2].
[1]Department of Transplantation Surgery, Sahlgrenska University Hospital, Gothenburg, Sweden; [2]Departement of Transplantation, Sahlgrenska academy, Gothenburg, Sweden.

Introduction: Improved surgical techniques have improved the outcome and the prospect for liver transplanted patients. However, technical complications leading to graft and patient loss still exist. Feared complications in liver transplantation are strictures of vascular anastomoses - especially in the upper cava. Caval stricture is a rare event and the management is not straightforward often necessitating a retransplant. Endovascular dilation may be attempted if not too temporally close to the transplant event, but results are often short-lived. Surgical repair is another option and this may include reanastomosis or a traditional vascular patch plasty – often with significant morbidity involved. We describe a novel technique simplifying these difficult procedures.
Method: From July 2010 to March 2014 three patients were treated with a new technique for strictures of the upper caval anastomosis after liver transplantation at Sahlgrenska University Hospital. Cavoplasty was performed with a donor derived vena caval bypassgraft using GIA stapling device with a vascular magazine. The new caval segment was placed parallel to the recipient cava and a long side to side anastomosis created across the narrowed portion of the cava thereby removing the stricture. All patients had elevated vena cava pressure gradients and symptoms of inferior vena cava syndrome. The time for intervention varied from one and a half and two months postoperatively. Data was assessed retrospectively. One further patient from our center with upper caval stricture was treated in 2001 with a suture-cavoplasty, five months after transplantation, and is therefore demonstrated as a control.
Results: Two of the three patients who underwent the new procedure are currently alive. One patient suffered from recurring upper caval obstruction and underwent balloon dilatation at the department of interventional radiology. No patient has required retransplantation and no patients experienced liver failure. Inferior vena cava syndrome, with symptoms like ascites and pleural effusion, was observed in all three patients preoperatively. During follow-up symptoms of vena cava syndrome were present in one patient. This patient suffered from lower extremity edema and was subsequently the one who was treated with endovascular procedures.
The control patient was retranplantated four years after suture-cavoplasty due to failure of management of the upper caval stricture. This patient underwent endovascular procedures three times after retransplantation due to recurrent caval strictures and developed liver cirrhosis.
Conclusions: A novel and simple use of a GIA stapler is described which can greatly reduce the risk of treating cumbersome caval strictures after organ transplantation.

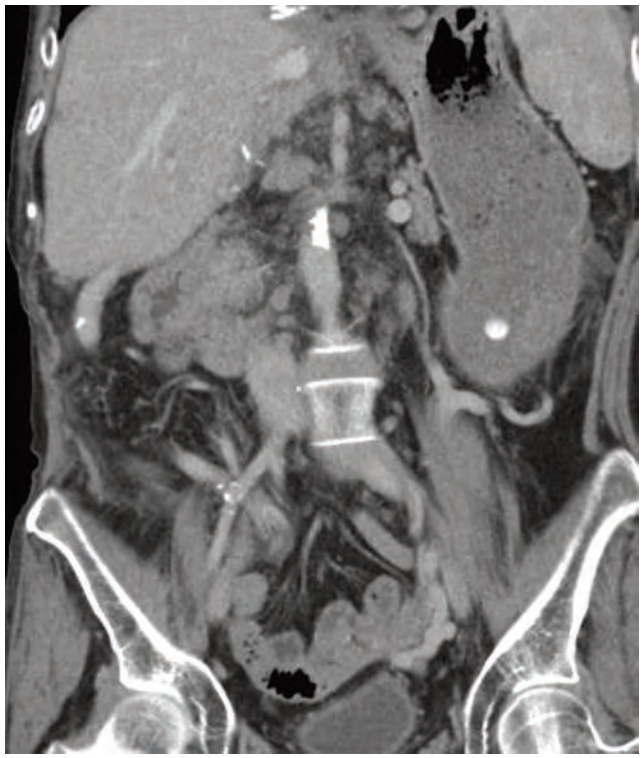


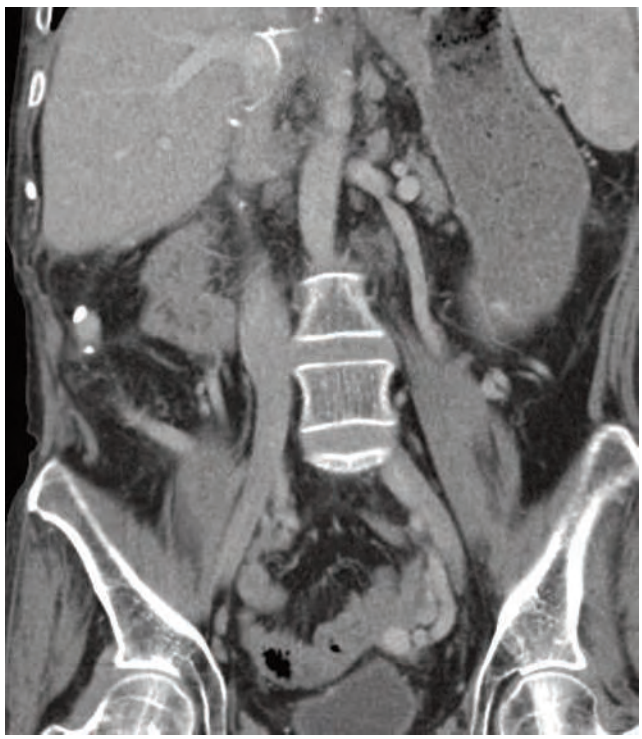
P.1155

Novel technique for arterial conduits in liver and multivisceral transplantation

Sofia Gyllenswärd[1], John Softeland[1,2], Niclas Kvarnström[1,2], Magnus Rizell[1], Malin Sternby-Eilard[1,2], Markus Gäbel[1,2], Michael Olausson[1,2].
[1]Department of Transplantation Surgery, Sahlgrenska University Hospital, Gothenburg, Sweden; [2]Departement of Transplantation, Sahlgrenska academy, Gothenburg, Sweden.

Introduction: Infrequently the recipient hepatic artery can be unsuitable for use and finding a site to anastomose a graft may be challenging. Examples can be retransplant cases, patients with previous surgery and a frozen hilum and patients with fragile or arteriosclerotic hepatic artery. The most frequently used alternative sites are the suprarenal or infrarenal aorta but these sites may also be inaccessible in certain cases. We describe another alternative that can be used both in liver and multivisceral transplantation.
Method: We describe a method where multiple donor iliac artery segments are used to create a conduit from the recipient iliac artery to secure arterial graft inflow in the upper abdomen.
A 53 year old woman with primary sclerosing cholangitis (PSC), transplanted for the first time in 2000 and retransplanted (ReTx) due to arterial thrombosis 2001. The patient was accepted for ReTx due to recurrent cholangitis and liver failure. The ReTx was complicated with portal vein thrombosis, resulting in a jumpgraft from the superior mesenteric vein (SMV), and an attempt to connect the donor artery to the old aorta conduit. The resulting flow was only 20ml/min and therefore a long arterial conduit from the right common iliac artery was used, connecting iliac artery segments from several donors. The resulting flow increased to 200ml/min.
A 47 year old man with neuroendocrine tumor was multivisceral transplanted (MVT) in 2000. Three years after MVT, this patient suffered from an aneurysm in the donor aortic conduit which was judged unsuitable for stenting. The patient was operated with an arterial conduit from the left iliac artery to superior mesenteric artery (SMA) and the graft coeliac trunk. The procedure was complicated by a rupture of the aneurysm leading to a complicated postoperative course.
Results: The 53 year old woman, despite a very complex transplantation and another ReTx due to primary non-function, was discharged to her home hospital after 4 months. She is still alive 5 years post transplantation, more than 15 years after the first transplantation. The 47 year old man was discharged to his home after 1,5 months. During follow-up no problems occurred that could be attributed to the intervention. However, this patient died 5 years after his surgical procedure due to a relapse of his cancer.
Conclusions: We describe an alternative site for an arterial conduit in complex liver or multivisceral transplant cases. In addition to establishing adequate arterial inflow another advantage is ease of access for interventional radiology postoperatively.





P.1156

Use stapling devices with caution in order to avoid cavastrictures in liver transplantation

Sofia Gyllenswärd[1], John Softeland[1,2], Niclas Kvarnström[1,2], Bengt Gustafsson[1,2], Michael Olausson[1,2].
[1]Department of Transplantation Surgery, Sahlgrenska University Hospital, Gothenburg, Sweden; [2]Departement of Transplantation, Sahlgrenska academy, Gothenburg, Sweden.

Introduction: Sustained high production of ascites after liver transplantation may be a diagnostic challenge that sometimes requires surgical intervention. One of the causes may be inferior vena cava syndrome resulting from a caval stricture. Untreated it may lead to thrombotic events. We describe a potential danger of using stapling devices to set off the hepatic veins during hepatectomy.
Method: A 56 year old woman with PBC and portal hypertension was transplanted with a liver from a 60 year old donor. The liver was transplanted using piggy back technique with a cavo-caval side to side anastomoses. The procedure was uneventful with a cold ischemia time of 8 hours. The recipient hepatic veins were divided using a GIA stapling device.
Results: Already on the first day after transplantation large volumes of ascites were collected from the drains. After two weeks, up to 9 liters/24 hours were collected and a cavography showed a stricture of the upper vena cava and a pressure gradient of 12mmHg. The patient was explored and no evident stricture could be detected initially – despite intraoperative ultrasound. The staplerow on the right liver vein looked like it could cause narrowing of the vena cava and was therefore removed. After surgery the ascites production was reduced to 1,5 liter and the patient was discharged to the referring hospital two weeks later. A cavography 3 months later could not detect any significant pressure gradient, and a 14mm balloon could easily pass through the previous stricture.
Discussion: We describe a potential danger of using stapling devices in liver surgery.

P.1157

Incidence and outcomes of de novo malignancies after living donor liver transplantation in comparison with the Japanese general population

Taizo Hibi, Chisato Takagi, Masahiro Shinoda, Osamu Itano, Hideaki Obara, Minoru Kitago, Yuta Abe, Hiroshi Yagi, Kentaro Matsubara, Yuko Kitagawa.
Department of Surgery, Keio University School of Medicine, Tokyo, Japan.

Background: De novo malignancies are a major cause of mortality in the long term after liver transplantation (LT). The incidences in comparison with the general population, surveillance and treatment strategies, and outcomes have rarely been described in a living donor (LD) setting.
Methods: A single-center, retrospective cohort analysis was conducted for 136 adult patients who underwent LDLT between 1995 and 2014. Our routine surveillance protocol to check for malignancies included annual upper gastrointestinal endoscopy, fecal occult blood, and chest/abdominal/pelvic computed tomography. Chemistry panel, complete blood count were monitored every 2−3 months at regular clinic visits.
Results: A total of 12 recipients developed 13 (10%) de novo malignancies (thyroid, 2; colorectal, 2; lung, 2; laryngeal, 1; gastric, 1; prostate, 1; and posttransplant lymphoproliferative disorders (PTLD), 4) were detected.
For solid tumors, the median duration from transplant to diagnosis was 6.3 years. Five patients have been followed more than 1 year after cancer treatment: One female noticed a neck mass which revealed to be papillary thyroid carcinoma. She is well >5 years after radical resection. One male presented with back pain caused by extensive sacral metastasis from follicular thyroid carcinoma and died of disease progression 1.1 year after cancer diagnosis. Another male developed melena from an early cancer in the rectum and underwent laparoscopic anterior resection. Third male with a fecal occult blood test positive had superficial cancer in the descending colon and underwent endoscopic mucosal resection. A laryngeal cancer was diagnosed in a female who presented with hoarseness. Although she remained disease free >4 years after definitive chemoradiation, she developed a primary lung cancer and underwent pulmonary resection. Three patients (lung, 1; gastric, 1; prostate, 1) all underwent curative resection and have been followed <1 year. Our surveillance program detected only 3 patients with malignancy (lung, 2; colorectal, 1).
For PTLD cases, the median interval from LT was 117 months. All 4 patients were EBV-PCR negative. The manifestation of disease was paraaortic lymph nodes for 1 and extranodal for 3. Pathological diagnosis was diffuse large B-cell lymphoma in 3 and Hodgkin lymphoma in 1. Complete response was achieved in 2 patients who underwent R-CHOP and both are alive >3 years. PTLD relapsed in 1 female who received rituximab alone because of recurrent hepatitis C: She died 4 months thereafter. Calcineurin inhibitor was reduced in 1 remaining patient who is currently under strict follow-up.
Overall, the incidence of all cancer types was significantly elevated in LT recipients compared with the Japanese population (standardized incidence ratio, 2.9; 95% confidence interval, 1.6−4.8); however, cancer mortality rate was comparable (standardized incidence ratio, 1.6; 95% confidence interval, 0.2−5.8)
Conclusions: Although cancer incidence in LDLT recipients is higher than the Japanese general population, cancer death was equivalent. Our surveillance program detected solid tumor in only 3 patients after LDLT and there is a pressing need to establish an effective protocol. In adult LDLT, the relation between PTLD and EBV infection is unclear: Risk factors and prognostic indicators remain to be elucidated.

P.1158

Management of CNI induced posterior reversible encephalopathy syndrome with everolimus change after living donor liver transplantation (case)

Geun Hong[1], Naroo Kim[1], Huisong Lee[1], Jae Hee Woo[2].
[1]Department of Surgery, EWHA Womans University, School of Medicine, Seoul, Korea; [2]Department of Anesthesiology and pain medicine, EWHA Womans University, School of Medicine, Seoul, Korea.

Introduction: Neurologic complications are not common in liver transplantation. Opportunistic infection and previous hepatic encephalopathy may be one of the reason. Sometimes immunologic neurotoxicity is related to cerebral damage with disastrous result. We present a patient with encephalopathy after liver transplantation.
Materials and Methods: The patient was thirty five years old female patient with alcoholic liver cirrhosis with jaundice and ascites. The liver has multiple dysplastic nodules. Her husband was donor and underwent donor right hemihepatectomy. Operation and immediate postoperative period was not eventful and immunosuppressants were triple regimen and simulect was used two times. CNI was started on postoperative 2 days, and the dose was increased gradually. Patient was moved to ward and routine liver biopsy was performed on postoperative 15 days. There was no event during liver biopsy. On the same night, general tonic clonic seizure was occurred abruptly. Neurological doctor managed the patient immediately with anti-epileptic drugs such as phenytoin. Two more seizure was found. After evaluation with brain CT and EEG, the impression was posterior reversible encephalopathy syndrome. The blood level of CNI was 7.10 ng/ml on the day of seizure. There was no triggering factor of seizure except the possibility of immunosuppressant neurotoxicity. Therefore, we changed immunosuppressants from CNI to everolimus.
Results: Follow up EEG showed decreased interictal epileptiform discharge frequency at left posterior temporo-parieto-occipital area one week after the initial event. MRI showed edematous lesion in both occipito-parietal cortex and subcortical white matter, left frontal lobe convexity, pons. The management for this event includes immunosuppressant change and anti-epileptic drug such as phenytoin and keppra. One month after the seizure, the patient shows no symptom with tapering of anti-epileptic drug.
Discussion: Posterior reversible encephalopathy syndrome (PRES) is a cerebropathy characterized by gray and white matter abnormalities in the area of temporal, parietal, and occipital lobes or intracerebral hemorrhage. The clinical sympotoms of PRES are headache, agitation, seizure, and focal neuropathy. The causes of PRES are immunosuppressants such as tacrolimus or cyclosporine, renal failure, and hypertension. This patient showed agitation and seizure postoperatively according to the elevation of tacrolimus serum level, even though not toxic level. Fortunately, a surgeon of transplantation team had an experience of CNI induced cerebral damage previously. We discussed about the cause of seizure and started everolimus instead of tacrolimus immediately.
Conclusions: CNI neurotoxicity can be occurred after liver transplantation. The transplantation team should know about this complication and manage the patient with immediate change of immunosuppressant and use of antiepileptic drugs.
Geun Hong

P.1159

Treatment of biliary stone and cast after liver transplantation using the percutaneous transhepatic cholangioscopy (PTCS); a case report

Ju Ik Moon, In Seok Choi, Chul Woong Kim, Min Kyu Kim, Sung Gon Kim.
Department of Surgery, Konyang University Hospital, Daejeon, Korea.

Introduction: Biliary complications remain highly prevalent among recipients after liver transplantion. Biliary stones and cast formation can result from biliary stricture. We present a case of biliary stone and cast after deceased donor liver transplantation successfully treated with PTCS.
Case: A 49-year-old female patient with alcoholic liver cirrhosis was referred for liver transplantation. From the deceased donor, the liver transplantation has been performed with reconstruction of bile duct with duct to duct anastomosis. After 8 months, we found common bile duct stone and stricture by Computed Tomography. Then, total bilirubin was normal range. So, we have done regularly follow-up. However, 21 months later total bilirubin level was elevated to 4.45mg/dl. We planned to remove common bile duct stone with PTCS. After dilating the sinus tract of PTBD using an 18-Fr catheter about 3 weeks after PTBD, PTCS was carried out through the sinus tract. We successfully removed common bile duct stone and cast using PTCS. After that, there were no remnant stone and filling defect on cholangiography. The patient was discharged without any complications and bilirubin level was normalized.
Conclusion: PTCS is considered one of the useful methods to use in the cases of biliary stones and cast formation result from biliary stricture.

P.1160

The impact of recipient's BMI at the deceased donor kidney transplantation

Ju Ik Moon, In Seok Choi, Chul Woong Kim, Min Kyu Kim.
Department of Surgery, Konyang University Hospital, Daejeon, Korea.

Background: The outcome of kidney transplant in obesity patient is still controversial. Furthermore, there are no many studies of these issue in Asian centers compared to the West. The aim of this study is to to investigate the association between pre-transplant resipient's body mass index (BMI) and their post-transplant outcomes.
Methods: Between May 2006 and Jan. 2016, 48 patients underwent DDKT at Department of Surgery at Konyang University Hospital, Daejeon, Korea. Recipients were divided into two groups; the under 25 kg/m2 of BMI (Group A : n = 36) and the exceed 25 kg/m2 of BMI (Group B : n = 12). The following characteristics and results were evaluated retrospectively through the medical records.
Results: BMI were significantly higher in group B than group A (21.9±1.7 kg/m2 vs. 28.1±3.0 kg/m2, p < 0.001). The demographic factors were not significantly different between A and B groups except BMI. Complications during the follow periods were not significantly different in both groups (83.3% vs. 83.3%, p = 1.000). The 1-year patient survival in the group A was 93.4% compared to 8.3% in the group B (p = 0.053). Graft survival at 1 year was 97.1% and 91.7% in the group A and the group B (p = 0.379) respectively. Delayed graft function (DGF) was more prevalent in the group B (8.3% vs. 33.3%, p = 0.055). Incidence of biopsy proven acute rejection (BPAR) episodes was not significantly different in both groups. (25.0% vs. 0.0%, p = 0.088). Glomerular filtration rate (GRF) level by period of 1 weeks, 1, 3, 6,and 12 months was not different in both group (12th months: 63.8±18.3 vs. 71.0±14.1, P = 0.263).
Conclusions: The results show that patient survival, graft survival and complications by resipient's BMI of the DDKT is not different. The findings of this study might have been influenced by the small sample size and short-term follow up length. We thought that long-term follow up and further studies are needed to evaluate outcomes after kidney transplantation.

P.1161

Outcome of deceased donor liver transplantation for alcoholic liver disease

Suk Kyun Hong, Nam-Joon Yi, Kyung Chul Yoon, Hyo-Sin Kim, Hyeyoung Kim, Kwang-Woong Lee, Kyung-Suk Suh.
Surgery, Seoul National University Hospital, Seoul, Korea.

Alcoholic liver disease (ALD) is the second leading indication for liver transplantation (LT) in the United States and Europe. In Korea, there has been a gradual increase in number of patients with ALD. Now ALD is the second most common indication for deceased donor LT (DDLT), but there is no strict guidelines or regulations such as minimum of 6 month's abstinence before transplantation. Moreover, little is known following the post-transplant outcomes for ALD in Asian countries. The aim of this study is to assess the post-DDLT survival outcome and evaluate the factors associated with survival rates of DDLT for ALD patients compared with HBV patients. The results were retrospectively reviewed from 272 patients, who underwent DDLT from January 2010 to Dec 2014 at Seoul National University Hospital. Alcohol group had less cases with HCC (8.8% vs. 46.4%, p<0.001), high MELD (25.6±8.0 vs. 22.2±8.8, p=0.046), and high CTP score (11.6±1.0 vs. 10.7±1.7, p<0.001). There were more admission cases for abnormal liver function test (30.3% vs. 14.4%, p=0.040) and more psychiatric problems (36.4% vs. 9.5%, p=0.001) in ALD group. There was no survival rate difference between two groups (p=0.907). In univariate analysis, post-LT 7day GGT was the only factor to be statistically significant (p=0.023). In non HCC group, the hospital day was longer (32.2±24.7 vs. 21.0±15.1, p=0.025), more cases show fatty change in post-LT 1yr biopsy (40.0% vs. 9.1%, p=0.040). In conclusion, despite no difference in overall survival, careful management after DDLT including psychiatric problem would be needed in ALD group.
References:
[1] G. Berlakovich. Challenges in transplantation for alcoholic liver disease. *World journal of gastoenterology*. 2014;20:8033-9
[2] P. Burra. Liver transplantation for alcoholic liver disease in Europe: a study from the ELTR(European Liver Transplant Registry). *American journal of transplantation*. 2010;10:138-48
[3] Hiroto Egawa. Risk factors for alcohol relapse after liver transplantation for alcoholic cirrhosis in Japan. *Liver transplantation*. 2007;13:465-466
[4] P. Iruzubieta. Long-term survival after liver transplantation for alcoholic liver disease. *World journal of gastroenterology*. 2013;19:9198-208
[5] S. Tome. Review: Timing of liver transplantation in alcoholic cirrhosis. *Journal of hepatology*. 2003;39:302-307
[6] D. Shawcross. Comment: The 6-month abstinence rule in liver transplantation. *Lancet*. 2010;376:216-7
[7] F. Stickel. Genetic variation in the PNPLA3 gene is associated with alcoholic liver injury in caucasians. *Hepatology*. 2011;53:86-95

P.1162

Graft versus host disease (GVHD) after liver transplantation (LT) focused on deceased donor LT (DDLT): A propensity score-matched study

Suk Kyun Hong, Nam-Joon Yi, Hyeyoung Kim, Hyo-Sin Kim, Kyung Chul Yoon, Kwang-Woong Lee, Kyung-Suk Suh.
Surgery, Seoul National University Hospital, Seoul, Korea.

Graft versus host disease (GVHD) is a rare (0.1-1%) but severe complication after liver transplantation. Due to rarity, few studies have been proposed the risk factors and yet they have been inconclusive. We performed a retrospective analysis of 1700 liver transplants (living donor : deceased donor = 1190 : 510) in Seoul National University Hospital in Korea, from March 1988 to October 2015, over a period of 27 years. Six patients (6/1700, 0.35%) with histologically diagnosed GVHD were included. One patient received living donor LT (LDLT) and found to be one-way HLA matching, which is generally known to be significant risk factor. Focusing on the other five DDLT cases, each of these five cases was matched to four controls based on the blood group, sex, and age difference between recipient and donor. Analyses were performed to identify risk factors associated with the development of GVHD after DDLT. Higher pre-LT AFP was identified to be a risk factor (p=0.002), until accompanying hepatocellular carcinoma identified not to be the risk factor (p=0.252). Although not statistically significant, operation time was potential risk factor (p=0.051). GVHD has been extremely rare and difficult in diagnosis and, what is more, fatal in outcome. Considering these risk factors when allocating, we may reduce further incidence of GVHD in DDLT.

References:

[1] S. Alexander. Chimerism and tolerance in a recipient of a deceased-donor liver transplant. *The new England journal of medicine*. 2008;358:369-374
[2] H. Kamei. Fatal graft-versus-host disease aftere living donor liver transplantation: differential impact of donor-dominant one-way HLA matching. *Liver transplantation*. 2007;13:465-466
[3] Y. Soejima. Graft-versus-host disease following living donor liver transplantation. *Liver transplantation*. 2004;10:460-4
[4] F. Abaalkhail. Graft-versus-host disease after liver transplantation: a single center case series. *Annals of transplantation*. 2015;20:397-401.

P.1163

Posttransplant mellitus (PTDM) after liver transplantation: Risk factors and outcome

Byeong Ju Kang, Yang Won Nah, Chang Woo Nam, Hyung Woo Park.
Departments of Surgery, Ulsan University Hospital, University of Ulsan College of Medicine, Ulsan, Korea.

Introduction: Posttransplant mellitus (PTDM) after liver Transplantation is common compliation. Several studies have shown that it increases rate of several complications, resulting in lower graft and patient survival. The goal of our study was to investigate the incidence, risk factors of PTDM and the effects of PTDM on outcome.
Patients and Methods: We retrospectively reviewed the medical records of the patients aged who underwent liver transplantation from March 2002 to September 2015. PTDM was defined as DM that newly developed after liver transplantation.
Results: We assigned 125 patients who had Liver transplantation in our center. Preoperative diabetes mellitus (DM) was diagnosed in 40 patients. Of the 85 selected patients, 13 patients (15%) developed PTDM after liver transplantation. We included Sex, Age, BMI, Family History of DM, underlying disease, type of Calcineurin inhibitor, and some factors of donor(BMI, DM) for risk factor. But we didn't find any significant risk factor for PTDM. And there was no difference morality and incidence of rejection between PTDM group and non-PTDM group.
Conclusion: The incidence of PTDM was 15% in our Hospital. And there was no significant factor that were associated with PTDM. Also PTDM was not associated with outcome.

P.1164

The influence of perioperative acute kidney injury for the prediction of decreased renal function after liver transplantation

Gun Woo Kang[1], Hong Ik Kim[1], Joo Dong Kim[2], Dong Lak Choi[2].
[1]Internal Medicine, Catholic University of Daegu School of Medicine, Daegu, Korea; [2]General Surgery, Catholic University of Daegu School of Medicine, Daegu, Korea.

Introduction and Aims: There is clearly a progressive deterioration in renal function after liver transplantation (LT). Perioperative acute kidney injury (AKI) is common, but a serious complication. The aims of the current study were to identify the association with perioperative AKI and decreased renal function after LT.
Methods: We reviewed medical records of 106 LT recipients without moderate to severe chronic kidney disease (CKD) (estimated glomerular filtration rate, eGFR <60 mL/min/1.73 m2). Patients with GFR ≥ 60 mL/min/1.73 m2 were assigned to group 1, and the remaining patients to group 2. We investigated eGFR changes pre-LT and 1-year post-LT using propensity score matching using age, gender and diabetes mellitus. Statistical significance of differences between clinical parameters and 1-year eGFR changes was assessed by univariate and multivariate analyses.
Results: Mean age was 49.5±10.9 years, and 66% patients were male. Mean differences in 1-year eGFR and serum creatinine were -32.0±29.2 mL/min/1.73 m2 and 0.3±0.3 mg/dL, respectively. Perioperative acute kidney injury (AKI) was observed in 43.4% of patients. There were significant differences in perioperative AKI (p=0.024), pre-LT eGFR (p=0.015), and hemoglobin 1-year (p=0.003) between the two groups. However, multivariate analysis revealed that there was no significant correlation between decreased renal function and perioperative AKI (OR 0.883, 95% CI 0.630-1.236, p=0.467).
Conclusions: Renal function significantly decreased the first year after LT. We couldn't find that perioperative AKI is a predictor of decreased renal function in LT recipients.
Keywords: perioperative acute kidney injury; renal function; liver transplantation

P.1165

Steal syndrome after liver transplantation: A single center experience

Aydincan Akdur[1], Emre Karakaya[1], Fatih Boyvat[2], Gokhan Moray[1], Mehmet Haberal[1].
[1]Transplantation Center, Baskent University, Ankara, Turkey; [2]Interventional Radiology, Baskent University, Ankara, Turkey.

Introduction: Arterial steal syndrome after orthotopic liver transplantation (OLT) is characterized by arterial hypoperfusion of the graft, which is caused by a shift in blood flow into the splenic or gastroduodenal arteries. In this report, we present mechanisms by which this syndrome caused ischemia in our patients.
Materials and Methods: We retrospectively investigated all 512 liver transplantation (LT) procedures that were performed between 8 December 1988 and 31 December 2015 in our center. Demographic features (including body weight, Child Pugh scores and etiology of liver disease), graft source, perioperative outcomes, long term results were evaluated retrospectively. Outcomes of management of steal syndrome were also evaluated. Steal was suspected by elevated levels of liver enzymes and the results of Doppler ultrasonography and CT angiography; it was confirmed by celiac angiography. Steal was treated by embolization with a coil or by placement of an endoluminal narrowing stent.
Results: 25 of 512 patients (4.8%) at our institution (17 men and 8 women; mean age, 22 years; range, 2 to 40 years) exhibited biochemical evidence of liver ischemia and graft failure at 1 to 170 days after having undergone orthotopic liver transplantation. 5 of them were deceased donor LT and 20 were living donor LT 22 of those patients had splenic steal, and 3 had both splenic and left gastric artery steal syndrome. None of the patients had gastroduodenal artery steal syndrome. Twenty one patients with splenic steal syndrome and 3 patients with both splenic and left gastric steal syndrome were treated by transcatheter occlusion with a coil. The remaining patient with splenic steal syndrome was treated with an endoluminal narrowing stent placement. All patients improved clinically within 24 hours after treatment, exhibiting significant changes in their biochemical and radiological parameters. We did not see any complications (such as splenic abscess) due to embolization of splenic artery.
Conclusions: We conclude that steal is a significant problem after OLT. Embolization and stenting are minimally invasive and successful treatments for steal, usually resulting early clinical improvement.

P.1166

Percutaneous ultrasound guided biliary interventions after pediatric liver transplantation using fine needle and 0.014 inch guide-wire

Fatih Boyvat[1], Ali Harman[1], Ebru H.Ayvazoglu Soy[2], Umut Ozyer[1], Mahir Kirnap[2], Gokhan Moray[2], Mehmet Haberal[2].
[1]Radiology, Baskent University, Ankara, Turkey; [2]Transplantation Center, Baskent University, Ankara, Turkey.

Background: After pediatric liver transplantation, percutaneous interventions to the biliary system may be difficult due to the small diameter of the biliary ducts. Using microguidewire and catheter system facilitates the procedural success.
Methods: We retrospectively analyzed biliary complications in 114 pediatric liver transplantation patients between July 2006 and August 2015. Biliary reconstruction was performed via duct to duct anastomosis in 79 (69%) patients and roux-en-y hepaticojejunostomy in 25 (31%) patients. During PTC, fluoroscopy and ultrasound guidance was performed using 0.014 inch guide wire (Terumo, Japan) and 21 G puncture needle.
Results: Post transplant biliary complications were observed in 42 (36%) patients. Biliary leak was encountered in 23 (55%) patients and stricture in 19 (45%) patients. Percutaneous transhepatic cholangiography (PTC) and drainage was successfully performed in all patients with biliary obstruction. Patients with biliary leak were managed surgically in 3 patients, ERCP in 1 patient, simple drainage in 6 patients and remaining again with PTC and drainage.
Conclusion: Percutaneous biliary intervention using fine needle and 0.014 inch guide wire increase the success and decrease complication rate even patients with minimal biliary dilation.

P.1167

Simultaneous percutaneous large profile multiple plastic stents for biliary anastomotic strictures after liver transplantation

Fatih Boyvat[1], Ali Harman[1], Aydincan Akdur[2], Cuneyt Aytekin[1], Mahir Kirnap[2], Gokhan Moray[2], Mehmet Haberal[2].
[1]Radiology, Baskent University, Ankara, Turkey; [2]Transplantation Center, Baskent University, Ankara, Turkey.

Objective: To evaluate the management of biliary anastomotic strictures with simultaneous multiple percutaneous plastic stents for liver transplants
Materials and Methods: Between 2004 and 2016, 13 patients with recurrent biliary anastomotic stenosis in whom prior balloon dilation and single plastic stent placement had failed and 25 patients with biliary anastomotic strictures with no previous intervention were included in this study. The patients were 22 females and 16 males, ages ranged from 5 to 66 years (mean age: 32). Percutaneous biliary drainage was performed then, 1 to 3 times, sequential dilation with conventional balloon was performed, then, two plastic stents were placed percutaneously through one tract (percutaneous tracts were between 8F to 14F). The size of the two plastic stents were 16F (n=11), 20F (n=22), 24F (n=4) and 28F (n=1).
Results: The median indwelling stent period was 6.3 months (3 months-15 months). In two patients, plastic stents were removed endoscopically at 25 days, and 3 months because of cholangitis. In 36 patients, no cholangitis or obstruction were observed and stents removed endoscopically.
Conclusion: Sequential percutaneous insertion of two plastic biliary stents through one percutaneous access, affords effective treatment of the anastomotic strictures that occured after liver transplantation. This technique enables large profile internal stent placement through a single small percutaneous hole. This technique has a high success rate and decrease the number of interventions and also the cost of the procedure.

P.1168

Successful percutaneous management of recurrent sclerosing cholangitis after liver transplantation with multiple intrahepatic plastic biliary stent placement with only one access

Fatih Boyvat[1], Cuneyt Aytekin[1], Aydincan Akdur[2], Gokhan Moray[2], Mehmet Haberal[2].
[1]Radiology, Baskent University, Ankara, Turkey; [2]Transplantation Center, Baskent University, Ankara, Turkey.

Background: Liver transplantation is the only treatment for patients with primary sclerosing cholangitis. However, there is a high rate of biliary strictures and recurrence has been reported. Percutaneous interventions help graft survival and resolution of strictures.
Methods: Liver transplantation was performed due to primary sclerosing cholangitis 8 years ago. Because of recurrent cholangitis, elevated liver enzymes and bilirubin levels, patient was evaluated and multiple intrahepatic strictures were diagnosed. Because of roux-en-y anastomosis, ERCP was failed for intervention. Percutaneous PTC and biliary dilatation was performed at 4 session and anastomotic and intrahepatic segment stenosis dilation was performed and then multiple intrahepatic plastic stents were placed via one percutaneous access for 6 months. These stents were sutured under the skin so that patient was followed only one external catheter but 3 internal plastic stent.
Results: Multiple intrahepatic stents were removed and patient was symptom free for the following 2 years without any intervention.
Conclusion: Percutaneous interventions with balloon dilation and multiple plastic stent placement is a good percutaneous management with only one access for recurrent sclerosing cholangitis after liver transplantation.

P.1169

Extensive indications for HCC in living donor liver transplantation

Mehmet Haberal[1], Aydincan Akdur[1], Gokhan Moray[1], Gulnaz Arslan[2], Figen Ozcay[3], Haldun Selcuk[4], Handan Ozdemir[5].
[1]General Surgery, Baskent University, Ankara, Turkey; [2]Anesthesiology, Baskent University, Ankara, Turkey; [3]Pediatric Gastroenterology, Baskent University, Ankara, Turkey; [4]Gastroenterology, Baskent University, Ankara, Turkey; [5]Pathology, Baskent University, Ankara, Turkey.

Introduction: Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and the third highest cause of death related to malignancy. Since HCC diagnosis is typically late, the median survival following diagnosis is approximately 6-20 months. The 5-year survival rate is reported as less than 12%. HCC typically arises in the background of cirrhosis. Liver transplantation is regarded as an optimal radical therapy for selected patients with HCC. Initial experiences with orthotopic liver transplantation were limited to patients with extensive unresectable tumors, and were marked by uniformly dismal outcomes due to high rates of tumor recurrence. Milan criteria are the gold standard for recipient selection. Since Milan criteria are restrictive for increasing candidates, they are expanded into alternative sets of criteria. We aimed to evaluate our LT indications and results for HCC.
Materials and Methods: Between 8 December 1988 and 31 December 2015 we performed 512 liver transplants at our centers. We developed our criteria for LT in HCC candidates at Baskent University and currently perform LT in all HCC patients without major vascular invasion and distant metastasis. We retrospectively reviewed our LT results of patients with HCC.
Results: 59 patients (59/512; 11.5%) had liver transplantation for HCC. 51 were male (86%) and 8 were female (14%). 11 of these patients were children and 48 were adults. We performed 39 living donor LT (10 pediatric, 29 adult), 20 deceased donor LT (1 pediatric, 19 adult). We had 16 patients (27%; 1 pediatric and 15 adults) who were beyond Milan criteria radiologically and pathologically. We performed 11 living donor LT and 5 deceased donor LT in these patients. All deceased donor LT had down staging therapy before LT. We had 15 patients (25.4%; 4 pediatric and 11 adults) who were within Milan criteria radiologically; but after LT, when pathologic specimens were evaluated, they were found to be beyond Milan. We performed 10 living donor LT and 5 deceased donor LT in these patients. We diagnosed HCC incidentally with pathological examination in 6 patients (10.1%) (4 pediatric, 2 adult). All of the 6 incidental HCC cases were still alive without HCC recurrence for 63-128 months. HCC recurrence was detected in 14 cases (23.7%). Disease free 5-year survival rates of LT patients beyond Milan criteria and within Milan criteria were 56.8% and 78.7%, respectively (p= .024).
Conclusions: LT within Milan criteria had better survival rates. However, Milan criteria can be safely and effectively expanded with promising results even in patients beyond Milan criteria.

P.1170

Renal cell carcinoma in transplanted kidney

Ebru H.Ayvazoglu Soy[1], Yalcin Kizilkan[2], Handan Ozdemir[3], Sedat Yildirim[1], Gokhan Moray[1], Mehmet Haberal[1].
[1]Transplantation Center, Baskent University, Ankara, Turkey; [2]Urology, Baskent University, Ankara, Turkey; [3]Pathology, Baskent University, Ankara, Turkey.

Introduction: Malignancy arising in transplanted tissue is rare. Renal cell carcinoma (RCC) more commonly arise in native kidneys and the reported incidence of RCC in normal population is 3%. Although incidence of RCC is up to 4.6% in kidney recipients, less than 10% of these RCCs are diagnosed in the transplanted kidney. In addition to other cancers, RCC incidence may increase in coming years due to prolonged graft survival and increased usage of elderly donors. Pathogenesis of RCC in graft kidney is still a subject of debate and there is no treatment consensus. In our study we reviewed our results of renal transplants with RCC.
Materials and Methods: The follow-up was standard for all recipients. In the first year of transplantation the patients were evaluated for every month with ultrasonography (USG) and renal function tests. After the first year we followed patients for every 6 month with USG and renal function tests. Each RCC was suspected by USG and confirmed by computed tomography (CT) or magnetic resonance imaging (MRI). All patients had nephron sparing surgery (NSS). The grafts were followed with USG and kidney function tests at 1,3,6 and 12 months, and then yearly.
Results: Between October 1985 and December 2015 we performed 2248 kidney transplantation procedures at our centers (1003 female, 1245 male). To date we follow 1285 renal transplant patients with normal graft functions. We retrospectively evaluated the data of these renal transplant recipients. The mean age of the patients is 41years (range 1.5-76 years). 85 (3.7%) patients were diagnosed with 86 malignancies. We diagnosed only 3 patients with RCC in transplanted kidney (0.2%). Two of these patient received kidneys from their relatives, the other patient had deceased-donor kidney transplantation. We performed NSS in all RCC (20mm, 15mm, 30mm) with nearest surgical margins of 0.5 mm, 1 mm, 1 mm, respectively. We followed them for 4, 14, 108 months with normal graft functions. Following NSS, serum creatinine levels were 0.85 mg/dL, 1.2 mg/dL, and 0.8 mg/dL in the third month. Living donors are also evaluated for RCC in their solitary kidney
Conclusions: RCC affecting graft kidney is reported in few case reports in literature with an incidence of nearly 0.5%. In our study our incidence is significantly less (0.2%). Transplantation improves life expectancy and quality of life so preservation of the graft may be considered. Although various treatment options are reported in studies, NSS for RCC provides functioning grafts and keeps patients independent of dialysis. However NSS is available only for low grade tumors. So, early diagnosis of RCC by graft screening with routine follow up must be the main concern.

P.1171

Dual stent placement for suprahepatic inferior vena cava stenosis after deceased donor liver transplantation with piggy-back technique

Tae-Seok Kim, Keun Soo Ahn, Yong Hoon Kim, Hyoung Tae Kim, Koo Jeong Kang.
Surgery, Dongsan Medical Center, Keimyung University School of Medicine, Daegu, Korea.

Introduction: Outflow obstruction after liver transplantation is uncommon but can have serious effects on the outcomes. Outflow obstruction caused by inferior vena cava (IVC) compression or kinking can be occurred sometimes after side-to-side piggy-back technique using large liver graft. Recently, outflow obstruction has been treated successfully by stent placement. However, IVC stent placement has some problems due to the overlapping IVC and the angle of approach in cases of side-to-side piggy-back technique. Herein we have reported successful treatment of outflow obstruction caused by suprahepatic IVC stenosis after deceased donor liver transplantation with side-to-side piggy-back technique.
Case: A 34-year-old woman underwent DDLT for alcoholic liver cirrhosis. The patient was slim with BMI 19. However, she had a large amount of ascites which was not controlled by diuretics and her liver was hypertrophied (2372 g). Although graft was relatively large weighted 1950 g, intraabdominal space was sufficient to implant graft. IVC anastomosis was performed by side-to-side piggy-back technique and other procedures were performed routinely without problem. After transplantation, laboratory test had been improved gradually and patent vascular flow was observed in Doppler ultrasound exam. However, abdominal drainage was significantly increased over 5 L/day. Computed tomography was performed on postoperative day 7 and suprahepatic IVC stenosis was shown. Venography showed suprahepatic stricture by IVC kinking. Measured pressure gradient between infrahepatic and right atrium was 17 mmHg and between hepatic vein and right atrium was 14 mmHg. Self-expandable metallic stent was placed across the stenotic area of recipient's suprahepatic IVC through right internal jugular vein and additional stent was placed across anastomosis to right hepatic vein through right femoral vein to prevent compression of hepatic vein outlet by IVC stent. After stent placement, pressure gradient was improved and there was no procedure-related complication. The amount of ascites and diuretics requirement was significantly decreased after procedure.
Conclusion: Percutaneous stent placement is a safe and effective procedure in patient with outflow stenosis, however this procedure should be performed with a well-designed plan.

P.1172

A case of early organizing portal vein thrombosis followed by arterio-portal shunt without hepatic dysfunction after liver transplantation

Tae-Seok Kim, Keun Soo Ahn, Yong Hoon Kim, Hyoung Tae Kim, Koo Jeong Kang.
Surgery, Dongsan Medical Center, Keimyung University School of Medicine, Daegu, Korea.

Introduction: Portal vein thrombosis developed in the immediate post-transplant period is detrimental to the graft and patient survival, and should be corrected by surgical or interventional procedure to maintain graft function. Here, we introduce our unusual experience of early portal vein thrombosis followed by arterio-portal shunt with preserving hepatic function which is corrected successfully by surgical procedure.
Case: A 41 year old male underwent orthotopic liver transplantation for hepatocellular carcinoma and liver cirrhosis caused by hepatitis B virus. Hepatic artery was anastomosed to the bifurcation of gastroduodenal artery using internal iliac artery graft conduit due to the short length of graft hepatic artery arised from superior mesenteric artery. Portal vein and hepatic vein was reconstructed using common manner. The intraoperative Doppler U/S performed after reperfusion showed good arterial and portal blood flow. The Doppler U/S performed at postoperative day 1, 2 and 4 also showed good blood flow in hepatic artery, portal vein and hepatic vein. The Doppler U/S performed at postoperative day 7, showed complete obstruction of extrahepatic portal vein with arterio-portal shunt in the hilar portion. However, there was no alteration of hepatic function in serologic test during the remarkable derangement of vascular inflow. Portal vein thrombosis and arterio-portal shunt were confirmed by CT angiogram and portal vein anastomosis was revised after removal of the organized thrombus at postoperative day 10. The patient has been followed up healthy with stable liver function until recently.
Conclusion: The early portal vein thrombosis followed by arterio-portal shunt preserving liver function is unusual complication after liver transplantation. However, this complication should be corrected by surgical procedure irrespective of hepatic function at that time to preserve long-term graft function.

P.1173

Immune modulation with CMV-hyperimmunoglobulin improves outcome in high-risk (MELD ≥ 30) liver transplant patients

Arno Kornberg[1], Ulrike Witt[1], Helmut Friess[1], Jennifer Kornberg[2].
[1]Surgery, Technical University, Munich, Germany; [2]Anesthesiology, LMU, Munich, Germany.

Introduction: Liver transplantation (LT) in patients with high model of end-stage liver disease (MELD) scores is associated with increased risk of infectious complications. Treatment with specific immunoglobulines (Ig) was shown to provide beneficial immuno-modulatory efficacies. The aim of this pilot trial was to assess the impact of early post-LT immune modulation with CMV hyperimmunoglobulin (CMVIg) on early post-LT outcome in high-risk (MELD ≥ 30) patients.
Materials and Methods: Thirty-two liver transplant patients with decompensated liver cirrhosis and a median MELD score of 38 (range: 30-40) were included. Based on CMV matching, CMVIg (Cytotect, Biotest, Germany) was administered at a dose of 3 x 5000IE during 1. week post-LT. The impact of CMV Ig treatment along with other parameters on post-LT immunology and outcome was analysed by uni- and multivariate analysis.
Results: Nineteen patients were ICU-bound (59.4%), 15 patients were under ventilation (46.9%), 11 patients received dialysis (34.4%), and 16 patients needed catecholamines (50%) at LT, respectively. Fatal risk trias (ventilation + dialysis + catecholamines) was evident in 11 patients (34.4%). Twenty-one patients have received CMVIg (65.6%), while 11 liver recipients did not. Overall 3- and 6-months post-LT survival was 81.3% and 68.8%. Infectious and septical complications were the main reasons for morbidity and mortality. In univariate analysis, patients' age ($P = 0.005$), ΔMELD ($P = 0.024$), lactate-level at LT ($P = 0.001$), ventilation at LT ($P = 0.009$), catecholamines at LT ($P = 0.023$), risk triad at LT ($P = 0.03$), CRP-level at LT and treatment with CMVIg ($P < 0.001$) were associated with poor outcome post-LT. Donor characteristics had no prognostic impact. In multivariate analysis, only pretransplant CRP-level ≤ 4mg/dl and treatment with CMVIg were independent promoters of survival. Overall survival rates at 3- and 6-months post-LT were 95.2% and 85.7% in patients receiving CMVIg treatment, but only 54.5% and 36.4% in those without CMVIg therapy ($P < 0.001$). Biopsy-broven rejection rate was 0% in the CMVIg-group but 18.2% in the non-treated subset ($P = 0.04$)
Conclusion: Early posttransplant treatment with CMVIg provides beneficial immuno-balancing properties in high-risk liver transplant patients with MELD ≥ 30.

P.1174

Liver transplantation for alcoholic liver disease without pre-transplant abstinence

Hae Won Lee[1,2], Young Joon Ahn[1,2], Hyeyoung Kim[1], Kyung-Suk Suh[1].
[1]Department of Surgery, Seoul National University College of Medicine, Seoul, Korea; [2]Department of Surgery, Seoul National University Boramae Medical Center, Seoul, Korea.

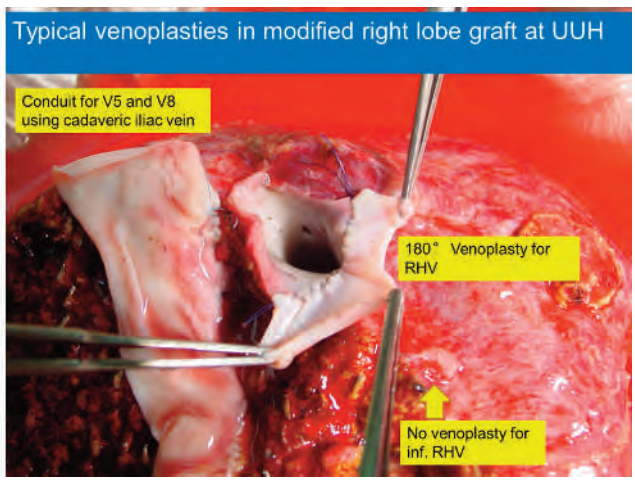
Background: Pre-transplant alcohol abstinence more than 6 months is generally recommended for liver transplantation (LT) for alcoholic liver disease (ALD). However, some patients undergo LT without sufficient abstinence period in Korea. We investigated the results of LT for ALD without pre-transplant abstinence.
Methods: We started a public hospital LT program to try to reduce patients' burden of expenses. Fifteen patients underwent LT in Seoul Metropolitan Government Seoul National University Boramae Medical Center between Jul 2011 and Sep 2015. Among them, 5 patients (33.3%) had heavy alcoholic history preoperatively. Pre-transplant abstinence was defined as no alcohol ingestion more than 6 months before LT. Four patients (80%) had no pre-transplant abstinence. The median follow-up duration was 13.5 (5.1-34.1) months.
Results: Four patients (80%) were male and one (20%) was female. The mean age of patients was 54.8 ± 7.8 years. Three (60%) patients underwent LT from deceased donor while two patients (40%) did from living donor. There was no operative or hospital mortality. One patient with pre-transplant abstinence had full post-transplant compliance with medical instructions including outpatient clinic visit and medications. He had no abnormal event during follow-up of 13.5 months except for biliary stricture, which was interventionally managed. Among of 4 patients with no pre-transplant abstinence, only one patient had full compliance after LT. The other three patients did not fully comply with medical instructions and restarted alcohol ingestion after discharge. All three patients experienced events of abnormal liver function. One female patient stopped the follow-up and medication after post-transplant 9 months. One month later, she was transferred to the emergent center and expired due to septic shock. Another patient irregularly took immunosuppressive agents and had acute cellular rejection at post-transplant 4 months. Thus, he needed steroid pulse therapy. Those three patients did not have good family support. However, one patient who had full compliance without pre-transplant abstinence had strong family support. The family support was very important to improve the post-transplant outcomes of alcoholic patients without pre-transplant abstinence.
Conclusions: The proportion of ALD was higher in the public hospital. Although LTs were performed without pre-transplant abstinence in some urgent cases, those outcomes were poor. The family support could be an important factor to improve the outcomes in those cases. In conclusion, the sufficient alcohol abstinence period should be strongly requested before LT. In addition, post-transplant supporting program are needed for patients who could not have sufficient abstinence period before LT.

P.1175

Surgical results of outflow reconstruction in living donor liver transplantation

Yang Won Nah, Hyung Woo Park, Byeong Ju Kang, Chang Woo Nam.
Department of Surgery, Ulsan University Hospital, Ulsan, Korea.

This study was done to assess the complication and patency rates of outflow reconstruction after living donor liver transplantation (LDLT) using modified right lobe (mRL) graft at a small volume center.
Materials: Consecutive 30 patients who received mRL graft during the period from April 2010 to December 2014 at Ulsan University Hospital were eligible for this study.
Surgical Techniques: In 28 cases, recipient right hepatic vein (RHV) was extended to the IVC to match the length of donor RHV which was widened by fencing with homograft. (Fig 1) Inferior right hepatic vein (iRHV) anastomosis was done in 80% (24/30). When 2 or more iRHVs were present (4 cases), the openings were unified with homograft fence. Middle hepatic vein branches of segment 5 (V5) and 8 (V8) were reconstructed in all the cases (V5 in 29 cases and V8 in 26) with homograft (26), autologous great saphenous vein (2) or Gortex (2). The authors tried to make the anastomosis as short as possible in every case.
Results: There was no hospital mortality. No intervention for problems related with outflow was done so far. RHV anastomosis was anatomically and functionally stable in 100% of the patients. Patency rate for iRHV anastomosis was 90% (19/21) at 6 months. Patency rate for V5/V8 anastomosis was 87% (26/30) at 1 month, during which the graft can regenerate enough to fulfill the metabolic needs of the recipients.
Conclusion: The complication and patency rates of outflow reconstruction in LDLT using modified right lobe graft at a small volume center seem to be not inferior to those of high volume center. International knowledge sharing and education can make surgical results of LDLT satisfactory even for a small volume center.



P.1176

Risk factors in primary liver transplantation - a retrospective single centre analysis

Manuel Maglione, Hannah Esser, Beatrix Mutschlechner, Benno Cardini, Rupert Oberhuber, Christian Margreiter, Dietmar Öfner, Stefan Schneeberger.
Department of Surgery, University Hospital for Visceral, Transplant and Thoracic Surgery, Medical University Innsbruck, Innsbruck, Austria.

Background: Liver transplantation represents the only treatment form for patients with irreversible liver failure. The aim of this study was to analyse the recent single centre experience for risk factors in primary liver transplantation.
Methods: This retrospective study includes all consecutively primary liver transplantations performed at our institution between January 2005 and December 2015. Statistical analysis was performed using Kaplan-Meier und Cox-Regression analysis.
Results: A total of 566 patients who underwent primary liver transplantation were included. The median follow up was 41,8 months (range 0 – 134 months). Alcoholic liver disease (42.6 %), hepatitis C virus infection (21.4 %) and non-alcoholic liver disease (15 %) were the most common indications for liver transplantation. The 1-, 3- and 5- year patient and graft survival was 92 %, 87 % and 81 %, and 89 %, 83 % and 77 %, respectively. 94 patients died, and there were 115 graft losses, resulting in 33 liver re-transplantations. Multivariate analysis revealed several risk factors associated with a worse patient and graft outcome. Among donor-associated factors, only positive hepatitis B core antibody negatively influenced patient and graft survival ($p = 0.01$ and $p = 0.002$, respectively). Both, inferior patient and graft survival, was also associated with recipient age >70 years ($p = 0.001$ and $p = 0.02$) and with recipients having previous major abdominal surgeries or complete portal vein thrombosis ($p = 0.05$ and $p = 0.015$). A higher MELD score at the time of transplantation resulted in worse graft survival ($p=0.034$), however, it did not reach statistical significance for patient survival ($p=0.06$) In contrast to cold ischemia time, which could not be identified as an independent risk factor in this cohort, prolonged vascular anastomosis time as well as the duration of the anhepatic phase resulted in lower patient and graft survival rates ($p=0.005$ and $p = 0.04$, and $p = 0.005$ and $p = 0.006$, respectively). Vascular and biliary complications were also identified as risk factors associated with graft ($p=0.003$ and 0.04) but not patient loss. In contrast, acute rejection episodes did not influence graft and patient outcome. Postoperative acute kidney failure and sepsis were negative predictors for both, patient ($p= 0.003$ and 0.001) graft survival ($p= 0.003$ and 0.001).
Conclusion: Surgical and infectiological issues are as important as meticolous donor and recipient selection for patient and graft survival following liver transplantation. All these factors have to be taken into account when listing patients for a liver transplantation, accepting a liver for transplantation or performing liver transplantation.

P.1177

Successful retransplantation of an allograft after early recurrence of focal segmental glomerulosclerosis in the first recipient

Katrin Kienzl-Wagner[1], Siegfried Waldegger[2], Rupert Oberhuber[1], Claudia Bösmüller[1], Manuel Maglione, Dietmar Öfner[1], Stefan Schneeberger[1].
[1]Visceral, Transplant and Thoracic Surgery, Innsbruck Medical University, Innsbruck, Austria; [2]Pediatrics I, Innsbruck Medical University, Innsbruck, Austria.

Background: Recurrent primary focal segmental glomerulcosclerosis (FSGS) develops in up to 55% after kidney transplantation and may lead to early graft loss. Experimental findings support the hypothesis of a circulating permeability factor in the pathogenesis of primary and recurrent FSGS. Here, we report the successful retransplantation of an allograft that failed in the first recipient due to early recurrence of primary FSGS.
Case Report: A 5 year old patient with end-stage renal disease caused by primary FSGS received a kidney transplant from a 31 year old cadaveric donor. Despite immediate graft function, the patient developed heavy proteinuria on day 2 (protein/creatinine ratio 32.5 g/g) and urinary output ceased, suggestive of early recurrence of FSGS. A series of plasmapheresis and rituximab was not effective. Repeat renal biopsies excluded rejection or severe damage of the graft. Severe proteinuria required graft nephrectomy in the first recipient on day 27 after kidney transplantation. After informed consent the allograft was retransplantated into a 52 year old recipient on the waiting list with vascular nephropathy. Immediately after retransplantation, the allograft regained function with a urinary output of 2800ml/24h on day 3, serum creatinine levels decreasing to 1.1mg/dl and proteinuria decreasing to 0.22 g/g. 9 months after retransplantation, the second recipient has excellent graft function with serum creatinine levels of 0.87mg/dl.
Conclusion: Retransplantation of a cadaveric allograft that failed in the first recipient due to recurrent primary FSGS into a second recipient was successful with excellent graft function at 9 months. Rapid recovery of the allograft supports the hypothesis of a circulating permeability factor in the pathogenesis of primary FSGS.

P.1178

Acquired haemostatic disorders by orthotopic liver transplantation

Eviatar Nesher[1], Eytan Mor[1,7], Sigall Nakav[2], Marius Braun[3], Eytan Cohen[4], Esther Rabizadeh[5,7], Dorit Blickstein[6,7].
[1]Transplantaion Department, Beilinson Hospital, Rabin medical center, Petach Tikva, Israel; [2]Coagulation Laboratory, Beilinson Hospital, Rabin medical center, Petach Tikva, Israel; [3]Liver Unit, Beilinson Hospital, Rabin medical center, Petach Tikva, Israel; [4]Medicine F-Recanati and Clinical Pharmacology Unit, Beilinson Hospital, Rabin medical center, Petach Tikva, Israel; [5]Hematology Laboratories, Beilinson Hospital, Rabin medical center, Petach Tikva, Israel; [6]Thrombosis and Hemostasis Unit, Beilinson Hospital, Rabin medical center, Petach Tikva, Israel; [7]Sackler Faculty of Medicine, Tel-Aviv University, Tel Aviv, Israel.

Introduction: Transmission of hemostatic disorders after orthotopic liver transplantation (OLT) is rare. FXI is produced by the liver and its deficiency may cause excessive blood loss. Ashkenazi/Iraqi ancestry Jews and Israeli Arabs are affected. FV is mainly synthesized by the liver.
A single point mutation in the FV gene at one of the cleavage sites for activated protein C (APC) is responsible for the coagulation defect (FV:Q506). This results in a properly activated FV, which is inactivated in a reduced rate by APC (APC resistance, APCR).
We report on two patients who underwent OLT and acquired hemostaic disorders.
Methods: APCR was done by using Factor V Leiden reagents, lupus anticoagulant (LAC) was detected by using Silica Clotting Time (SCT) and dilute Russell's viper venom time (dRVVT) screen and confirm reagents and FVIII, FIX, FXI activity was detected by using a specific factor deficient plasma.
Mixing study was done by mixing patient's plasma 1:1 with commercial normal plasma.
Specific coagulation factor inhibitor detection was done by incubation of this mixture for 2 hours at 37°C.
FV gene mutation was done by real time PCR.
Antiphospholipid antibodies (APLA) IgG, IgM, anti β2GP1 IgA ,anti-β2GP1 IgG, IgM and anti cardiolipin IgA were assessed.
Results: *Patient 1:* A 61 years old man of Libyan Jewish ancestry, referred before lung biopsy because of prolonged partial thromboplastin time (PTT, 73.6 sec, normal <40 sec) two years after OLT for cirrhosis and hepatic carcinoma. The patient's PTT before transplantation was 35.2 sec.
Mixing study demonstrated complete correction of PTT (35.2 sec). Antiphosholipid antibodies (APLA+β2GP1) and LAC dRVVT/SCT were not detected, FVIII-208%, FIX-121%, FXI-4%(normal > 50%) with no specific inhibitors. Treatment with parenteral 1 gram tranexamic acid (hexakaprone®) was introduced before the biopsy and the patient underwent lung biopsy without complications.
Patient 2: A 48 years old woman with right jugular and subclavian deep vein thrombosis two months after OLT because of fulminate hepatic failure. Screening for inherited and acquired thrombophilia revealed low APCR (1.49, n>2.2) without detected mutation in the FV gene. The patient received treatment-dose enoxaparin for 7 months.
Conclusion: Transmitted hemostatic disorders after OLT depend on the genotypes of the recipient/donor livers, and may risk the graft and cause severe/life threatening complications.
Being aware of the potential donor transmitted inherited coagulation defects and their timely detection is critical in the management of transplant recipient.
Before liver transplantation is done, one should take detailed history of the donor/donor's family for any hemostatic disorders so that prophylactic measures can be implemented.
Any donor with hemostatic disorder such as FV Leiden mutation or FXI deficiency should not be excluded from liver donation.

P.1179

Percutaneous transhepatic bililary drainage after living donor liver transplantation resulting in acute hepatic failure: a case of hepatic compartment syndrome

Je Ho Ryu, Kwangho Yang, Young-Mok Park, Byunghyun Choi, Chongwoo Chu.
Liver Transplantation Division, Surgery, Pusan National University Yangsan Hospital, Yangsan, Korea.

Introduction: Subcapsular hematoma after percutaneous transhepatic biliary drainage(PTBD) in living donor liver transplantation(LDLT) is rare complication. Expanding hematoma can cause parenchymal compression and resulting in acute liver failure. It may cause fulminant hepatic failure requiring emergent liver transplantation. We therefore diagnosed a "hepatic compartment syndrome", because of its similarities with mechanisms of abdominal compartment syndrome. We report a case of subcapsular hematoma after PTBD in LDLT causing hepatic compartment syndrome.
Case: A 46-year-old man underwent LDLT with modifted Right lobe graft for hepatitis B-related cirrhosis. He admitted for biliary stricture at 9 months later after LDLT. Endoscopic retrograde biliary drainage to resolve biliary stricture was not failed, and then, percutaneous transhepatic biliary drainage(PTBD) was done. After PTBD, The patient presented with dizziness, abdominal pain. Computed tomography(CT) showed intrahepatic and subcapsular hematoma, stenosis between right hepatic vein and IVC. There was a progressive increase in liver enzymes, bilirubin, and INR and a decline in hemoglobin. We inserted right hepatic vein stent to resolve outflow disturbace. And then, pig-tail catheter drainage of subcapsular hematoma was done. However, the patient's clinical course progressed to hepatic failure. The serum bilirubin was increased up to 31.4 mg/dl. We decided to perform explorative laparotomy for evacuation of subcapsular hematoma at 20 days later after PTBD. In operative finding, large amount of subcapsular hematoma was seen. It seemed 'hepatic compartment syndrome'. Subcapsular hematoma evacuation was done and multiple drainage catheters around liver graft were inserted. After decompression of subcapsular hematoma, the patient's condition was improved gradually. The patient discharged at 3 weeks later after re-operation. Liver enzyme and serum bilirubin decreased to nearly normal range at 3 months later after re-operation. Now the patient is doing well.

P.1180

Surgical techniques and outcome of autologous portal vein interposition for anomalous right portal veins in living donor liver transplantation

Young-Mok PARK[1], Je Ho Ryu[1], Kwangho Yang[1], Byunghyun Choi[1], Chongwoo Chu[1], Jeonghee Kang[2].
[1]Transplantation Division, Surgery, Pusan National University Yangsan Hospital, Yangsan, Korea; [2]Coordinator, Organ Transplantation Center, Pusan National University Yangsan Hospital, Yangsan, Korea.

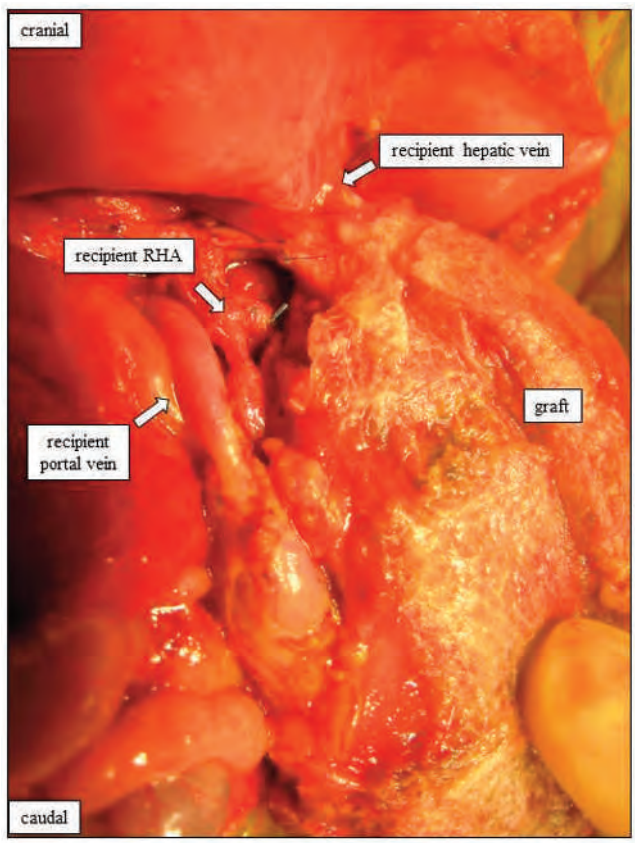
In living donor liver transplantation (LDLT) with right lobe (RL) grafts, variations of right portal vein (PV) are very clinical significance. This study was intended to describe in detail our PV reconstruction techniques in RL grafts with anomalous PV and evaluate the impact of accompanying biliary variations on recipient outcomes.
Methods: From May 2010 to December 2014, 109 patients underwent LDLT with RL grafts at our institution. According to the types proposed by Cheng et al, the PV anatomy of donor livers was classified as type I in 94 donors (86.2%), type II in 3 patients (2.8%), and type III in 12 patients (11.0%). We retrospectively analyzed characteristics of the patients according to variations of PV and divided two group (type I : group1 versus type II & III : group2).
Results: The PV reconstruction techniques utilized in group 2 included Portal Y-graft interposition (n= 4), Quilt-plasty by autologous PV with PV interposition (n=9), cryopreserved iliac vein graft interposition (n=2). In median follow-up of 24 months, four recipients underwent PV stenting within postoperative 1 week. Group 2 was associated with a high (73.3%) incidence of biliary variations. In group 1 and group 2, biliary complication rates were 18.1%, 26.7% respectively, but this difference was not of statistical significance.($P<0.434$) Overall 1-and 3-year survival rates of recipients were 90.8 %, 89.4%, respectively.
Conclusions: Anomalous PV and biliary variations make LDLT technically more challenging. By applying appropriate PV reconstruction techniques to LDLT, it is possible to reach to successful outcome in LDLT with anomalous PV without endangering recipient and donor safety.

P.1181

Surgical technique for hepatic arterial reconstruction in pediatric patients undergoing monosegmental living donor liver transplantation

Yukihiro Sanada[1], Noriki Okada[1], Naoya Yamada[1], Takumi Katano[1], Yuta Hirata[1], Yoshiyuki Ihara[1], Taizen Urahashi[1], Shuji Hishikawa[2], Koichi Mizuta[1].
[1]Transplant Surgery, Jichi Medical University, Shimotsuke, Japan; [2]Center for Development of Advanced Medical Technology, Jichi Medical University, Shimotsuke, Japan.

Introduction: When living donor liver transplantation (LDLT) is performed for neonatal and small infant patients, the incidence of hepatic artery complication (HAC) is high due to the size discrepancy between the graft and the recipient's abdominal cavity, as well as differences in anastomotic vessel diameters. Here, we present a retrospective analysis with a focus on our surgical procedure for hepatic arterial reconstruction and the outcomes of monosegmental LDLT.
Materials and Methods: Of 275 patients undergoing LDLT at our institution between May 2001 and December 2015, 13 patients (4.7%) underwent monosegmental LDLT. Hepatic artery reconstruction was performed using interrupted 9-0 monofilament nylon sutures and a double clip under a microscope. The size discrepancy between a graft and the recipients' abdominal cavity was defined as the graft-to-recipient distance ratio (GRDR) between the left hepatic vein and the portal vein (PV) bifurcation on a preoperative computed tomography. When the GRDR was small, the anastomotic field was usually selected the ventral position of the PV anastomosis. When the GRDR was large, the anastomotic field was selected the dorsal position of the PV anastomosis, with pulling of the graft's round ligament in the caudal direction.



HACs were defined as hepatic arterial hypoperfusion caused by anastomotic factors such as stenosis or thrombosis, and external factors, such as hematoma or inflammation around the anastomotic field.
Results: Recipient hepatic arteries were selected for branch patch technique in five cases (38.5%), RHA in five, LHA in two, and PHA in one, and the diameter was 2.2±0.6 mm. The anastomotic fields were selected the dorsal position of the PV in seven cases (53.8%) and the ventral position in six, and the GRDR was 2.8±0.4 and 1.9±0.5, respectively (p=0.012). In receiver operating characteristic curve analysis, the cutoff value of GRDR for the selection of the dorsal position of the PV was 2.4 (area under the curve: 0.929). The incidence of HACs was significantly higher in monosegment graft recipients versus those receiving non-monosegment grafts (23.1% vs. 5.3%, p=0.010); however, the incidence rate of HACs due to external factors, such as compression or inflammation around the anastomotic field was significantly higher in monosegment graft recipients versus those receiving non-monosegment grafts (15.4% vs. 1.1%, p<0.001).
Conclusion: Although monosegment graft recipients suffered from HACs caused by external factors, such as hematoma and inflammation around the anastomotic field, hepatic arterial reconstruction could be safely performed using our technique. An important component in the success of monosegment graft hepatic arterial reconstructions was the employment of the branch patch technique and the selection of the dorsal position of the PV as the anastomotic field.

P.1182

Postoperative outcomes of Controlled Donation after Cardiac Death compared with Donation after Brain Death : a meta-analysis

Jianxin Tang, Nan Jiang.
Department of Hepatic Surgery, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, People's Republic of China.

Aim: Live transplantation (LT) from controlled donation after cardiac death (DCD) donors has increased because of the donor shortage. The outcome of using DCD organs remains unclear ,especially among recipients with hepatits C virus (HCV) infection. We aimed to compare recipient postoperative outcomes from controlled DCD donors and these with donotion after brain death (DBD) donors.
Methods: We searched the databases PubMed, Embase,and The Cochrane Library from January 2000 to January 2016, for studies reporting on post-transplantation outcomes after Maastricht category III and IV DCD liver transplantation which were screened for inclusion.We appraised studies using the Newcastle-Ottawa scale and meta-analyzed using a fixed or random effects model.
Results: The analysis included 12984 patients (controlled DCD = 2217 and DBD =10767) enrolled in 24 trials,including 23 retrospective and 1 prospective cohort studies. There was no significant difference in patient survival up to five years (HR:1.21; 95% CI,0.99-1.48; P = 0.06) ,graft survival up to five years (HR =1.24; 95% CI, 0.99-1.56; P = 0.06), hepatic artery thrombosis (OR = 1.11; 95% CI,0.76-1.63; P = 0.59), portal vein thrombosis (OR = 1.09; 95% CI,0.55-2.19; P = 0.80), HCV recurrence(OR = 2.27; 95% CI,0.66-7.88; P = 0.20),length of hospital stay (WMD: 0.41; 95% CI, -0.56-1.38; P = 0.41) or rejection episodes (OR = 0.88; 95% CI, 0.62-1.24; P = 0.46). We detect a significant difference in primary nonfunction (OR:2.47; 95% CI,1.68-3.65; P < 0.00001), biliary complications (OR =2.42; 95% CI, 2.01-2.92; P < 0.00001), retransplantation (OR = 2.49; 95% CI,1.95-3.18; P < 0.00001) or peak ALT (WMD: 330.88; 95% CI,259.88-401.87; P < 0.00001).However, subgroup analysis found the biliary complications (P = 0.16), retransplantion (P = 0,15) and peak ALT(P = 0.06) were no significant higher in recipients with hepatits C virus between the controlled DCD and DBD groups.
Conclusions: Using controlled DCD, liver transplantation is a viable alternative to DBD transplants although it is marred by inferior outcomes including higher rates of primary nonfunction and biliary complications.Besides, the use of controlled DCD grafts in HCV-positive recipients do not impact on patient and graft survival compared with DBD grafts. This latent benefit of controlled DCD liver donation requires further study.
References:
[1] Doyle, M.B, et al. Outcomes Using Grafts from Donors after Cardiac Death. *J Am Coll Surg*.2015; 221(1): 142-152.
[2] Grewal, H.P, et al. Liver transplantation using controlled donation after cardiac death donors: an analysis of a large single-center experience. Liver Transpl. 2009;15(9): 1028-1035.
[3] Singhal, A, et al. Hospital Resource Use with Donation after Cardiac Death Allografts in Liver Transplantation: A Matched Controlled Analysis from 2007 to 2011. *J Am Coll Surg*. 2015. 220(5): 951-958.
[4] Yamamoto, S, et al. Liver transplantation with grafts from controlled donors after cardiac death: a 20-year follow-up at a single center. *Am J Transplant*, 2010; 10(3): 602-611.
[5] Abt, P, et al.,Liver transplantation from controlled non-heartbeating donors: An increased incidence of biliary complications. *Transplantation*, 2003.;75(10):1659-1663
[6] Skaro, A, et al. The impact of ischemic cholangiopathy in liver transplantation using donors after cardiac death: the untold story.*Surgery*, 2009; 146(4): 543-552
[7] Croome, K.P, et al.Endoscopic management of biliary complications following liver transplantation after donation from

cardiac death donors. *Can J Gastroenterol*, 2012; 26(9): 607-610.
[8] D'Alessandro, A.M, et al. Liver transplantation from controlled non-heart-beating donors. *Surgery*, 2000;128(4): 579-588.
[9] de Vera, M.E, et al. Liver transplantation using donation after cardiac death donors: long-term follow-up from a single center. *Am J Transplant*, 2009; 9(4): 773-781.
[10] DeOliveira, M.L, et al. Biliary complications after liver transplantation using grafts from donors after cardiac death: results from a matched control study in a single large volume center. *Ann Surg*, 2011; 254(5): 716-722
[11] Dezza, M.C, et al. The choice of recipient does not have a bearing on early outcome in liver transplant patients receiving grafts from non-heart-beating donors: a reappraisal? *Transplant Proc*, 2007;39(8): 2675-2677
[12] Dubbeld, J, et al. Similar liver transplantation survival with selected cardiac death donors and brain death donors. *Br J Surg*, 2010;97(5): 744-753
[13] Foley, D.P, et al.Biliary complications after liver transplantation from donation after cardiac death donors: an analysis of risk factors and long-term outcomes from a single center. *Ann Surg*, 2011; 253(4): 817-825
[14] Fujita, S, et al. Liver transplantation from donation after cardiac death: a single center experience. *Transplantation*, 2007; 84(1): 46-49
[15] Hernandez-Alejandro, R, et al. Increased risk of severe recurrence of hepatitis C virus in liver transplant recipients of donation after cardiac death allografts. *Transplantation*, 2011; 92(6): 686-689
[16] Jay, C.L, et al. The increased costs of donation after cardiac death liver transplantation: caveat emptor. *Ann Surg*, 2010; 251(4): p. 743-748
[17] Manzarbeitia, C.Y, et al. Long-term outcome of controlled, non-heart-beating donor liver transplantation. *Transplantation*, 2004;78(2): 211-215
[18] Meurisse, N, et al. Outcomes of liver transplantations using donations after circulatory death: a single-center experience. *Transplant Proc*, 2012;44(9): 2868-2873
[19] Pine, J.K, et al. Liver transplantation following donation after cardiac death: An analysis using matched pairs. *Liver Transplantation*, 2009; 15(9): 1072-1082
[20] Pine, J.K, et al. Liver transplantation following donation after cardiac death: An analysis using matched pairs. *Liver Transplantation*, 2009; 15(9): 1072-1082
[21] Taner, C.B, et al.Use of liver grafts from donation after cardiac death donors for recipients with hepatitis C virus. *Liver Transpl*, 2011; 17(6): 641-649
[22] Tao, R, et al. Hepatitis C recurrence is not adversely affected by the use of donation after cardiac death liver allografts. *Liver Transpl*, 2010;16(11): 1288-1295
[23] Vanatta, J.M, et al. Liver transplant using donors after cardiac death: a single-center approach providing outcomes comparable to donation after brain death. *Exp Clin Transplant*, 2013; 11(2): 154-163
[24] Yagci, G, et al. The impact of donor variables on the outcome of orthotopic liver transplantation for hepatitis C. *Transplant Proc*, 2008; 40(1): 219-223
[25] Callaghan, C.J, et al. Outcomes of transplantation of livers from donation after circulatory death donors in the UK: A cohort study. *BMJ Open*, 2013:3(9)

P.1183

Analysis of initial 100 cases of a new liver transplantation program : the Korea University Anam hospital experience

Young-Dong Yu, Dong-Sik Kim, Sung-Won Jung, Jae-Hyun Han.
Department of Surgery, Division of HBP Surgery and Liver Transplantation, Korea Univeristy Medical College, Seoul, Korea.

Background: To evaluate patient outcomes according to types of liver transplantation as part of a new liver transplant program developed in an East Asian country with a limited number of deceased donors.
Methods: Medical records of initial 100 liver transplantations were reviewed retrospectively. Also we compared the outcome between the initial and latter 50 cases.
Results: Sixty-one patients underwent deceased donor liver transplantation (DDLT) and 39 patients underwent living donor liver transplantation (LDLT). Mean model for end-stage liver disease scores of recipients of DDLT and LDLT were 23.18 ± 11.30 and 12.1 ± 5.5, respectively ($P < 0.001$). Fifty-four patients had HCCs and 30 of them (55.5%) underwent LDLT. There were 5 cases of perioperative mortality; 4 and 1 patient from DDLT and LDLT, respectively. Median follow-up was 25 months. Overall patient and graft survival rates at 6 months, 1 and 3 years were 95.8%, 93.6%, and 89.7%, respectively. There was no significant difference in survival between DDLT and LDLT. Overall recurrence-free survival rates of hepatocellular carcinoma (HCC) patients at 6 month, 1, and 3 years were 99.0%, 96.9%, and 93.4%, respectively. However, recurrence free survival was lower in LDLT cases possibly due to larger tumor burden. When comparing the latter 50 cases to the initial 50 cases, anastomosis time, operation time and amount of blood transfusion decreased with experience.
Conclusion: With experience, anastomosis time, operation time and amount of blood transfusion decreased. As a new liver transplant program with limited resource and waiting list, patients with critical condition could undergo DDLT whereas relatively stable patients with HCCs were mostly directed to LDLT. We recommend a balanced approach between DDLT and LDLT for initiating liver transplant programs.

P.1184

Testosterone deficiency a diagnosis to consider: In adult male post liver transplant (LTx) recipients with failure to thrive, muscle wasting or sarcopenia is observed

Ashokkumar Jain[1], Zakiyah Kadry[1], Ali (Riaz) Shah[1], Tracey Valania[3], Thomas Riley[2], Karen Krok[2], Ian Schreibman[2].
[1]Surgery (Transplant), Hershey Medical Center, Hershey, PA, United States; [2]Medicine (Gastroenterology), Hershey Medical Center, Hershey, PA, United States; [3]Pharmacy, Hershey Medical Center, Hershey, PA, United States.

Introduction: Testosterone deficiency with chronic liver failure is well described. De mineralization of bone, loss of muscle mass, sarcopenia, osteopenia, anemia, decreased energy, negative mood state are the common observations with advance decompensated cirrhosis. Yet, routine measurement of testosterone in this patient not included in standard of care. Furthermore there is a systemic lack of data on role of replacement therapy in this situation. We, at our institution have measured serum concentration of testosterone in clinically suspected post liver transplant (LTx) male patients. If the testosterone level was lower than lowest physiological range, patients were treated with replacement therapy.
Aim: of the present study is to examine the subsequent outcome of post LTx patients who had testosterone deficiency and received testosterone replacement therapy.
Patients and Methods: From February 14 to May 2015, post LTx eight male patients (mean age 56.5± 70.1) were clinically suspected for hypo testosterone deficiency based on failure to thrive and or sarcopenia. Plasma concentration of testosterone was measured. There renal function, hospital stay, post discharge placement readmission rate and current status was examined.
Results: ALL eight patients and very low level of testosterone level; mean 29.6±17.88 (median 22.5) ng/mL. All patients received 200mg testosterone intra muscular inject, one patient received twice two weeks apart. All 8 patients had hepatorenal syndrome and 7 of them required perioperative renal replacement therapy. Three patients required tracheostomy and one of them expired a year later from LTX. Two other patients expire in post LTx follow-up period. The mean hospital stay was 33.4± 22.6 days. The rate of readmission was 100 %. 2(25%) were discharge home (one of them subsequently transferred skilled nursing facility). Remaining six was discharged to acute rehab or skilled nursing facility. At the last follow up 5 patients (62.5%) were alive 3 of which them are at home.
Conclusion: Male Patients with high MELD score, renal impairment, failure to thrive, muscle wasting, sarcopenia, may have associated testosterone deficiency; a diagnosis to be considered. These patients often have:
1. prolonged hospital stay,
2. discharged to rehab or nursing facilities
3. requires readmissions and
4. have high mortality in first year post LTx. Benefit of testosterone replacement therapy needs prospective evaluation in a large population with well-defined parameters.

P.1185

Measurement of creatinine clearance in immediate post kidney transplant (KTx) period with functioning allograft

Ashokkumar Jain[1], Zakiyah Kadry[1], Ali (Riaz) Shah[1], Farooq Umar[2], Fareeha Khalil[2], Narsollah Ghahramani[2], Steven Wassner[3].
[1] Surgery (Transplant), Penn State Hershey Medical Center, Hershey, PA, United States; [2]Medicine (Nephrology), Penn State Hershey Medical Center, Hershey, PA, United States; [3]Medicine (Pediatric Nephrology), Penn State Hershey Medical Center, Hershey, PA, United States.

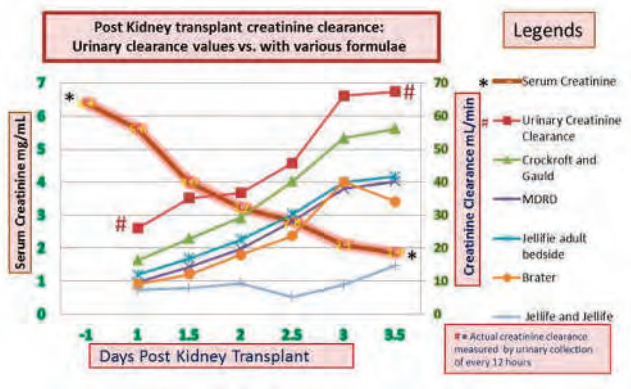
Introduction: There are several formulae available to estimate creatinine clearance based on serum creatinine. However, there is not a single specific formula to estimate creatinine clearance with functioning renal allograft post KTx. Most of these formulae use one serum creatinine value and incorporate a fraction to account for gender, body mass index, ideal body weight index, body surface area or serum albumin differences. There are formulae which are more suitable for children and some formulae which are more suitable for obese patients. There are also a few formulae which take into account a change in the serum creatinine to estimate progression of chronic renal disease. However, these formulae are not designed to estimate creatinine clearance immediately post KTx, where serum creatinine decreases every day at a different rate.
Aim: of the present study is to compare the actual creatinine clearance measured by established urinary collection every 12 hours and compare with estimated creatinine clearance by various formulae which takes in to account either a single creatinine value or changes two serum creatinine values over time.
Patients and Methods: Between Oct.2015 and Jan. 2016, six consecutive consenting post KTx subjects (2 male, 4 female, mean age 53.4 ± 6.4 years, mean wt. 94.0 ± 24.4 Kg, mean BMI 30.2± 5.6 wt./ht. m.2) with primary allograft function were included in the study. Every 12 hours urinary was collected via a Foley catheter (total 45 collections) to measure creatinine concentration. The creatinine clearance was calculated with simultaneous serum creatinine value using standard calculation. This was compared with 1) Crockroft and Gauld 2) MDRD, and 3) Jellife adult bedside (formulae where creatinine clearance is estimated with single serum creatinine value) and with 4) Brater and 5) Jellife and Jellife (formulae where creatinine clearance is estimated by the change between two consecutive serum creatinine values over time).
Results: Figure 1 shows, a) Mean value of serum creatinine mg/dL before KTx and every 12 hours after the primary allograft function and b) Calculated creatinine clearance by urinary excretion and estimation by various formulae at the same time points for competitions.

Figure 1: Post KTx Serum creatinine
Urinary creatinine clearance v/s. estimated creatinine by various formulae

Days Post KTx	-1	1	1.5	2	2.5	3	3.5	4	4.5	5
Serum Creatinine mg/dl	6.4	5.6	4.0	3.2	2.8	2.1	1.9	1.4	1.3	1.5
Method	Creatinine Clearance ml/min									
Urinary Creatinine Clearance $		26.0	35.1	36.7	45.7	66.1	67.4	79.3	71.7	59.8
Crockroft and Gauld *		16.2	22.8	29.2	40.1	53.3	56.1	71.3	69.3	61.8
MDRD *		9.6	14.3	19.7	28.0	38.0	40.1	53.7	51.6	45.2
Jellife adult bedside *		11.8	16.8	22.5	30.3	40.0	41.6	53.2	51.5	44.0
Brater #		9.1	12.0	17.9	23.7	40.0	34.1	40.2	51.6	59.7
Jellife and Jellife #		7.32	7.88	9.28	5.12	8.85	14.61	13.68	7.38	1.29

$= actual urinary, *= one serum creatinine based formulae, #= two consecutive serum creatinine based formulae

Figure 2, shows graphic comparisons of the data in first figure.



Conclusion: The preliminary observations suggest that current formulae are not applicable for post-KTx patient with primary allograft function. They grossly underestimate the creatinine clearance until serum creatinine is stabilized below 1.5 mg/dL.

There is a need to develop a new formula to estimate creatinine clearance in functioning allografts post-KTx. This will allow clinicians to adjust the dosage of various medications based on renal function to improve the efficacy and reduce drug-related toxicities in post KTx recipients.

References:

[1] Buron F, Hadj-Aissa A, Dubourg L et al. Estimating glomerular filtration rate in kidney transplant recipients: performance over time of four creatinine-based formulas. Transplantation 2011; 92: 1005-1011.

[2] Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976; 16: 31-41.

[3] Levey AS, Bosch JP, Lewis JB et al. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med 1999; 130: 461-470.

P.1186

Incidence and management of bile stone after liver transplantation: Report of a single center

Mahir Kirnap[1], Ebru H.Ayvazoglu Soy[1], Aydincan Akdur[1], Fatih Boyvat[2], Gokhan Moray[1], Mehmet Haberal[1].
[1]Transplantation Center, Baskent University, Ankara, Turkey;
[2]Interventional Radiology, Baskent University, Ankara, Turkey.

Introduction: Bile stones (BS) after liver transplantation (LT) is rare (2-6%). However, BS cause various complications like recurrent cholangitis, biliary strictures, secondary biliary cirrhosis, sepsis, graft loss and even patient mortality. In this study we aimed to evaluate our incidence, pathogenesis and management of BS following LT.

Materials and Methods: Between 8 December 1988 and 31 December 2015 we performed 512 LT procedures to 492 patients at our centers. We excluded early postoperative mortality, patients with insufficient data records and patients that we performed last year to reach a reasonable follow up period for developing de nova BS. We retrospectively evaluated demographic features, LT indication, blood lipid profile, bile reconstruction technique, postoperative complications, time of BS diagnosis and treatment modalities.

Results: We retrospectively evaluated 352 liver transplant patients (169 adult, 186 pediatric). 299 living donor LT and 56 deceased donor LT. We diagnosed BS in 18 LT patients (5.1%) (11 adults, 7 pediatric). Six of these patients had LT from deceased donors. We performed 13 duct to duct (DD) (13/18, 72.2%) for bile reconstruction. Seventeen patients with BS had biliary complications prior to BS development; 7 biliary stricture (7/18, 38.9%), 6 biliary leakage (6/18, 33.3%), 4 biliary strictures secondary to biliary leakage (4/18, 22.2%). 7 patients (7/18, 38.9%) had hepatic artery complications in early postoperative period. It is statistically significant that previous biliary complications and recurrent cholangitis increase BS in transplanted liver. There is a significant difference between patients with triglyceride levels higher than 250mg/dL and patients with triglyceride levels lower than 250mg/dL (p=0.0001). Besides cold ischemia time is statistically different between BS and non BS patients (p=0.001). Three BS patient (3/18, 16%) were treated by endoscopic tools, the others (15/18, 84%) were managed via percutaneous procedures.

Conclusions: BS following LT is rare, but it affects graft and patient survival, it should not be ignored especially in LT patients with previous history of biliary complications, hepatic artery problems, long cold ischemia time, and high cholesterol levels. BS management can be successfully done by endoscopic and percutaneous techniques.

P.1187

Management of early hepatic arterial thrombosis after liver transplantation

Ebru H.Ayvazoglu Soy[1], Mahir Kirnap[1], Aydincan Akdur[1], Fatih Boyvat[2], Gokhan Moray[1], Mehmet Haberal[1].
[1]Transplantation Center, Baskent University, Ankara, Turkey;
[2]Interventional Radiology, Baskent University, Ankara, Turkey.

Introduction: Hepatic artery thrombosis (HAT) is a severe complication with an overall incidence varies between 2.6% to 20%, after LT. Early diagnosis and revascularisation via surgery or interventional techniques is important to avoid graft loss. The choice of therapy depends on a variety of factors, including the timing of thrombosis, the graft function, the underlying cause and the availability of organs for retransplantation. In this study, we present our diagnosis, treatments and outcomes of early HAT after LT.
Materials and Methods: Between 8 December 1988 and 31 December 2015 we performed 512 LT procedures to 492 patients at our centers (age range, 6 months to 64 years). We included hepatic arterial thromboses (HAT) that are developed in the early postoperative period of these patients, within 30 days. Diagnosis of HAT were done by doppler ultrasonography (USG) and confirmed by conventional arterial angiographyAll biliary and vascular anastomoses were performed with loop magnification (x2.5). The arterial anastomosis was performed with 7-0 or 8-0 monofilament suture placed between the recipient common hepatic artery, gastroduedonal artery junction or hepatic artery branches and the hepatic artery of graft.
Results: Fifty of 512 LTs (9.7%) were diagnosed with HAT (20 adult, 30 pediatric). Four patients with HAT received whole liver graft from cadaveric donors. 18 patients received a right lobe, 8 patients received a left lobe and 24 patients received a left lateral lobe. 24 HAT went directly to surgery and artery was reconstructed successfully. Percutaneous tools were chosen as the first treatment modality in 26 HAT patients and recanalisation is achieved in 18 of them. Percutaneous tools failed in 8 HATs and recanalisation was achieved by surgery in 3 of them. Retransplantation was done after failed percutaneous procedures and surgical reconstruction in 5 HATs. We lost two patients due to sepsis in early postoperative period.
Conclusion: Interventional radiology is a very important tool for early diagnosis of HAT. In very selective cases it can also be used for treatment. However it is mostly helpful for late postoperative period. In early postoperative period, the choice of percutaneous interventions and surgery as the first line treatment of HAT should be done very carefully and individually.

P.1188

Unusual indications for a liver transplant: A single-center experience

Aydincan Akdur[1], Mahir Kirnap[1], Ebru H.Ayvazoglu Soy[1], Figen Ozcay[2], Gokhan Moray[1], Gulnaz Arslan[3], Mehmet Haberal[1].
[1]Transplantation Center, Baskent University, Ankara, Turkey;
[2]Pediatric Gastroenterology, Baskent University, Ankara, Turkey;
[3]Anesthesiology, Baskent University, Ankara, Turkey.

Objectives: This study sought to evaluate the efficacy of liver transplant for unusual liver diseases.
Materials and Methods: The results of 476 patients who underwent liver transplant from 1988 to January 2015 were retrospectively analyzed. Two hundred forty-five of them were adult patients and 231 of them were pediatric. Thirty-one of them had unusual liver disease.
Results: Of the 31 patients, 9 (29%) were adult and 22 (71%) were pediatric. In the pediatric group, indications for liver transplant were Alagille syndrome (n = 5), Crigler– Najjar syndrome type 1 (n = 5), glycogen storage disease (n = 3), oxalosis (n = 3), familial hypercholesterolemia (n = 2), alpha-1-antitrypsin deficiency (n = 2), Caroli disease (n = 1), and cystic neuroblastoma metastasis (n = 1). Six patients (27.2%) had acute rejection episodes and were successfully treated with pulse steroids. In 2 patients, retransplant was performed for chronic rejection. Three patients died during follow-up (13.6%). Two patients died because of sepsis, and 1 died because of cranial hemorrhage. In the adult group, indications for liver transplant were neuroendocrine tumor metastasis (n = 1), liver angiosarcoma (n = 1), familial hypercholesterolemia (n = 2), alveolar hydatid disease (n = 2), cystic fibrosis (n = 1), congenital hepatic fibrosis (n = 1), and oxalosis (n = 1). Four patients (44.4%) had acute rejection episodes and were successfully treated with pulse steroid. One patient died due to the recurrence of primary disease (liver angiosarcoma) during follow-up (11.1%).
Conclusions: Advances in liver transplant and our understanding about unusual liver disease have led to significant improvements in managing these diseases. Liver transplant effectively treats the underlying defect and the complications of portal hypertension, or risk of malignancy for those disorders, in which the liver is affected.

P.1189

Effects of natural immune system on acute rejection of living donor renal transplantation

Mahir Kirnap[1], Yaprak Yalcin[2], Umit Ozcelik [3], Yahya Ekici [1], Belgin FAtac[2], Gokhan Moray[1], Mehmet Haberal [1].
[1]General Surgery, Baskent University, Ankara, Turkey; [2]Medical Biology, baskent university, Ankara, Turkey; [3]General Surgery, Baskent University, Istanbul, Turkey.

Background and Aims: One of the most common problems encountered in renal transplant patients is allograft rejection. Acute rejections seen in long term are caused by incompliance of patient to immune suppressive therapy. Unlike long term in early period from transplantation, acute rejection are mostly related with natural immune response rather than immune suppressive therapy. Variations in TLR2, TLR4, CD14, and mbl2 expressing genes could have an effect on acute allograft rejection due their functions in natural immune response.
It has been suggested that various molecular and epigenetic factors may modify allograft rejection. Moreover the polymorphisms either single nucleotide polymorphisms (SNPs) or microsatellite polymorphisms of genes often affect expression levels and can serve as disease modifiers. In view of the location and proposed biologic effect of natural immune response, we thought that it would be prudent to further evaluate the association between CD14 (159C/T), TLR4 (Asp299Gly and Thr399Ile), TLR2 (Arg753Gly) and mbl2 codon 52, 54 genotypes and allograft rejection in live renal transplantation.
Materials and Methods: We have analyzed consecutive 53 renal transplant patients and their donars. Written informed consent was obtained from all participants. The isolated genomic DNA was used as a template for polymerase chain reaction of CD14 (159C/T), TLR4 (Asp299Gly and Thr399Ile), TLR2 (Arg753Gly) and mbl2 codon 52, and 54. Genotyping was performed by using real time PCR and the results were analyzed by melting curve.
Plasma creatine levels on first, 6th and 12th month after transplantation were recorded and graft rejection events were also evaluated. Graft rejection diagnosis was confirmed with percutaneous renal biopsy.
Results: No significant associations were found between CD14 (159C/T), TLR4 (Asp299Gly and Thr399Ile), TLR2 (Arg753Gly) and mbl2 codon 52, and 54 genotypes and allograft rejection in this series.
Conclusion: Here we present our preliminary results. We still keep in working by increasing the study population. Despite this, it may be possible that other known, or as yet unknown, SNPs within these or other immunological genes could still be important in the pathogenesis of graft rejection. Further studies with other genes are required for the identification of molecular predisposing factors for graft rejection that may help in the development of new treatments and hence might allow for targeting of this treatment to a "high-risk" subgroup, reducing unnecessary exposure to potentially harmful therapies.

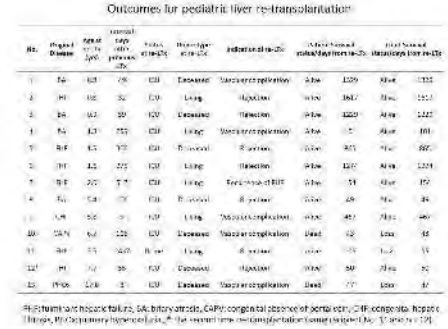
P.1190

Outcomes for pediatric liver re-transplantation at a single center

Akinari Fukuda, Seisuke Sakamoto, Kengo Sasaki, Hajime Uchida, Takanobu Shigeta, Soichi Narumoto, Mureo Kasahara.
Organ Transplantation Center, National Center for Child Health and Development, Tokyo, Japan.

Background: The only therapeutic option for patients with a failing allograft is re-transplant. Although outcomes have improved over the last decade, currently published pediatric patient and graft survival (47.5-73.0%) remain lower after re-transplantation than after primary transplant.
Objectives: The aim of this study was to examine outcomes in our patients and to analyzed risk factors for patient and graft survival after pediatric liver re-transplantations
Patients and Methods: A total of liver transplantations were performed in 366 children (less than 18 years old) at National Center for Child Health and Development in Japan, a total of 13 (3.6%) re-transplantations were performed in 12 recipients (re-transplantation performed twice in 1 patient) from November 2005 to January 2016. Patient survival was analyzed with respect to various preoperative factors, such as age group at re-transplantation; within 6 year old and over 6 year old, functional status, cause of primary graft failure, interval from previous to subsequent transplantations; within 30 days and more than 30 days, and donor type of re-transplantation.
Results: The median age at re-transplantation was two (range 0.8-17.8; within 6 yrs was nine cases and over 6 yrs was four cases) year-old. The most common primary diagnosis was fulminant hepatic failure (n=6; 46.2%). The median interval days after previous liver transplantation to re-transplantation was 106 (range 8-2437; within 30 days was two cases and more than 30days was 11 cases) days. Seven donor grafts were from deceased donors, the oter six donor grafts were from living donors. Pathologically proven major causes of primary graft failure were rejection (n=7; 53.8%), vascular complications (n=5; 38.5%), and recurrence of fulminant hepatic failure (n=1; 7.7%). Of the two patients who died, these cause of death were sepsis and veno-occlusive disease.

Outcomes for pediatric liver re-transplantation



Kaplan-Meier estimated patient and graft survival rate from the date of re-transplantation to one and three year were 84.6%/76.9% and 76.9%/84.6%. There were no significant differences in preoperative functional status, interval to re-transplant, indication for re-transplantation and donor type of grafts on survival after re-transplant. Age of patients at the re-transplantation significantly differed between within 6 yrs (n=9; one and three year patient and graft survival 100%/100%) and over 6 yrs (n=4; one and three year patient and graft survival 50%/25%) on survival after re-transplantation (patient survival: p=0.027 and graft survival: p=0.004, Log-Rank test).
Conclusions: Re-transplant can save patients with a failing allograft. There is a trend toward improving results. Liver re-transplantation makes an important contribution to improve overall survival in children. The age of patient over 6 year old at re-transplantation was the most adverse impact factor for survival rate after re-transplantation.

P.1191

Retransplantation of the liver: single center results

Maxim Kornilov, Sergey Gautier, Ekaterina Kornilova, Olga Tsirulnikova.
Federal Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation.

Liver transplantation is a recognized treatment for patients with end-stage and focal liver diseases, metabolic disorders and cases of acute liver failure. In the case of irreversible graft dysfunction in the early or late postoperative period and retransplantation of the liver (reOLT) is the only possible potential treatment method. From December 2004 up to November 2015, our experience is 260 cadaveric liver transplants, 15 of them (5,7%) are reOLT. In the early postoperative period reOLT were performed to 5 recipients. In one case in the absence of initial graft function; in two cases due to thrombosis of the hepatic artery and two other cases due to severe graft dysfunction in the early postoperative period. In far postoperative period made 10 operations. The indications for surgery were: the recurrence of the underlying disease - 6, transplant dysfunction associated with HCV infection (in one case de novo) - 2, biliary complications (ischemic cholangiopathy) - 2. The median MELD was 30 points [interquartile interval 23-37] among the patients operated in the earlier period, and 27 points [interquartile interval of 17.2-36] in the later period. Among recipients who were operated in the early postoperative period signs of infection prior to surgery was observed in 2 (40%). The efferent blood flow was restored by the same procedure as that of primary transplantation. Certainly arterial reconstruction within reOLT is the most difficult part of the implant. Mobilization and formation of anastomosis at primary OLT, the adhesion process in the postoperative period, disruption of the afferent blood flow and possible endovascular intervention involve changes of the vascular wall of arteries, making the formation of the anastomosis is very difficult, and frequently impossible. Among the 15 reOLT in 2 cases (13.3%), we resorted to the use of the arterial conduit. Preferential option for biliary reconstruction was choledochojejunostomy on intestine disabled by Roux (66%). When reOLT were performed in short term - survived only 2 patients (40%) with thrombosis of the hepatic artery. While of the 10 patients who underwent reOLT in the long terms - survived 6 patients (60%).
To conclude: our experience of retransplantation in the early postoperative period before the development of multiorgan failure and infectious complications demonstrates the validity of the urgent retransplantation.

P.1192

Poor patient outcome in combined liver-kidney transplant: A fifteen years experience from a single centre

Sonsoles Martinez, Imeshi Wietjunga, Vivek Upasani, Ecuyer Claire, Willis Sean, Magdy Attia, Niaz Ahmad.
Division of Surgery, Department of Transplantation, Leeds Teaching Hospitals, Leeds, United Kingdom.

Introduction: Increasing number of Combined Liver and Kidney Transplant (CLKT) are being performed since the introduction of MELD system for allocation of deceased donor liver. The indications and timing for this type of transplant, however remain unclear. The aim of this study was to evaluate and compare overall survival, kidney graft survival and number of preemptive transplants after CLKT in our centre.
Methods: Patients receiving a combined liver and kidney transplant from 2000 to 2015 were included in this study. Data was collected from a prespectively maintained database of liver and kidney transplant in our centre. Missing data were completed from patient's paper based records, and the hospital electronic record. Comparison made with patients receiving kidney transplant only matched by gender, age, ethnicity and date of transplant. We compared overall survival, graft survival and mortality between the two groups. Published liver transplant outcome from NHSBT was used as reference.
Results: 20 patients received combined liver and kidney tranpslant between 2000 and 2015. The most common indication was Hyperoxaluria (25%) followed by Polycystic Kidney and Liver Disease (20%). Eleven kidneys (55%) were transplanted pre-emptively.
One year overall survival for the CLKT group was 80% with 4 deaths within the first postransplant year, and remained 80% after 5 years. All deaths happened with functioning kidney graft. For kidney only transplant in the matched group, 1 year and 5 year survival remained at 100% (p=0.006). In the CLKT group 1 yr and 5yr graft survival was 75% compared with kidney only group where this was 93% and 85% repectively (p=0.361). NHSBT published data records 1 and 5 years after liver transplant as 92.1% and 78.8% for our centre.
Discussion: Our data reflect and increased postoperative mortality in the patients receiving combined liver and kidney transplant. A majority of patients received preemptive kidney transplant in this group. A kidney transplant may have been added because of a favourable bias in the allocation of a kidney to these patients. Adding a second procedure may have contributed to increased mortality in this group. A lover kidney graft attrition rate (not significant), may reflect better quality graft allocated with CLKT. We propose a multicentre review of CLKT to confirm these results and review the policy based upon the findings of the study.
References:
[1] Gonwa TA, McBride MA, Anderson K, Mai ML, Wadei H, AhsanN. Continued influence of preoperative renal function on outcome of orthotopic liver transplant in the US: Where will MELD lead us? Am J Transplant 2006;6:2651-9
[2] Nadim MK, Sug RS, Davis CL, et al. Simultaneous Liver-Kidney Transplant summit: Current state and future directions. Am J Transplant. 2012;12:2901-8
[3] Puri V, Eason J. Simultaneous Liver-Kidney Transplantation. Curr Transpl Rep (2015) 2:297-302
[4] NHSBT Annual Report on Liver Transplantation. Report for 2013/2014 (1 Apr 2005 – 31 March 2015) Published September 2015

P.1193

Pediatric living donor liver transplantation at KFSH&RC – implementation of the laparoscopic retrieval in a high volume center

Markus Boehnert, Yasser El Sheikh, Mohammad Shagrani, Talal Al Goufi, Dieter Broering.
Organ Transplant Centre, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia.

Since the first successful pediatric liver transplantation was performed in 1967 the survival rate increased from 30 % to currently above 90 %. This is owed to the introduction of cyclosporine in the early eighties. Another problem was the lack of available size matched liver donors, which caused high death rates on the waiting list of pediatric recipients. Technical innovations based on the segmental anatomy of the liver, e.g. reduced liver grafts, split grafts and living liver transplantation addressed this problem. The last big advantage was the implementation of FK 506 Tacrolimus, which allows steroid withdrawal within the first year post-transplant and preserves the growth potential of children. The new task in living related pediatric liver transplantation is the implementation of the laparoscopic retrieval technique.

Between 2011 and 2015 we performed 198 pediatric liver transplantations with a 92 percentage of living related donors. In our series 38.4 % of the children were below one year old with a predominance of genetic-metabolic diseases of 48.2 %. In contrast to other countries biliary atresia was the indication for transplant only in 29.5 %, 7.1 % showed end stage liver disease of unknown reason.

Our results show a recipient and graft survival of 93 and 89 % respectively. The morbidity was 17 % for surgical and 18 % for medical complications. The biopsy proven rejection rate was 7 %. Six children died after discharge at home or in peripheral hospitals for unknown reasons.

In accordance with other centers the rate of grafts from living donors clearly prevails deceased grafts. We implemented the laparoscopic approach in left lateral living donation. The donor complication rate was similar between the minimal invasive and open procedure, while the post donation hospital stay was reduced in the minimal invasive group.

Therefore the laparoscopic retrieval procedure should become standard on the donor side in pediatric living donor liver transplantation.

P.1194

Right-sided diaphragmatic hernia after living donor right hepatectomy: A rare complication after living donor

Gyu-seong Choi, Yoo Dong-Do[2], Jeea Lee.
Surgery, Samsung Medical Center, Seoul, Korea; [2]Saint Vincent Hospital, Suwon, Korea.

Background: Right-sided diaphragmatic hernia after living donor Right hepatectomy has been rare complication. we report a Right-sided diaphragmatic hernia in a living donor.

Methods: A 26-year-old man underwent donor right hepatectomy for living donor liver transplantation. Three months after liver donation, he presented with upper abdominal pain and vomitting. Computed Tomography revealed a diaphragmatic hernia of the right thorax with stragnulation.

Results: He underwent laparotomy and a 3cm defect in the right diaphragm was identified and repaired with interrupted polypropylene suture, after operation, the patient remains well.

Conclusions: Throughout the expansion of living donor liver transplantion, the safety of the donor is most important thing. Diaphragmatic hernia is a rare complication of right donor hepatectomy but may occur. And early diagnosis and prompt management is needed for donor safety.

P.1195

Living donor liver transplantation in a Jehovah's Witness - the first adult living donor liver transplantation in Korea

JeeA Lee[1], Yoo Dong-Do[2], Gyu-seong Choi[1].
[1]Surgery, Samsung Medical Center, Seoul, Korea; [2]Saint Vincent Hospital, Suwon, Korea.

Purpose: Orthotopic liver transplantation is typically associated with large volume blood loss. Technological and pharmacological advances permit liver transplantation in patients who formerly were not candidates for this surgery because of strict limitations on blood product administration. We describe a liver transplant in a Jehovah's Witness with liver cirrhosis related with HBV infection.
Clinical features: A 52-year-old Jehovah's Witness with liver cirrhosis related with HBV infection and end stage liver disease presenting with uncontrolled ascites underwent orthotopic liver transplantation. MELD score was 18. Recombinant human erythropoietin (10,000 IU sc every two days for four weeks, then 10,000 IU sc every day for one week) established a normal hemoglobin concentration preoperatively (>13.1g/dL compared with 7.3 g/dL baseline). Intraoperatively, strategies for reducing risk of blood product transfusion included avoidance of hypothermia (temperature>35°C), minimal blood sampling (1 ml samples only four times), normovolemic hemodilution (3 units) and return of blood (300 ml) scavenged from the operative field. Estimated blood loss was 800 ml. The preoperative and postoperative hemoglobin concentration was 13.4 g/dL (hematocrit 0.38) and 11.4 g/dL (hematocrit 0.32), respectively. No blood products were required and he was discharged 15 days postoperatively without complication.
Conclusion: Technological and pharmacological advances allow patients to undergo surgery traditionally associated with large volume blood loss with reduced risk of blood product administration.

P.1196

Anastomosis of type 2 portal vein variation and low-lying right posterior duct anomaly in living donor liver transplantation

Kwangsik Chun, Jongduk Kwan, Hyemi Ko, Insang Song.
Surgery, Chungnam National University Hospital, daejeon, Korea.

Introduction: We report our experiences of type 2 portal vein and bile duct anastomosis during living donor liver transplantation
Case: Forty-four years old man was admitted for generalized weakness. He suffered from chronic viral hepatitis for 20 years (hepatitis B) and 2years ago diagnosed liver cirrhosis with hepatocellular carcinoma. Primary HCC was treated by percutaneous RFA and recurred HCC was by TACE twice. After recent TACE generalized weakness, ascites were progressed. Hepatic encephalopathy was developed. Living donor liver transplantation was decided. Donor was 27-year-old his younger son. GRWR was 1.48. Preoperative donor abdomen CT scan was revealed trifurcation of portal vein and low-lying right posterior hepatic duct. Middle hepatic vein branches were double in S5 and single in S8 level. Donor hepatectomy was performed as modified extended right hepatectomy (weight = 850gm). During bench operation neo-middle hepatic vein was reconstructed by use of iliac vein allograft. Lumens of graft portal vein were double. So left saphenous vein autograft patch was fenced to the graft portal veins for making single lumen. Graft was transplanted to recipient from right hepatic vein, portal vein, neo-middle hepatic vein and then right hepatic artery. Bile ducts were make common cannel in manner of V-shaped plasty then anastomosed to recipient bile duct. Total operation time was 632 minutes cold ischemic time was 40 minutes for bench operation. Maximal AST/ALT was 230/207IU/ml at POD #1 then normalized at POD #5 and #15 each. Postoperative abdomen CT revealed patent portal vein, neo-middle hepatic vein and hepatic artery. There was no congestion area in the transplanted liver. Patient was discharged at POD #34. There was no stricture or stenosis in anastomosis site in veins, artery and bile duct.
Conclusion: In the living donor liver transplantation, there were many anatomical difficulties in anastomosis due to anatomical variation especially in portal vein and bile duct. Portal vein fencing and bile-ductoplasty can be a good choice of anastomotic manners.

P.1197

More than 16 years follow up of living donor liver transplantation for two cases of homozygous familial hypercholesterolemia from the heterozygous donor

Naoki Kawagishi, Kenji Shimizu, Satoru Yoshida, Kai Maida, Kohji Miyazawa, Yasuyuki Hara, Chikashi Nakanishi, Shigehito Miyagi, Noriaki Ohuchi, Susumu Satomi.
Division of Transplantation, Reconstruction and Endoscopic Surgery, Tohoku University Hospital, Sendai, Japan.

Introduction: Homozygous familial hypercholesterolemia (FH) is a rare, genetic disorder due to mutations in the low-density lipoprotein (LDL) receptor gene. The incidence of this disease is approximately one per million in the Japanese population. Severe hypercholesterolemia from the time of birth induces the atherosclerosis in childhood and even death due to myocardial infarction before the age of twenty. So far, LDL apheresis and gene therapy have been attempted but the outcome is less satisfactory than liver transplantation. The aim of this study is to review our experiences of FH for living donor liver transplant recipients and analyze the long-term outcome.
Materials and Methods: We experienced two pediatric siblings with FH who received living donor liver transplantation (LDLT) from their parents who were heterozygous for FH. Case 1 ; The elder brother presented orange cutaneous xanthomas and was diagnosed homozygous FH at the age of one. The plasma lipidogram showed that total cholesterol was 898 mg/dl, LDL cholesterol was 756 mg/dl and triglyceride level was 60 mg/dl. There were no living donors with a normal LDL receptor in their family, and it was difficult to find a deceased donor in Japan; thus he underwent LDLT with his father as the donor. Case 2 ; The younger sister was born 2 years after her brother's LDLT. The plasma lipidogram showed that total cholesterol was 857 mg/dl just after birth. She was once listed on the brain dead donor series of Japan, but no donor appeared for one year. She underwent ABO-incompatible LDLT with her mother as the donor at the age of 2.
Results and Discussion: Case 1; The liver function tests (LFTs) of the elder brother were normalized immediately after transplantation, and his cholesterol has remained controlled at around 240 mg/dl with HMG-CoA reductase for 16 years after LDLT. Case 2; We performed plasma exchange twice before Tx to reduce the IgM and IgG hemagglutinin titers. One week after Tx IgG titers were elevated associated with a slight deterioration of LFTs, but graft needle biopsy showed drug induced liver dysfunction. Twelve years after LDLT the cholesterol of her remained stable at around 240 mg/dl. At present the four patients including the two heterozygous donors are leading a normal daily life. This is the first time in the world that grafts from donors with heterozygous FH are effective in patients with homozygous FH even for a period of over 16 years.
Conclusion: Our patients showed that LDLT from a heterozygous or even an ABO-incompatible donor is effective for patients with homozygous FH, provided they are cholesterol-lowering drugs after transplantation.

P.1198

Renal function status in liver transplant patients in the first month post-transplant is associated with progressive chronic kidney disease

Naoki Kawagishi, Ken-ichi Shimizu, Satoru Yoshida, Kai Maida, Koji Myazawa, Yasuyuki Hara, Chikashi Nakanishi, Shigehito Miyagi, Kazushige Sato, Noriaki Ohuchi, Susumu Satomi.
Division of Transplantation, Reconstruction and Endoscopic Surgery, Tohoku University Hospital, Sendai, Japan.

Introduction: Long-term deterioration of renal function is common in LT patients, and can often lead to chronic renal failure. An important cause of chronic kidney disease (CKD) among LT patients is calcineurin-inhibitor (CNI)-based immunosuppression, although the etiology of CKD after LT is multifactorial, including old age, diabetes mellitus, hepatitis viral-associated glomerulonephritis, hepatorenal syndrome, pretransplant renal dysfunction and acute kidney injury. This study was done to investigate the association between renal functional status soon after LT and the development of CKD.
Materials and Methods: We retrospectively evaluated 63 patients who were aged 18 years or older, and underwent LT at Tohoku University Hospital from 2000 to 2010. The initial immunosuppressive regimen consisted of CNI and steroids with or without basiliximab. To facilitate the decrease in CNI doses, mycophenolate mofetil was administrated to patients who had repeated rejection episodes or those who developed renal insufficiency. The estimated glomerular filtration rate (eGFR) was calculated by the Modification of Diet in Renal Disease study equation for Japan. The eGFR at 1 week, and 1, 2, 3 and 5 years was based on an average of at least five measurements. In this study, CKD was defined as an eGFR of less than 60 mL/min per 1.73 m2 for 3 months or more.
Results and Discussion: Before transplantation, 25 patients (39.7%) were diagnosed with CKD. The incidence of CKD was 22.4% (13/58) at 2 years, 23.2% (13/56) at 3 years and 22.7% (12/54) at 5 years. The patients with CKD at 2 years post-transplant were more likely to have a history of glomerulonephritis, and were significantly older at the time of LT, compared to those without CKD. CKD in the first month post-transplant and a volume of intraoperative blood loss of more than 300 mL/kg were predictive factors for the development of CKD at 2 years post-transplant and thereafter. Therefore, a careful management of the renal function in the early post-transplant period is indispensable for the prevention of the long-term CKD.
Conclusion: Regardless of the presence of pre-existing CKD, both renal function status at the first month post-transplant and a volume of intraoperative blood loss were predictive factors for the development of CKD at 2 years post-transplant and thereafter.

P.1199

Early experience of living donor liver transplantation at Dong-A University Hospital: Initial 24 cases

Kwan Woo Kim, Sung-Hwa Kang, Young-Hoon Roh, Young-Hoon Kim.
Division of Hepato-Biliary & Pancreatic Surgery, Department of Surgery, University of Dong-A College of Medicine, Dong-A University Hospital, Busan, Korea.

Background: Living donor liver transplantation has been progressed dramatically in Asia because of the scarcity of deceased donor. We present the initial experience of living donor liver transplantation (LDLT) at Dong-A university hospital during 24 months.
Materials and Methods: From November 2013 to November 2015, 24 LDLTs were performed at our hospital. There were 22 cases of modified right lobe graft, 1 case of extended right lobe graft and 1 case of dual grafts (two left lobe grafts). Middle hepatic vein in 22 cases of modified right lobe grafts were reconstructed using cryopreserved iliac vein (n=10), iliac artery (n=3) and expanded Polytetrafluoroethylene(ePTFE, GORE-TEX)grafts (n=9).
Results: The mean age of recipient was 52.5 years and mean hospital stay was 22 days. The mean CTP and MELD score were 7.5 and 13.3, respectively. The most common indication of liver transplantation was hepatocellular carcinoma (n=16, 75%). Among them, only one patient was out of Milan criteria. The mean GRWR was 1.13 and mean graft weight was 701.5g. One patient (4.3%) died because of severe sepsis on postoperative 15th days. Morbidity occurred in 8 (33%) patients ; biliary stricture (n=4), right hepatic vein stenosis (n=2), postoperative bleeding (n=2). Primary tumor recurrence occurred in 3 recipients (12.5%) that are all alive. There were no specific complications related using PTFE graft and cryopreserved iliac vessels. There were no serious donor complication or mortality, but one donor experienced large amount biloma around cut surface which was treated by percutaneous drainage.
Conclusion: Although small cases, early result of our experience of LDLT was acceptable. Careful donor and recipient evaluation, meticulous operation, proper postoperative management are guarantee for the safety of donor and recipient in LDLT.

P.1200

Right lobe living donor liver transplantation with middle hepatic vein for treatment end stage liver diseases

Oleg Kotenko, Denis Fedorov, Alexander Korshak, Alexander Grinenko, Marat Grigiryan, Akexey Popov, Andrey Gusev, Alexander Ostapishen, Artem Minich.
Liver Surgery and Transplantation, National Institute of Surgery and Transplantology, Kiev, Ukraine.

Background: Harvesting of right hemilver graft (RHG) without middle hepatic vein (MHV) could lead to graft Sg 5, 7 venous congestion and small for size syndrome (SFSS) in recipients. However, right hepatectomy with MHV in donor could evoke posthepatectomy liver failure (PLF). Reconstructions of Sg 5 and 8 hepatic veins (HV) are not always possible, resulting in restricting abilities of patient to be transplanted in countries where living related donor liver transplantation (LRDLT) is only option.
Objectives: the aim of this study was evaluate recipient benefits and donor risks from LRDLT of right hemiliver with MHV.
Materials and Methods: Medical data of 77 recipients who underwent LRDLT and their 77 living donors was evaluated. In 36 (46,7%) case of LRDLT RHG was harvested with MHV and in 41 (53,2%) cases without MHV. Perioperative data and CT-anatomy where collected and compared in MHV and non-MHV group. RHG was harvested with MHV only in presence of scissural Sg 4 HV. Thick (over 5 mm) inferior right hepatic vein or middle inferior hepatic vein was compulsory reconstructed. PLF was estimated by international consensus criteria.
Results: SFSS developed in 6 (16,6%) recipients of MHV group and in 17 (41,4 %) recipients of non-MHV group (difference is significant, $p = 0,029$). Blood lose (1130 ± 43 ml vs. 1105 ± 36 ml, $p = 0,55$), operative time (624 ± 22 min vs. 530 ± 39 ml, $p = 0,43$), ischemia time (88 ± 14 min vs. 76 ± 12 ml, $p = 0,34$) and vascular complications rates (3 vs. 5, $p = 0,97$) were not different in groups. In MHV group 60-days mortality was 11,1%(4 cases) vs. 14,6%(6 cases) in non-MHV group (p=0,7). Donors presented PLF in 4(11,1%) cases of MHV group and in 4(9,75%) cases of non-MHV group (difference insignificant, $p = 0,72$). All donors recovered on 7 – 14 day p.o. Other characteristics like operation time, blood lose, infection and biliary complications rates in donors were also not significantly different in groups. There was not donor mortality in both groups.
Conclusion: Harvesting of donor right hemiliver with MHV could be safely performed in presence of adequate Sg4 venous drainage with minimal PLF rate 11,1% in donors. Transplanting RHG with MHV lead to less SFSS rate (16,6 % in MHV vs. 41,4 % in non-MHV) with better mortality outcome (11,1% in MHV vs. 14,6 % in non-MHV) in recipients.

P.1201

Early term results of biliary reconstruction using microsurgical outer knotted suture technique in living donor liver transplantation

Shigehito Miyagi, Koji Miyazawa, Satoru Yoshida, Kai Maida, Toshiaki Kashiwadate, Kazuaki Tokodai, Yasuyuki Hara, Chikashi Nakanishi, Hitoshi Goto, Takashi Kamei, Naoki Kawagishi, Noriaki Ohuchi, Susumu Satomi.
Division of Transplantation, Reconstruction and Endoscopic Surgery, Tohoku University, Sendai, Japan.

Objectives: In living donor liver transplantation (LDLT), the recipient bile duct is small and short. Bile duct complications often occur in LDLT. Small size and multiple ducts, particularly in right lobe liver grafts, are major factors that contribute to biliary complications in LDLT. Recently, we began to perform routine microsurgical biliary reconstruction using outer knotted suture technique in LDLT. The purpose of this study was to investigate the effects of this technique and the early complications of biliary reconstruction.
Methods: From 1991 to 2015, we performed 164 LDLTs (pediatric:adult = 91:73, left lobe:right lobe = 97:67). For the present cohort study, patients were divided into 2groups. Group I; we performed conventional biliary reconstruction (n=152). Group II; we performed microsurgical biliary reconstruction using outer knotted suture technique (n=12). We investigated all biliary complications in both groups. The identification of complications included only up to 12 months of follow-up for each recipient in both groups.
Results: The most frequent early complication was biliary stricture (15/164=9.3%), followed by biliary leakage (13/164=7.9%). In the group I, total biliary complications occurred in 23 cases (15.1%, biliary stricture 15/152(9.9%); biliary leakage 12/152(7.9%), include both complications.). In the group II, those complications occurred in only one case that had small size and multiple bile ducts (8.3%, only biliary leakage). Total complication rate in the group II was slightly lower than that in the group I, but there was no significant difference between two groups.
Discussion: In this study, biliary reconstruction using microsurgical outer knotted suture technique was not risk factors for biliary complications. However, the number of cases is small, so further investigation is needed in the future.
Conclusion: In conclusion, this technique is safety and might contribute to more satisfactory results.
References:
[1] Freise CE, Gillespie BW, Koffron AJ, Lok AS, Pruett TL, Emond JC, Fair JH, Fisher RA, Olthoff KM, Trotter JF, Ghobrial RM, Everhart JE; A2ALL Study Group. Recipient morbidity after living and deceased donor liver transplantation: findings from the A2ALL Retrospective Cohort Study. AmJ Transplant 2008; 8(12):2569-79.
[2] Saha A, Naidu C, Ramesh G, Chatterjee J, Puri P, Nandi B, Nambiar P, Madan R. Liver transplantation in Indian Armed Forces—initial experience. Med J Armed Forces India 2012; 68:110-17.
[3] Reichman TW, Katchman H, Tanaka T, Greig PD, McGilvray ID, Cattral MS, Renner EL, Selzner M, Ghanekar A, Levy G, and Grant DR. Living donor versus deceased donor liver transplantation: a surgeon-matched comparison of recipient morbidity and outcomes. Transplant Int 2013 Aug; 26(8):780-7.
[4] Lin TS, Concejero AM, Chen CL, Chiang YC, Wang CC, Wang SH, Liu YW, Yong CC, Jawan B, Cheng YF. Routine microsurgical biliary reconstruction decreases early anastomotic complications in living donor liver transplantation. Liver Transpl. 2009; 15: 1766-75.

P.1202

Prior kidney donation does not impact the outcome of subsequent living right liver lobe donation

Amit Sharma[1], Adrian Cotterell[1], Marlon Levy[1], Robert Fisher[2].
[1]Transplantation Surgery, Virginia Commonwealth University, Richmond, VA, United States; [2]Transplantation Surgery, Beth-Israel Deaconess Medical Center, Boston, MA, United States.

Background: Organ shortages remain the most formidable obstacle to widespread application of organ transplantation. In countries where deceased organ donation rates are low, there are reports of a parent sequentially donating both a left lateral liver segment and a kidney to pediatric recipients[1],[2]. Sequential donation of a kidney and right lobe of liver (SKL) is rare. We have previously reported on the psychological profiles of Good Samaritans SKL donors at our center[3]. In this study, we hypothesized that living right lobe (RL) donors with previous history of kidney donation (SKL donors) would have inferior outcomes as compared to RL donors.
Materials and Methods: Retrospective data analysis of all potential living liver donors at our center (1998 – 2013) was done. SKL donors were compared to RL only donors.
Results: Out of 126 right lobe donors, there were 4 donors who had donated a kidney in the past. There were no donor deaths. There was no difference in hospital stay, incidence of bile leak, wound infections or renal failure (not shown in Table 1). There was no significant difference in serum bilirubin, AST or ALT levels 1-year post-donation. The serum creatinine levels in SKL donors were significantly higher in comparison to right lobe only donors. However, the 1-year serum creatinine continued to be stable in SKL donors compared to pre-operative values.

Table 1 Comparison of donor outcomes

	Sequential kidney-liver (n = 4)	Right lobe only (n = 122)	p-value
Age at liver donation	39.6 ± 8.4	37.4 ± 9.2	0.83
Males	2 (40%)	72 (59%)	0.41
Caucasians	5 (100%)	105 (86.1%)	0.37
Resected right lobe weight (g)	758.6 ± 58.7	853.3 ± 178.9	0.25
Hospital stay (d)	6.0 ± 0.7	6.2 ± 1.5	0.48
S. Creatinine (mg/dl)			
Pre-operative	1.36 ± 0.3	0.90 ± 0.19	0.02
At discharge	0.99 ± 0.3	0.79 ± 0.20	0.03
1-year	1.13 ± 0.1	0.91 ± 0.17	0.005
S. AST			
Pre-operative	24.6 ± 8.0	25.6 ± 11.1	0.71
At discharge	113 ± 22	150 ± 113	0.47
1-year	29.5 ± 8.4	31.3 ± 14.5	0.78
S. ALT			
Pre-operative	26.8 ± 14.0	32.5 ± 17.7	0.46
At discharge	207.4 ± 83.6	245 ± 147	0.58
1-year	22.8 ± 9.4	31.4 ± 21.4	0.38
S. Bilirubin (total)			
Pre-operative	0.8 ± 0.27	0.64 ± 0.29	0.23
At discharge	1.16 ± 0.54	1.89 ± 1.77	0.36
1-year	0.58 ± 0.36	0.67 ± 0.21	0.39
Complications (n)	3	39	
Patients (n)	2	31	
Clavien Grade			
1	3	11	
2	-	14	
3	-	13	
4	-	1	

Conclusions: In carefully selected living donors with previous history of kidney donation (sequential kidney-liver donors), subsequent donation of right lobe does not appear to confer any extra risk of surgical complications. In our small cohort, these 'double donors' had stable renal function one year after right lobe donation.
References:
[1] Sakamoto S, Kasahara M, et al. Pediatric liver-kidney transplantation for hepatorenal fibrocystic disease from a living donor. *Pediatr Transplant*. 2012 Feb;16(1):99-102.
[2] Nakamura M, Fuchinoue S, et al. Three cases of sequential liver-kidney transplantation from living-related donors. *Nephrol Dial Transplant*. 2001 Jan;16(1):166-8.
[3] Sharma A, Ashworth A, Cotterell A, Posner M and Fisher R. Men may be from Mars. Women May be from Venus: Good Samaritan Liver-Kidney Living Donors are from Earth. (2012), The ILTS 18th Annual International Congress. *Liver Transpl*, 18: S1–S306. doi: 10.1002/lt.23435

P.1203

The study of the measurement of liver volume by methods of multi-slice spiral CT and displacement method

Chao Sun, Wei Gao, Zhongyang Shen.
Transplantation Department, Tianjin First Center Hospital, Tianjin, People's Republic of China.

Objective: To compare the methods of liver volume measurements by multi-slice spiral CT (MSCT) and displacement method in vitro.
Method: Eight abandoned donor liver were selected. The whole and half liver volume were measured by MSCT and displacement method in vitro separately. Correlation coefficient was used for statistical analysis.
Result: The volume measured by MSCT was relative to the volume measured by displacement method. There was no significant difference between the whole liver volumes measured by the two methods (R square=0.9173, P<0.05). And there was also no significant difference between the right liver volumes measured by the two methods (R square=0.7728, P<0.05). The liver volume measured by MSCT was smaller than the liver volume measured by displacement method.
Conclusion: The volume measured by MSCT was accurate and relative to the volume measured by displacement method in vitro.
Cheng Yue; Shen Wen
References:
[1] Sakamoto, S., M. Kasahara, Y. Ogura, et al., Current status of deceased donor split liver transplantation in Japan. J Hepatobiliary Pancreat Sci, 2015.
[2] Ringe, K.I., B.P. Ringe, C. von Falck, et al., Evaluation of living liver donors using contrast enhanced multidetector CT - The radiologists impact on donor selection. BMC Med Imaging, 2012. 12: p. 21.
[3] Mokry, T., N. Bellemann, D. Muller, et al., Accuracy of estimation of graft size for living-related liver transplantation: first results of a semi-automated interactive software for CT-volumetry. PLoS One, 2014. 9(10): p. e110201.
[4] Wathen, C.A., N. Foje, T. van Avermaete, et al., In vivo X-ray computed tomographic imaging of soft tissue with native, intravenous, or oral contrast. Sensors (Basel), 2013. 13(6): p. 6957-80.
[5] Hori, M., K. Suzuki, M.L. Epstein, et al., Computed tomography liver volumetry using 3-dimensional image data in living donor liver transplantation: effects of the slice thickness on the volume calculation. Liver Transpl, 2011. 17(12): p. 1427-36.
[6] Suzuki, K., M.L. Epstein, R. Kohlbrenner, et al., Quantitative radiology: automated CT liver volumetry compared with interactive volumetry and manual volumetry. AJR Am J Roentgenol, 2011. 197(4): p. W706-12.
[7] S. M. Nichues, J.K.U., M. Malinowsik, J. Neymeyer, B. Hamm, M. Stockmann, Liver volume measurement: reason of the difference between in vivo CT-volumetry and intraoperative ex vivo determination and how to cope it. Eur J Med Res, 2010. 15.
[8] D'Onofrio, M., R. De Robertis, E. Demozzi, et al., Liver volumetry: Is imaging reliable? Personal experience and review of the literature. World J Radiol, 2014. 6(4): p. 62-71.
[9] Goumard, C., F. Perdigao, J. Cazejust, et al., Is computed tomography volumetric assessment of the liver reliable in patients with cirrhosis? HPB (Oxford), 2014. 16(2): p. 188-94.
[10] Kianmanesh, R., T. Piardi, E. Tamby, et al., Liver angulometry: a simple method to estimate liver volume and ratios. HPB (Oxford), 2013. 15(12): p. 976-84.

P.1204

Successful living donor liver transplantation for primary biliary cirrhosis with ABO blood type incompatibility and strongly positive donor-specific HLA antibodies

Yuki Takano, Takeshi Gocho, Hiroaki Shiba, Yuichi Shiba, Kenei Furukawa, Masaru Kanehira, Shinji Onda, Yuichi Ishida, Tadashi Uwagawa, Katsuhiko Yanaga.
Surgery, The Jikei University, Tokyo, Japan.

Humoral rejection remains the key factor to overcome ABO blood-type incompatible liver transplantation for which routine use of preoperative anti-CD20 antibody (rituximab), plasma exchange for high anti-donor blood type titer and splenectomy in selected patients have been introduced for live donor liver transplantation (LDLT) since 2004 in Japan. With such a regimen, the three years survival rate of ABO blood-type incompatible LDLT has remarkably improved to approximately 80%. Recently, ABO blood-type identical or compatible LDLT with elevated donor-specific HLA antibodies has been identified to be associated with poor outcome due to humoral rejection, for which successful management with the regimen adopted for ABO blood-type incompatible LDLT has been reported. We report a case of ABO blood-type incompatible LDLT for a recipient with elevated donor-specific HLA antibodies.
The recipient was a 65-year-old female with primary biliary cirrhosis (PBC), blood type A, with a history of gastrointestinal bleeding due to angioectasia of the small intestine presumably caused by portal hypertension in 2013, and prophylactic EVL and EIS in 2014 for esophagogastric varices. Her liver function deteriorating gradually and a 2-cm hepatocellular carcinoma (HCC) was detected by enhanced abdominal CT. Although her Child-Pugh Score was 8 points for PBC, she was judged to be a good candidate for liver transplantation due to portal hypertension with a history of gastrointestinal bleeding and a HCC within Milan criteria. The only potential donor was her 58-year old sister, who was blood type B. As the recipient was positive for donor-specific HLA antibodies, she was given rituximab 21 days preoperatively, and treated with three courses of plasmapheresis for high anti-B antibody titer (IgG x1,024 and IgM x256) on 7 days preoperatively, which was reduced two days before transplant to IgG x128 and IgM x64, respectively. Mycophenolate mofetil (MMF) was started 7 days preoperatively. After LDLT, the recipient was given three immunosuppressive drugs including tacrolimus, MMF and prednisolone without showing humoral or cellular rejection, while the anti-B antibody titer remained very low. The recipient was discharged without complications on Postoperative day 21.
To our knowledge, this is the first reported case of successful ABO blood-type incompatible LDLT for a recipient with elevated donor-specific HLA antibodies.

P.1205

Rapid preparation for ABO-incompatible living donor liver transplantation in acute liver failure with high isoagglutinin titers

Wei-Chen Lee, Tsung-Han Wu, Yu-Chao Wang, Chih-Hsien Cheng, Chen-Fang Lee, Ting-Jung Wu, Hong-Shiue Chou, Kun-Ming Chan.
Liver and Transplantation Surgery, Chang-Gung Memorial Hospital, Taoyuan, Taiwan.

Background: Liver transplantation is the only effective treatment for the patients with acute liver failure. However, deceased liver donors are always short in Asia and living donor is one of the ways to save the patients. When ABO-compatible donors are not available, crossing the ABO blood type barrier becomes the only chance for the patients to receive a new liver. However, it becomes troublesome to perform ABO-incompatible living donor liver transplantation when the acute liver failure patients with high isoagglutinin titers need urgent liver transplantation. Herein, we introduced a regimen to prepare ABO-incompatible living donor liver transplantation rapidly for acute liver failure patients.
Materials and Methods: This regimens were designed to prepare ABO-incompatible living donor liver transplantation within 3 days if the patients' anti-A/anti-B isoagglutinin IgG and IgM titers >64. Bortezomib was given intravenously and followed by 3 courses of once-a-day plasma-exchange to reduce isoagglutinin titers below 64. Then, living donor liver transplantation was performed. Rituximab (375 mg/m2) to deplete B-cells was administered on postoperative day 1. Post-transplant immunosuppression was achieved by administration of mycophenolate mofetil, tacrolimus, and steroids.
Results: Three male patients with acute hepatitis B and liver failure received this regiment to prepare ABO-incompatible living donor liver transplantation (two A to O ad one B to O). Their pre-transplant MELD scores were 35, 37 and 40, respectively. Their IgG isoagglutinin titers were 512, 128 and 256, respectively. IgG isoagglutinin titers were reduced to 8, 16 and 64 before transplantation. All three liver transplantation were achieved successfully. During postoperative follow up, one patient died of pneumocystis jiroveci pneumonia 4 months after transplantation. One patient had biliary stenosis at anastomotic site and resolved by endoscopic stenting. No ischemic biliary complication occurred in these patients.
Conclusion: It is troublesome to perform urgent ABO-incompatible living donor liver transplantation for acute liver failure patients with high isoagglutinin titers. Our regimen can prepare the patients rapidly to undergo ABO-incompatible living donor liver transplantation and achieve liver transplantation successfully.

P.1206

ABO incompatible living donor liver transplantation in patient with fulminant hepatic failure

Sam-Youl Yoon.
Surgery, Korea University Medical Center, Seoul, Korea.

ABO-incompatible (ABOi) living donor liver transplantation (LDLT) in adult patients with severe infection and high MELD score has been controversial because of the high risk of aggravation of infection.
We present a case of successful ABOi living donor liver transplantation (LDLT) for alcoholic cirrhosis with ABO-incompatible graft. The patient was a 33-year-old man (RH+, O) who had a history of hepatic encephalopathy. The patient waited for cadaver donor, but he had no chance for cadaveric liver transplantation. During 2 months, he showed no mentality, severe pneumonia and deep sore on sacrum area. With careful management for pneumonia and sore, the decision was made for ABO-incompatible adult living donor liver transplantation (ALDLT) from his wife (Rh+, A)
He was administered preoperative rituximab once and plasma exchanges according to the hemagglutinin titer. The spleen was preserved and no local infusion therapy was required. After ALDLT, it took 3 months for rehabilitation because he was intubated status during preoperative 2month. The successful results of this case has been due to the use of appropriate management infection and planed immune suppression for ALDLT.
Preoperative poor mentality, high MELD score and severe infection was usually contraindicated for ABOi LDLT. Adequate infection control and appropriate management of immune suppression could result in successful ALDLT even with high MELD score and severe infection.

P.1207

The Czech single centre experience with split liver transplantation: 3 years of the program, 37 splits and 72 transplants

Jiri Fronek[1], Libor Janousek[1], Matej Kocik[1], Milos Kucera[1], Martin Oliverius[1], Pavel Trunecka[2].
[1]Transplant Surgery Department, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [2]Hepatology Department, Institute for Clinical and Experimental Medicine, Prague, Czech Republic.

Background: Split liver transplantation can help with organ shortage and may serve some small adults and also pediatric recipients. On average, there are some 200 DBD donors in Czech every year. The pediatric recipients used to wait for either pediatric graft, only few received reduced graft. For these reasons the pediatric recipients used to wait for lon, with related waiting list morbidity and mortality. The split liver program has been introduced at single institution in Czech Republic in January 2013.
Methods: Retrospective analysis of 37 split liver procedures performed at our institution. Initial experience with split liver at our institution in 1998 included two classical splits and three transplants, two pediatric recipients died from graft disfunction early after the the transplant, one adult lived for 6 years. One classical split was done in 2009, both recipients are alive. Split liver program has been set up at our unit in 2013, this means compulsory split in case of donor within criteria.
Results: There were 37 split liver procedures performed since 2013, of those 24 classical for child and adult, 13 full left/full right for 2 adults. All split procedures we performed in vivo except one. All recipients except 5/74 are alive and well, all recipients developed prompt graft function. Some 5 patients were re-transplanted, for either biliary leak or severe rejeciton. In some cases, partial graft wa used for second transplant, ion one case for third transplant, in few cases for fluminant liver failure patients, some even AB0i. Since 2013 the average waiting time for pediatric recipients has changed from 271 days down to 5 weeks. There was no death of the pediatric recipient on the waiting list since the introduction of the program.
Conclusions: Split liver transplantation has been introduced as program at our institution quite late. It helped to decrease significantly the waiting time for pediatric transplant, it may help to avoid deaths on the adult waiting list as well. Even full left/full right split liver for two adults is duable, partial grafts can be successfully used even for fulminant cases, AB0i cases and also re-transplants.

P.1208

Deceased donor uterus retrieval as part of uterus transplant trial – the first Czech experience

Jiri Fronek[1], Libor Janousek[1], Eva Honsova[3], Roman Chmel[2], Michael Olausson[4].
[1]Pathology Department, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [2]Gynaecology Department, Motol University Hospital, Prague, Czech Republic; [3]Pathology Department, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [4]Transplantation Department, University of Gothenburg, Gothenburg, Sweden.

Background: Uterus transplantation (UTx), may serve some of the patients with Absolute uterine factor infertility (AUFI). There were only some 12 uterus transplant cases performed worldwide only. There is only one UTx trial performed, in Sweden, where 9 UTx where done, of those 7 were long-term successful, 5 recipients already delivered healthy child. UTx might become another solid organ transplant within next few years.
Methods: UTx human transplant trial has been setup in Czech Republic, permitted by Ministry of Health, officially started on 9th November 2015. There will be some 20 UTx procedures performed, of those 10 from live donors, 10 from deceased donors. There were already the first 2 deceased donor retrievals performed. Retrieval details follow.
Results: The 2 deceased donor uterus retrievals were performed as a part of multi-organ retrieval including liver, pancreas, hear, lung and kidneys. The uterus was dissected together with uterine artery and vein, ovarian vein. The uterus dissection followed after all the other organ transplant dissection, prior to the cannulation. Perfusion was made using cannulation of both external iliac arteries simultaneously, using HTK perfusion solution. On back table, uterus was assessed for perfusion and vessel length/quality, after the retrieval the uterus was stored in cold preservation solution and histology was performed on regular basis to assess the retrieval and preservation organ damage and potential viability.
Conclusions: Both uterus retrievals went uneventful, there were no delays to the other organ preparation and retrievals, the uterus dissection took about 30-45 minutes. The histology verified minimal retrieval and preservation injury of the uterus graft. More retrievals will follow shortly, the UTx procedures will follow later on this year.

P.1209

AB0-incompatible liver transplantation, full size, partial, auxiliary – single centre experience

Jiri Fronek[1], Libor Janousek[1], Martin Oliverius[1], Milos Kucera[1], Matej Kocik[1], Eva Kieslichova[2], Ondrej Viklicky[3], Pavel Trunecka[4]. [1]Transplant Surgery Department, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [2]Anesthesia Department, IInstitute for Clinical and Experimental Medicine, Prague, Czech Republic; [3]Nephrology Department, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [4]Hepatology Department, Institute for Clinical and Experimental Medicine, Prague, Czech Republic.

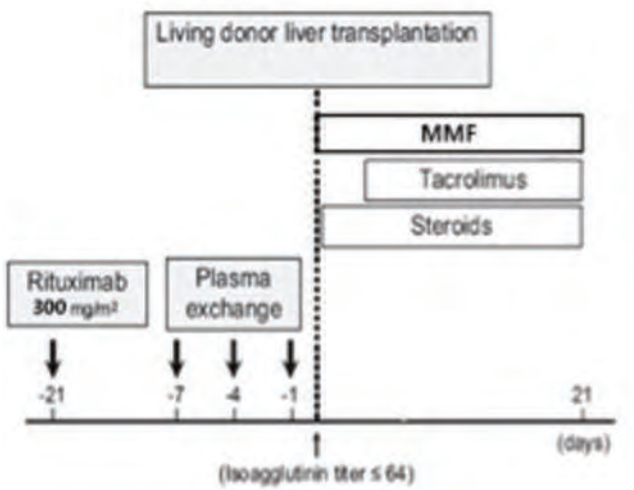
Introduction: The AB0-incompatible liver transplantation (LTx) is alternative method which can be used in some of the fulminant liver failure (FLF) cases, in some countries even for elective transplants. For overcoming the blood group barrier various techniques can be used.
Methods: Czech Republic is country with 10 million inhabitants and some 200 deceased donors per year. At our institution we performed some 137 LTx in 2015, our LTx program counts over 1200 LTx since 1995. To increase the chance for survival, in some of the fulminant liver failure cases we used the AB0i graft. In all except two cases we used plasma-exchange, in one case non-specific immunoadsorption, in one specific.
Results: In total 16 patients received AB0i liver graft, 6/16 received hemiliver, 2 patients as auxiliary graft, 4/16 died shortly after the LTx, none due to the incompatibility-related problems, 2/16 were re-transplanted, 12/16 patients are alive with well functioning graft.
Conclusions: In a small country with limited number of liver grafts per year, the AB0i liver transplantation is justified in FLF setting. Such technique gives reasonable chance for survival, the final outcome depends on severity of the FHF as well as primary diagnosis. Both apheretic as well as plasma-based techniques can be used with success to overcome the AB0i barrier. Both full size and hemiliver grafts can be used for transplant, partial or full size graft can be used as auxiliary, either as bridge to transplant or even as bridge to recovery.

P.1210

ABO incompatible living donor liver transplantation in patient with history of platelet transfusion related anaphylaxis

Won-Bae Chang, Bo-Ram Lee, Jai Young Cho, Ho-Seong Han, Yoo-Seok Yoon, YoungRok Choi, Jae Yool Jang, Hanlim Choi, Jae Seong Jang, Seong Uk Kwon.
Surgery, Seoul National University Bundang Hospital, Seongnam, Korea.

Anaphylaxis or severe allergic transfusion reactions (ATR) are not common complication after blood transfusion. The results are very critical such as dyspnea, shock and death. The patients with severe liver disease who need liver transplantation have bleeding tendency due to splenomegaly induced thrombocytopenia or decreased coagulation factor production from liver. Therefore, it is commonly required to transfuse platelet and other blood products for them. We report a successful case of ABO incompatible living donor liver transplantation in a patient with history of platelet transfusion related anaphylaxis.



Patient's serum isoagglutinin titers were measured (Anti-A IgG: 128, Anti-A IgM: 64) and total two times of plasma exchange were performed until Anti-A Immunoglobulin titers reduced less than 1:32 (Anti-A IgG: 16, Anti-A IgM: 8) before transplantation. A single dose of rituximab (300mg/m2) was given at 2 weeks before transplantation. During the operation, we decided to remove spleen because his low platelet level was not fixed by multiple times of preoperative platelet transfusion so a splenectomy was performed. He was transfused 23 units of RBC, 27 units of FFP and 3 units of apheresis platelet intra-operatively and there was no transfusion related allergic reaction. His surgical procedure was done without complication.

P.1211

Liver transplantation in a small volume center; initial outcome

Ho Joong Choi[1], Jin Beom Jo[1], Gun Hyung Na[2], Il Young Park[1].
[1]Surgery, Bucheon St. Mary's hospital, Catholic University of Korea, Bucheon, Korea; [2]Surgery, Seoul St. Mary's hospital, Catholic University of Korea, Seoul, Korea.

Introduction: Case volume is popularly being used as a quality indicator for high-risk and complex surgical procedures. Especially, liver transplantation surgery is a challenging procedure that is associated with high perioperative morbidity and mortality, due to patient comorbidities and technical demands of the procedure. So it is justifiable to perform such a procedure in high-volume procedure centers. However, small volume center can provide a service for a local population that would have limited opportunity for LT in large volume center due to the cost or poor conditions for transfer. Herein, we report our initial experiences and outcomes of liver transplantation as a small volume center.
Patients and Methods: In our hospital, we performed 13 liver transplantations from July 2014 to January 2016. Among 13 patients, we performed 9 deceased donor liver transplantations (DDLT) and 4 living donor liver transplantations (LDLT). Indications for LT were primarily hepatitis B (n=7), alcoholic (n=5) and autoimmune hepatitis (n=1). Among 13 patients, 3 patients were accompanied by HCC and one patient was accompanied by intrahepatic cholangiocarcinoma. For deceased donors, inferior vena cava anastomosis was performed by piggy-back technique and for living donors, modified Rt. graft was used with middle hepatic vein reconstruction by Dacron graft. Liver transplant patients were treated in the ICU postoperative day 6. The immunosuppressant regimen consisted of conventional triple regimen (FK506, MMF and steroid).
Results: In this series, the 90-day mortality was 0%. Also, there was no graft failure in 13 recipients. There were 2 patients mortality in the follow up period. The causes of mortality in each patient were cardiac arrest on postoperative 4 months and HCC recurrence on postoperative 14 months. Three patients (23%) required biliary stent due to bile duct anastomosis site stricture. There was not any vascular complication during postoperative period. The acute cellular rejection (ACR) was occurred in 2 patients, and they all recovered from ACR by steroid pulse therapy. In LDLTs, there were no donor mortality and postoperative complication.
Conclusion: Although there is no long-term data yet, liver transplantation results in this series as a small volume center were comparable. Other major hepatobiliary procedures can help the surgeons maintain their operative skills. A smaller LT program may require more efforts, but it can provide a good service for a local population.

P.1212

Urgent living-donor liver transplantation in a patient with concurrent active tuberculosis: a case report

Bo-Hyun Jung[1], Jeong-Ik Park[1], Sung-Gyu Lee[2], Hyucksang Lee[3].
[1]Department of Surgery, Inje University Haeundae Paik Hospital, Busan, Korea; [2]Division of Hepatobiliary Surgery and Liver Transplantation, Department of Surgery, Asan Medical Center, Seoul, Korea; [3]Department of Surgery, Inje University Seoul Paik Hospital, Seoul, Korea.

Background and Objective: Although active tuberculosis(TB) is considered a contraindication for liver transplantation(LT), LT is the only treatment in patients with liver failure and concurrent active TB. We report a case with successful urgent living-donor liver transplantation(LDLT) for irreversible liver failure in the presence of active TB.
Materials and Methods: A 48-year-old male was transferred with stupor mentality. He had followed with alcoholic decompensated liver cirrhosis. At the admission, he became stupor to semicoma status and developed hepatorenal syndrome. A preoperative CT scan of chest showed several small cavitary lesions in both upper lobes and AFB stain from sputum revealed 2 positive. ADA from ascites was elevated to suggest TB peritonitis.
Results: We immediatedly had started anti-TB regimen (rifampin, isoniazid, levofloxacin, amikacin) 2 days before operation. He registered cadaveric donor waiting list, however, we should perform urgent LDLT because of rapid mental deterioration. After LT, we changed to 2nd line regimen(amikacin, levofloxacin, closerin, pyridoixine) and sputum AFB stain converted to negative after 10 days of therapy. His liver function is well-preserved even after the change to 1st-line anti-TB treatment again. Now his general condition is getting better after treatment and he has a plan to discharge.
Conclusion: LT can be perforemed carefully as a life-saving option particularly for the patients with liver failure and concurrent active TB.
Key Words: Liver transplantation, living-donor, liver failure, active tuberculosis

P.1213

Living donor liver transplantation in over 70 years old patients

Jae Hyun Kwon, Sung-Gyu Lee, Shin Hwang, Ki-Hun Kim, Chul-Soo Ahn, Deok-Bog Moon, Tae-Yong Ha, Gi-Won Song, Dong-Hwan Jung, Gil-Chun Park, Jee Yeon Kim, Min Kyu Sung.
Division of Hepatobiliary Surgery and Liver Transplantation, Department of Surgery, Asan Medical Center, Seoul, Korea.

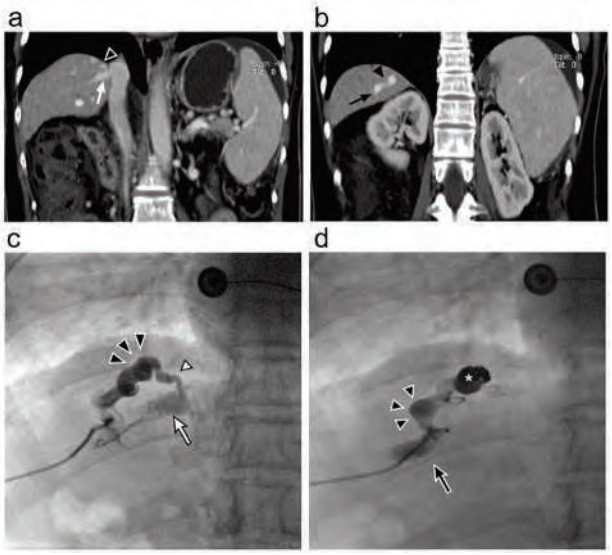
Introduction: Over the past two decades there has been a steady increase in the age of potential recipients for liver transplantation. In this study, we reviewed our experience for patients over 70 years old who underwent liver transplantation. To our knowledge, this study is the first report of LDLT in elderly patients above 70 years old.
Method: From 2000 January to 2015 October, 4529 cases of liver transplantation (including both LDLT and DDLT) were performed in our institution. Among them, 20 patients were over 70 years old at the time of liver transplantation. We analyzed clinical course of these patients retrospectively.
Results: Mean age was 71.5± 2.2 years old, ranged from 70 to 78 years old. Mean MELD score was 17.9± 11.7. According to Child-Turcotte-Pugh (CTP) scoring, 5 patients belonged to Child class A, 8 patients to Child class B, and 7 patients to Child class C. Mean CTP score was 8.9 ± 2.7. 8 patients received single living donor LT, 5 patients had dual living donor LT, and 7 patients underwent cadaveric donor LT. Mean GRWR was 1.46 ± 0.44, and mean SLV/GV (%) was 81.1% ± 26.3 %. 1-year survival rate was 85.0% and 5-year survival rate was 77.3%. Mean survival time was 42.2 ± 4.4 months (33.7~50.8 months) (3.5 years). Mean post-operative hospital days were 63.0 ± 42.1 (range; 21~164) days. 4 cases of biliary strictures occurred after 1 ~ 5 months post-transplant, and 1 case of acute rejection developed 1 month after LDLT.
Conclusion: Liver transplantation should be considered judiciously in elderly recipients. Old age per se is not a contraindication for deciding liver transplantation in these patients. LDLT in recipients aged over 70 years old is safe and feasible.

P.1214

Embolization of an intrahepatic portal systemic shunt for the treatment of hepatic encephalopathy to defer urgent liver transplantation

I Ha Lao[1,3], Hsiao Han Chiang[1], Yu Fan Cheng[2].
[1]Medical Imaging Center, Chi Mei Medical Center, Tainan, Taiwan; [2]Department of Diagnostic Radiology, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan; [3]Biomedical Sciences, National Sun Yat-sen University, Kaohsiung, Taiwan.

Liver transplantation is a recognized treatment option for patients with end-stage liver disease (ESLD).
But, if the recipient has life-threatening problem, bridging therapy may be necessary while preparing the donor for the transplantation procedure.
A 59 y/o woman with chronic hepatitis B-related cirrhosis and hepatocellular carcinoma was successfully treated with hepatectomy and transarterial embolization previously. However, a decade after the treatment, her liver function started to deteriorate. She presented to the emergency department with hepatic encephalopathy and sudden loss of consciousness with head injuries for several times.
A complete imaging survey for LDLT did not identify recurrence of hepatocellular carcinoma but showed an intrahepatic portosystemic venous shunt between the right posterior portal vein and right hepatic vein (Fig. 1a). Non-contrast computed tomography of the potential donor liver showed moderate fatty content at approximately 35%. Therefore, LDLT was proponed and diet and lifestyle modifications were suggested to decrease the fatty content of the donor liver.
The patient experienced an episode of syncope again two months later. Therefore, embolization of the intrahepatic portosystemic venous shunt for bridging therapy was performed.
After embolization, her condition improved, and the patient did not experience any further episodes of syncope and serum ammonia levels decreased to normal range. Mild esophageal variceal bleeding was noted after embolization, which indicate there was still liver decompensation and was controlled medically. Four months after embolization, with a reduction in the fatty content of the donor liver, the patient underwent LDLT. Her condition has great improvement after the successful transplantation.
Portosystemic shunts are congenital or acquired, and can lead to hepatic encephalopathy even in non-cirrhosis patients. Pathophysiology of hepatic encephalopathy involves the direct movement of substances from the portal venous system into systemic circulation without being metabolized by the liver[3].
Embolization has been shown to be an effective treatment for non-cirrhotic patients[4]. However, post-embolization portal hypertension has always been a concern in cirrhosis patients and may lead to variceal bleeding[5]. After the embolization procedure, syncope and hepatic encephalopathy resolved in our patient; however, she experienced minor variceal bleeding, which was controlled medically. The bleeding indicated that the patient had decompensated liver function; therefore, LDLT was performed after a reduction in the fatty content of the donor liver. After the successful LDLT, her condition was good and she did not have any symptoms.
In conclusion, in an ESLD patient with intrahepatic portosystemic venous shunt causing life-threatening hepatic encephalopathy, percutaneous transhepatic embolization is a feasible bridging therapy that allows urgent LDLT to be deferred.



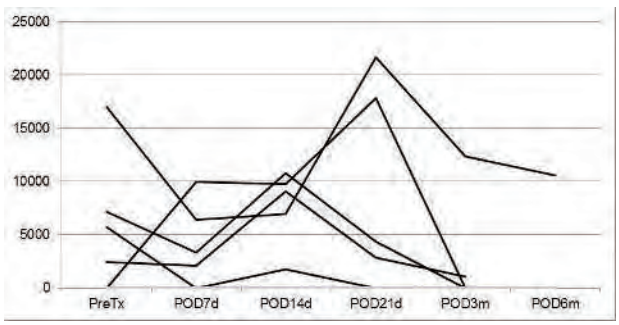
References:
[1] Vathsala A. Improving cadaveric organ donation rates in kidney and liver transplantation in Asia. *Transplant Proc*. 2004; 36(7):1873-1875.
[2] Wong VW. Nonalcoholic fatty liver disease in Asia: a story of growth. *J Gastroenterol Hepatol*. 2013;28(1):18-23.
[3] Mori H, Hayashi K, Fukuda T, et al. Intrahepatic portosystemic venous shunt: occurrence in patients with and without liver cirrhosis. *AJR Am J Roentgenol*. 1987;149(4):711-714.
[4] Laleman W, Simon-Talero M, Maleux G, et al. Embolization of large spontaneous portosystemic shunts for refractory hepatic encephalopathy: a multicenter survey on safety and efficacy. *Hepatology*. 2013;57(6):2448-2457.
[5] Tanoue S, Kiyosue H, Komatsu E, Hori Y, Maeda T, Mori H. Symptomatic intrahepatic portosystemic venous shunt: embolization with an alternative approach. *AJR Am J Roentgenol*. 2003;181(1):71-78.

P.1215

Impact of serial change of donor specific antibodies on the graft outcomes after liver transplantation

Jung Jun Lee[1], Jae Geun Lee[2,3], Juhan Lee[2,3], Seung Hwan Song[2], Jee Youn Lee[2], Su-kyung Kwon[2], Myoung Soo Kim[2], Soon Il Kim[2,3], Dong Jin Joo[2,3].
[1]Department of Surgery, CHA Bundang Medical Center, CHA University, Seongnam, Korea; [2]Department of Surgery, Yonsei University College of Medicine, Seoul, Korea; [3]Research Institute for Transplantation, Yonsei University College of Medicine, Seoul, Korea.

Introduction: This study aimed to investigate the acute rejection rate and graft function after liver transplantation according to pre-transplantation donor specific antibody (DSA) status and changing pattern of DSA after liver transplantation.
Patients and Methods: This study was prospectively designed observational study from March 2011 to October 2013. Forty patients were enrolled in this study. Singe antigen bead assays were performed to detect DSA all patients at pre-transplantation, post-transplant-7, 14, 21, 3rd month and 6th month.
Results: There were four patients (10%) with acute rejection and eleven patients (27.5%) with biliary complication. Four patients (10%) were positive on DSA before transplantation and one of them was converted to negative on DSA after transplantation. Three patients who had no DSA before transplantation showed de Novo DSA after liver transplantation. Two of four patients with acute rejection were positive on DSA. One of four patients with acute rejection showed pre-transplant DSA and de Novo DSAs were detected in another one acute rejection patient. Seven patients of eleven patients with biliary complication showed no DSA and the others demonstrated de Novo DSA after transplantation (p=0.039). Biliary complications occurred in two of three patients with de Novo DSA. Figure showed the change pattern of mean fluorescent intensity (MFI).
Conclusions: De Novo- DSA after transplantation can be associated biliary complication and the patients with high MFI showed more biliary complication. Further study with large sample size and long-term follow-up are needed.



P.1216

Living Donor Liver Transplantation in Patients Older Than 60 Years : Single center experiences

Byunggon Na[1], Gyowon Lee[2], Donggyu Oh[2], Chanwoo Cho[2], Nuri Lee[2], Wontae Cho[2], Jinyong Choi[2], Seunghwan Lee[2], Gyusung Choi[2], Choon Hyuck David Kwon[2], Jongman Kim Kim[2], Jaeberm Park[2], Jaewon Joh[2], Sungjoo Kim[2], Sukkoo Lee[2].
[1]Vascular & transplant surgery, Dong-san medical center, Dae-gu, Korea; [2]Transplantation surgery, Samsung Medical Center, Seoul, Korea.

Liver transplantation is life-saving intervention in the management of end-stage liver disease. There has been an increased demand to consider old patients to have access to liver transplantation. The purpose of this study is to compare outcomes of older recipients (>60 years) with younger recipients. The total 331 recipients of single institution from Feb. 2010 to Feb. 2013 were reviewed and dichotomized to group I (<60years); n=252 and group II (≥60years); n=79. The rate of mortality was 18.7% and 24.1% (p=0.295). The graft failure rate was 4.4% and 7.6% (p=0.253). There was no significant difference of postoperative stays in ICU, total hospital stays, death and graft failure cases during follow up periods. The patient survival rate of 1, 3, 5 year were 88.9, 82.1, 79.2% of group I and 81.7, 76.2, 72.3% of group II (p=0.114). The death censored graft survival rate of 1, 3, 5 year were 96.8, 95.2, 95.2% of group I and 93.3, 90.7, 90.7% of group II (p=0.17). We concluded that older recipients showed short-term outcomes comparable with younger recipients.

P.1217

Survival analysis for patients with MELD score < 15

Wong Hoi She, See Ching Chan, Albert C.Y.Chan, Kenneth S.H.Chok, Tan To Cheung, James Y.Y.Fung, Chung Mau Lo.
Surgery, The University of Hong Kong, Hong Kong, Hong Kong.

Background: This study aimed to perform the survival analysis for patient who had MELD score < 15 with or without undergoing liver transplantation (LT), and compared those patients who had undergone LT with various MELD score.
Methods: Patients who suffered from live failure with various MELD scores were included in the study. For those patients who suffered from diseases which would benefit from extra bonus MELD point such as hepatocellular carcinoma, Carol's disease, polycystic liver disease, familial amyloid polyneuropathy and hepatopulmonary syndrome were excluded. They were accepted on the waiting list between 01/01/07 to 31/07/15. Survival was calculated from the time of acceptance. The Kaplan-Meier method was used for survival analysis. The log-rank test was used for survival comparison.
Results: Total 349 patients were included in the study. 49 patients had MELD score < 15 did not undergo liver transplantation, while 345 patients underwent either deceased donor (DDLT) or living donor liver transplantation (LDLT). Hepatitis B infection was the main etiology for liver failure. Median MELD score was 12 at the time of listing. Median survival was 47 months with 5-year survival of 43.5%. This result was significant poor (p<0.001) than other patients who had undergone LT with various MELD scores, in particular MELD > 40 (median survival 99 months, 5-year survival 60.3%). On the other hand, for those MELD < 15 who were transplanted, they did well with a median survival of > 103 months and 5-year survival of 82%.
Conclusions: Patients who suffered from liver failure with low MELD score (<15) might never get transplanted, and unfortunately resulted in poorer prognosis.
Keywords: Liver failure, liver transplantation, low MELD

P.1218

Role of liver transplantation in patients of acute liver failure with hepatic coma requiring mechanical ventilation: Is it justified? Single center successful experience

Ashok Thorat[1], Long-Bin Jeng[1,2], Horng-Ren Yang[1,2], Ping-Chun Li[1,3], Chun-Chieh Yeh[1,2], Te-Hung Chen[1,2], Shih-Chao Hsu[1,2], Lorena Noriega[1], Kin-Shing Poon[1,4].
[1]Organ transplantation centre, China Medical University Hospital, Taichung, Taiwan; [2]Department of Surgery, China Medical University Hospital, Taichung, Taiwan; [3]Department of Cardiovascular surgery, China Medical University Hospital, Taichung, Taiwan; [4]Department of Anaesthesiology, China Medical University Hospital, Taichung, Taiwan.

Background: The prognosis of the patients of acute liver failure (ALF) with onset of hepatic coma is often dismal. ALF is a well accepted indication for liver transplantation (LT) and has markedly improved the prognosis of the patients. However, its role in ALF patients with onset of hepatic coma has never been elucidated before. The aim of our study is to analyze the outcome in 11 patients of ALF with hepatic coma that underwent LT and the guidelines about the timing of the LT in such patients.
Materials and Methods: From January 2002 to December 2015, a total of 726 LT surgeries were performed at the institute of China Medical University Hospital, Taiwan. The hospital database was retrospectively analyzed for 59 ALF patients that underwent LT during this period. Eleven ALF patients (n=11) with the onset of coma requiring mechanical ventilatory support were included in study. The demographic data, perioperative details, and postoperative follow up records were studied. The patients were further sub grouped depending upon the timing of LT and the onset of hepatic coma: **Group A**- LT within 48 hours of onset of coma (n=8). **Group B**- LT after 48 hours of onset of coma (n=3). The immunosuppressive therapy was given as per the institutional protocol[1].
Results: The average age of the study cohort [Male:Female, 8:3] was 39.63 ± 13.95 years (range, 13 to 63). Ten patients received living donor liver transplantation (LDLT) whereas deceased donor liver transplantation was done in 1 recipient. Three patients had fulminant hepatic failure, 1 patient of alcoholic liver cirrhosis developed acute hepatic decompensation whereas 7 patients developed ALF due to hepatitis B virus infection flare up. The pretransplant mean ammonia levels were 179.62 µg/dL (range, 111 to 242). All the recipients required mechanical ventilatory support prior to LT. Significant cerebral edema was present in 4 recipients. For LDLT subgroup, 9 patients received right liver allografs while 1 patient received left liver with a mean graft to recipient weight ratio of 0.95%. The average model for end-stage-liver disease score was 31 (range, 16 to 40). All the patients in group A were extubated within 48 hours after LT whereas for group B patients the extubation was delayed for several reasons. **Table 1** shows postoperative blood parameters and liver function profile of the recipients. At median follow up of 18 months (range, 6 to 60 months), the overall survival achieved was 81%. For group A the survival was 100% while for group B it was 33 %. Infectious complications were present in 5 of the recipients.
Conclusions: Our experience proves the safety and feasiblity of LT for patients of ALF with acute hepatic coma that are mechanically ventilated prior to transplantation. The timing of LT appeared to be an important determinant of successful outcome in this study. If LT is performed within 48 hours of onset of coma, the chances of survival are 100%, but recovery may be delayed.

Table 1. Pre and post-transplant laboratory parameters in recipients of acute liver failure with hepatic coma.

	At admission	Before liver transplantation	7th Post operative day	30th Post operative day	6th Post operative month
Hb (g/dL)	11.85 ± 3.32 (Range, 7.5-16)	10.67 ± 2.17 (8.3-13.2)	10.16 ± 0.51 (9.8-11.3)	12.10 ± 1.40 (9.9-13.6)	13.41 ± 1.68 (11.4-15.4)
WBC (x 10^3/mm^3)	10.0 ± 4.86 (Range, 3.3-16.8)	10.13 ± 2.81 (7.15-15.50)	11.62 ± 6.12 (4.1 ± 21.8)	8.07 ± 6.12 (4.1-21.8)	7.26 ± 2.92 (4.69-12.2)
INR	2.75 ± 0.91 (Range, 1.32-4.42)	2.63 ± 0.78 (1.42-3.99)	1.15 ± 0.19 (0.87-1.41)	1.07 ± 0.10 (0.97-1.24)	1.02 ± 0.04 (0.99-1.11)
AST (IU/L)	904.71 ± 1140.3 (Range, 50-2733)	105 ± 33.48 (45-148)	108.5 ± 95.33 (56-312)	43.37 ± 18.32 (20-80)	30.5 ± 17.27 (19-71)
ALT (IU/L)	814 ± 770.08 (Range, 34-1771)	163.14 ± 151.20 (36-449)	175 ± 130.16 (39-326)	58 ± 27.51 (17-103)	37.75 ± 31.95 (19-116)
T. Bilirubin (mg/dL)	23.65 ± 9.15 (Range, 10.6-31.59)	23.37 ± 6.02 (13.7-34.2)	5.46 ± 2.80 (1.2-9.5)	1.52 ± 0.53 (1-2.39)	0.76 ± 0.21 (0.35-1)
S. Creatinine (mg/dL)	1.10 ± 0.73 (Range, 0.51-2.58)	1.39 ± 1.07 (0.4-3.31)	0.89 ± 0.27 (0.57-1.32)	1.06 ± 0.18 (0.7-1.28)	1.16 ± 0.16 (0.9-1.27)
S. Albumin (g/dL)	2.98 ± 0.47 (Range, 2.1-3.4)	2.92 ± 0.24 (2.6-3.3)	3.23 ± 0.51 (2.6-4)	3.74 ± 0.77 (2.6-4.6)	-

* **Abbreviations:** Hb, Hemoglobin; WBC, White blood cells; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase.

Long-Bin Jeng. Email: otc@mail.cmuh.org.tw Organ Transplantation Center, China Medical University Hospital, 2, Yuh-Der Road, Taichung, Taiwan, 40447 TEL□04-22052121 Ext.1765, FAX□04-22029083

References:

[1] Jeng LB, Thorat A, Hsieh YW, et al. Experience of using everolimus in the early stage of living donor liver transplantation. Transpl Proc 2014; 46:744-748.

P.1219

Successful living donor liver transplantation in a case of pulmonary hypertension

You Youngkyoung[1], Jun Suh Lee[2], Gun Hyung Na[1], Sung Ho Choi[1], Tae Ho Hong[1], Young Chul Yoon[2], Ho Joong Choi[3], Soo Ho Lee[4], Dong Goo Kim[1].
[1]HBP Surgery, The Catholic University, Seoul St. Mary's Hospital, Seoul, Korea; [2]General Surgery, Incheon St. Mary's Hospital, Incheon, Korea; [3]General Surgery, Bucheon St. Mary's Hospital, Bucheon, Korea; [4]General Surgery, Uijeongbu St. Mary's Hospital, Uijeongbu, Korea.

Introduction: Portopulmonary hypertension (POPH) is defined as pulmonary hypertension coexisting with portal hypertension. It is a serious complication of chronic liver disease, and severe POPH, defined as a mean pulmonary artery pressure (mPAP) of over 45mmHg, is thought to be a contraindication of liver transplantation (LT). We report a case of successful liver transplantation that was performed on a patient considered to have moderate POPH preoperatively, but was diagnosed with severe POPH intraoperatively.
Case Report: A 58 year old woman diagnosed with liver cirrhosis (non-hepatitis B, non-hepatitis C) was referred to our hospital for liver transplantation. Echocardiography revealed pulmonary hypertension, with an RVSP of 59mmHg. Sildenafil was started, and given for 5 days preoperatively. At beginning of the operation, a Swan Ganz catheter was inserted, and the systolic PAP was over 110mmHg, with a mPAP of 70. These findings were consistent with severe POPH, but the decision was made to proceed with LT, given response to therapy, as demonstrated by a decrease in mPAP. IV infusion of nitroglycerine and milrinone and iloprost inhalation was started. MPAP decreased to 48mmHg, and LT was performed uneventfully. Postoperatively, the mPAP was 50 to 70mmHg. Sildenafil, dilatren and ambrisentan were used for maintenance therapy. The patient was discharged on postoperative day 28. Only sildenafil was prescribed as discharge medication. Follow up echocardiography performed one week after discharge showed a decreased RVSP of 54mmHg, and much improved pulmonary hypertension.
Conclusion: When the mPAP can be controlled by medication, severe POPH may not be an absolute contraindication for LT. We suggest prompt control of mPAP as a predictive factor of successful LT.

P.1220

Outcome results of cardiac surgery and cardiac intervention after liver transplantation

Mitsuru Nakatsuka[1].
[1]Department of Anesthesiology, Virginia Commonwealth University Health System/MCV, Richmond, VA, United States; [2]Department of Anesthesiology, Virginia Commonwealth University Health System/MCV, Richmond, VA, United States.

Introduction: Cardiac complications and adverse cardiac events are one of major causes of morbidity mortality in liver transplantation. This review is to investigate feasibility and safety for cardiac surgical procedure and intervention in post liver transplant patients.
Materials and Methods: We retrospectively reviewed the outcome results of cardiac surgery and cardiac intervention in 858 poetoperative liver transplantation patients from 1995 to 2012.
Results: Among 905 cases of liver transplantaion from 1995 to 2012, there were 30 documented cases of cardiac-related causes of death (3.3 %) 27 cases were due to cardiac arrest/myocardial infarction (2.98 %) and 3 cases were due to endocarditis/sepsis. 13 cases were within 6 months and 17 cases were after 6 months after liver transplantation. The incidence of atrial fibrillation seemed to be fairly common without any mortality (5.8%). Among patients who were re-admitted for cardiac events,11 cases were due to congastive heart failure (1.2%), 1 case due to cardiac arrest and 7 cases with myocadial infarction (0.77%). Among 858 patients, 7 cases underwent coronary artery bypass graft procedures (0.77%) and 3 cases underwent PTCA/STENTS (0.33%), in both cases without mortality.
Conclusion: Cardiac complications and adverse cardiac events were one of the major causes of morbidity and mortality after liver transplantation. Even from our limited experience, our study data showed no significant morbidity and zero mortality with cardiac surgery procedures and cardiac interventions in our post-liver transplant patients. It appears that cardiac surgery and cardiac intervention can be performed safely in liver transplant recipients with extrenely low morbidity and mortality.
References:
[1] 1. G.Prabhaker,G.Testa,O.Abbasoglu. et al. Ann Thorac Surg 1998,65:1060-1064
[2] 2. J.Pruthi,K.A.Medikiff,K.T.Esraon, et al. Liver Transplantation 2001,7:811-815
[3] 3. S.D.Johnston,J.K.Morris,R.Cramb. et al Transplantation 2002,73:901-906

P.1221

Safety and complications of living donor hepatectomy

Mitsuru Nakatsuka[1], Jiepei Zhu[2].
[1]Department of Anesthesiology, Virginia Commonwealth University Health System/MCV, Richmond, VA, United States; [2]Department of Anesthesiology, Virginia Commonwealth University Health System/MCV, Richmond, VA, United States.

Introduction: Because of shortage of available cadaveric liver donors,Living donor liver transplantation has become to play an important role in treating patients with end-stage liver disease. After several deaths among otherwise healthy donors were reported ,living donor hepatectomy has been performed more cautiously for living donor liver transplantation.
Materials and Methods: We evaluated safety and complications of living donor hepatectomy for total 148 patients, retrospectively from 1977 to 2014 in our institution.
Results and Discussion: There were no mortality among 148 patients with living donor hepatectomy. 128 cases were with right lobe hepatectomy and 20 cases with left sgmental hepatectomy. Demographic data is shown in Table 1. Overall,complicattion rate were 36%, Major compications and Clavien classified complicatios are shown in Table 2 Common complications. included incisinal hernia,wound infection,neuropraxia,pleural effusion in addition to biliary compolications. Neuropraxia was only encountered in patients with right lobe hepatectomy. Biliary complications were in 8 cases but only one case required choledochojejunostomy following right lobe hepatectomy. 2 cases with right lobe hepatectomy required exploratory laparostomy with 1 case with left segmental lobe hepatectomy. According to Clavien complication scores, Grade 1 was16.2%,Grde 2 of 14,8% and Grade 3 of 11.5% but none in Grade 3 and 4. Righy lobe hepatectomy seemed to have more complications than left segmental hepatectomy.
Conclusion: Adult living donor hepatectomy were associated with some complications,mostly with lower grades without any mortality. Since donor safety is the highest priority, low morbidity and satisfactory long-term recovery should be achieved when living donor hepatectomy is contemplated for living donor liver transplantation.

Table 1 Demographic data

	R. lobe hepatectomy	L segmental hepatectomy
number	128	20
Female/male ratio	54-74	10-10
Mean age	38.9 (18-55)	34.2(21-50)
meanDonor weight (gm)	879 (470-1535)	152 (135-383)

TABLE 2 Major complications

	R lobe	L segmental lobe	Clavien grade classification
Number of cases	128	20	
Neuropraxia	9	0	Grade 1
Incisional hernia	10	3	Grade 3
Biliary leak	5	2	Grade 2
Biliary stenosis	1	0	Grade 5
Woundinfection	6	1	Grade 2
UVI	5	0	Grade 2
P valuePulmonary emboli	2	0	Grade 2
Exploratory lap bleed	2	1	Grade 3
IPortal vein thrombosis	1	0	Grade 3
Pleural effusion	8	0	Grade 1

TABLE 3 Overall Clavien Classification for Complications

Grade 1	16.2%
Grade 2	12.8%
Grade 3	11.5%
Grade 4	0%
Grade 5	0%

References:
[1] K.L.Beavers,R.S Sandler,R.Shrestha. LIverTranspl 2002:116-117
[2] R.M.Ghobrial,C.E.Freise,J.F.Trotter,et al. Gastroenterology 2008,135:468-476
[3] C.B.Taner,M.Dayangac,B.Akin. et al. Liver Transplantation 2008,14:1174-1179

P.1222

Current status of cadaveric and living donor kidney transplantation of the last 6 years, compared with previous 6 years experience

Mitsuru Nakatsuka[1], Jiepei Zhu[2].
[1]Department of Anesthesiology, Virginia Commonwealth University Health System/MCV, Richmond, VA, United States; [2]Department of Anesthesiology, Virginia Commonwealth University Health System/MCV, Richmond, VA, United States.

Introduction: We investigated any significant differences in our current status of cadaveric and living donor kidney transplantation in comparison with previous 6 year experience in our institution.
Materials and Methods: We compared retrospectively the results of kidney transplantation of current 6 years experience with previous 6 years in cadaveric and living donor kidney transplantation.
Results and Discussion: Detailed demographic data in 2 separate periods are shown in tables 1 and 2. Waiting list times were much shorter for LD in both periods($p<0.05$). Extended criteria donors(ECD) and donation after cardiac death(DCD) were more frequently used for current 6 year period compared with previous 6 years($p<0.005$). Incidence of delayed graft function(DGF), acute cellular rejection(ACR), postoperative acute tubular rejection(ATN), postoperative antibody mediated rejection(AMR) and Creatinin levels at discharge were significantly higher in cadaveric kidney transplant(CKT) than living donor kidney transplant(LKT) in both periods($p<0.05$). Postoperative complications are shown in table 3. Patient survival rate and graft survival rate in 5 years after kidney transplant were better in LKT in both groups($p<0.05$).Outcome results in both periods are shown For CKT and LKT in table 4.
Conclusion: LKT showed overall better results than CKT. 5 year follow up for patient survival and graft survival were better in LKT,compared with CKT($p<0.05$). ECD and DCD were more current cadaveric kidney transplantation.

Table 1. Demographic data of recipients for two periods

	2000-2006		2007-2012	
Recipient facts	CD (356)	LD (252)	CD (371)	LD (223)
age	47.5 +/- 12.1	48.7 +/- 13.9	50.6 +/- 12.4	45.7 +/- 13.9
BMI	26.8 +/- 5.1	26.2 +/- 4.7	28.0 +/- 5.0	27.5 +/- 4.8
Male	193 (54.3%)	135 (53.0%)	351 (61.4%)	138 (61.9%)
Pre kidney tx.	49 (14.5%)	17 (7.5%) (*1)	65 (11%)	21 (9.4%) (*1)
Pre TX dialysis	332 (95.5%)	173 (74.6%) (*1)	521 (91.1%)	157 (70.4%) (*1)

Table 2. Demographic data of donors

	2000-2006		2007-2012	
	CD	LD	CD	LD
Donor age	39.8 +/- 16.7	39.1 +/- 10.6	39.0 +/- 17.7	41.7 +/- 12.0
HLA mismatch	4.1 +/- 1.7	3.1 +/- 1.5	4.7 +/- 1.3	3.7 +/- 1.7
CIT	1020 +/-429	26.9 +/-16.3 (*1)	1194 +/- 445	38.1 +/- 52.5 (*1)
WIT	32.5 +/- 10.0	28.0 +/-5.8	32.3 +/-7.0	29.2 +/-4.0
ECD	40 (11.8%)		128 (22.9%) (*2)	
DCD	16 (4.7%)		9 (15.7%)	
Male	191 (56.7)	99 (42.7%)	354 (61.9%)	80 (35.9%)

Table 3 Post transplant complication

	2000-2006		2007-2012	
	CD	LD	CD	LD
Creatinin @ distch	4.7 +/-3.2	1.8 +/- 1.8 (*1)	4.1 +/- 2.7	1.5 +/- 1.2 (*1)
DGF	158 (47.8%)	13 (5.6%) (*1)	310 (54.2%)	9 (4%) (*1)
ACR in 1st year	47 (13.9%)	17 (7.3%) (*1)	31 (5.4%)	8 (2.7%) (*1)
AMR in 1st year	25(6.8%)	4 (1.7%) (*1)	24 (4.2%)	3 (1.5%) (*1)
Postop ATN	57 (16.9%)	8 (3.4%) (*1)	3 (0.5%)	0 (*1) (*5)
Wound inf 1st yr	26 (7.7%)	5 (2.2%) (*1)	30 (6.6%)	4 (1.8%) (*1)

Table 4 Outcome results

	2000-2006		2007-2012	
Nephrectomy 1st y	18 (5%)	1 (0.4%) (*1)	4 (0.7%)	
Nephrectomy 5 yr	18 (5.5%)	7 (3.0%) (*1)	10 (1.7%)	
1 yrpt survival	95.3 +/- 1.2	98.2 +/-0.9	96 +/- 0.8	
3 yrpt survival	82.8 +/- 2.1	89.6 +/- 2.0 (*1)	85.8 +/-2.0	
1st graft survival	89.6 +/- 1.7	97.8 +/- 1.0 (*1)	91.5 +/- 1.2	
5 yrgraft survival	68.1 +/- 2.6	81.7 +/- 2.6 (*1)	70.8 +/- 2.5	

*1 – between CD and LD – p<0.05
*2 – between CD (2006) and CD (2012) – p<0.05
*3 – between LD (2006) and LD (2012) – p<0.05

References:
[1] D.H.Koo,K.I.Welsh,A.J.Mclaren. et al. Kidney International. 1999,56:1551-1559

P.1223

Neoplasms or cancers in HCVab-positive OLT recipients with hepatocellular carcinoma: the analysis of the outcomes

Ekaterina Kornilova, Olga Tsirulnikova, Maxim Kornilov, Sergey Gautier.
Federal Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation.

From January 2006 to November 2015 in our transplantation center were made OLT to 21 HCVAb-positive recipients with hepatocellular carcinoma (HCC).To the moment of transplantation 4 recipients were HCV RNA - negative as a result of antiviral treatment (AVT) (1 - LDV-SOF; 3 - PEGIFN+RBV) and 3 of them developed SVR which persists to the present time, in one case we had detected HCV RNA in serum during the first month after OLT. 4 recipients died in the early postoperative period and 2 patients died within the first year of observation from the recurrence of HCC. Both patients died due to HCC recurrence had G3 of hepatitis C virus; antiviral therapy with PEG IFN+ RBV was initiated on the 30th day post-transplant period and immunosupression was conversed to everolimus when we noticed mild elevation of AFP. In one case patient developed rapid and early virologic response, but at 16 week of AVT treatment was discontinued because of verified return HCC with distant metastases; recipient received treatment with sorafenib but unfortunately it has not brought results and the patient died 13 months after OLT. In the other case patient too developed rapid and early virologic response, the treatment lasted 20 weeks, with the achievement of EVT, but at the 9 month after OLT revealed metastatic lesion in lungs and at 12-month follow-up - metastatic liver lesion.The patient died at 13 month follow-up of stroke metastatically etiology.
Currently 15 patients are alive and continue their observation. In 11 patients post-transplant period is not complicated by HCC recurrence or development neoplasms or cancers, but at the same time in 4 recipients registered the following complications: multiple myeloma - 1; basal cell carcinoma of the skin - 1; metastatic involvement of the sternum - 1; metastatic involvement of the pelvic bones - 1. It is important to note that patient with basal cell carcinoma of the skin at the time of development this complication more than 8 years had a sustained virologic response, her post-OLT follow-up period is 4,5 years and during 51 months she has had immunosupressive regimen based only on everolimus. At least 2 other recipients at the time of detection neoplastic or cancer complications were HCVRNA-positive but not recieved steroids.
To conclude: spectrum of neoplastic or cancer complications in recipients with HCC and their correlation with hepatitis C virus requires further study and analysis.

P.1224

HCV treatment after liver transplantation 2006-2016: result assessment

Ekaterina Kornilova, Olga Tsirulnikova, Maxim Kornilov, Sergey Gautier.
Federal Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation.

In 2006 OLT program to HCVAb-positive recipients had started in our transplant center.
Within these years we had performed liver transplants to 72 HCVAb-positive recipients. To the moment of transplantation 5 recipients were HCV RNA - negative as a result of antiviral treatment (AVT) (1 - LDV-SOF; 4 - PEGIFN+RBV) and 4 of them developed SVR which persists to the present time, in one case we had detected HCV RNA in serum during the first month after OLT.
7 patients died in the early postoperative period (2 patients died of fibrosing cholestatic viral hepatitis C) but unfortunately antiviral therapy (AVT) was not performed due to the severity of their condition, contraindications and/or unavailability of modern well-tolerated drugs. Another 1 patient (who died in the third year of follow-up from sudden cardiac death) had severe renal insufficiency and was not able to tolerate IFN-based antiviral treatment.
At the present 40 patients were cured and a further 20 are in the process of treatment or expected at the beginning.
Of the 40 treated patients prevalent amount (29) had HCV G1B, 4 patients had G2 and 7 recipients had G3 of hepatitis C virus. Our first attempts of AVT started in 2008 with the use of PEG IFN + RBV combination in 30 patients and we reached rate of SVR in patients with genotype 1 was 87% and in patients with 2 and 3 genotypes 100% and 89%, respectively. The obtained results are in part a result of aiming the selection of patients and personalized therapy. In 5 recipients with G1B who prior to OLT were non-responders we performed AVT which consisted of PEG IFN+SIM+RBV, and we reached rate of SVR 100%. In others 5 recipients with G1B we performed AVT which based on IFN-free regimen: 2- SOF+SIM-12 wk; 1- SOF+SIM+RBV-12wk; 2-DAC+SOF+RBV-24 wk. It is obvious that in this group we have achieved SVR in 100% of cases.
The frequency and nature of adverse events (AE) in patients who received IFN-based therapy, did not differ from those in the population; AE within IFN-free AVT, we were not registered.
To conclude: in our time antiviral therapy in recipients after liver transplantation has become a logical therapeutic option to improve survival and prognosis of patients with hepatitis C.

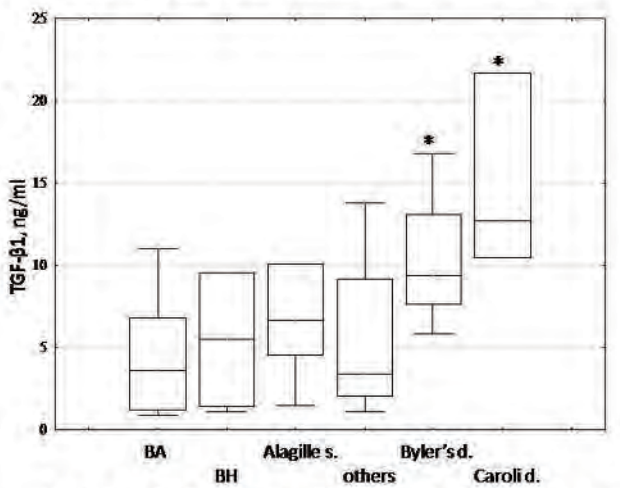
P.1225

Transforming growth factor beta 1 blood level relates with liver disease etiology and fibrosis severity in pediatric liver recipients

Rivada Kurabekova[1], Irina Tsirulnikova[2], Galina Olefirenko[1], Olga Gichkun[3], Natalia Mozheyko[4], Olga Tsirulnikova[2], Olga Shevchenko[1], Sergey Gautier[2].
[1]Regulatory Mechanisms in Transplantology, V.I. Shumakov Federal Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation; [2]Pediatric Liver Transplantation, V.I. Shumakov Federal Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation; [3]Laboratory of Immunological Monitoring, V.I. Shumakov Federal Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation; [4]Pathological Anatomy, V.I. Shumakov Federal Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation.

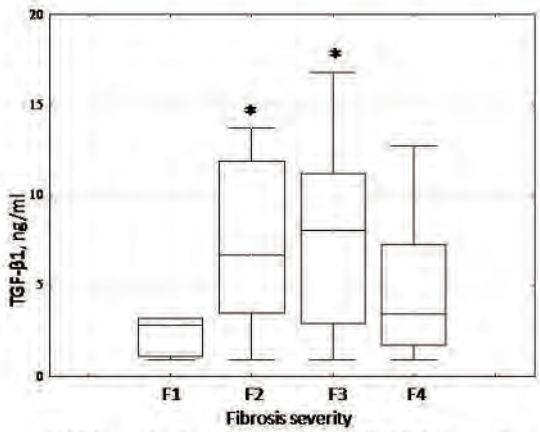
Introduction: Transforming growth factor β1 (TGF-β1) is a pleiotropic cytokine with profibrotic activity in different tissues, but it is still not clear how TGF-β1 blood level relates with liver graft function in pediatric recipients. The aim of the study is to analyze the relationship of TGF-β1 blood level before and after liver transplantation with disease etiology and hepatic fibrosis severity in pediatric recipients.
Materials and Methods: The study involved 128 children (58 boys) aged from 3 to 73 (median 8) months with end stage liver disease (ESLD) caused by biliary atresia (BA) (71 pts), biliary hypoplasia (BH) (10 pts), Alagille syndrome (9 pts), Byler's disease (10 pts), Caroli disease (7 pts) and other rare diseases (21 pts). They underwent orthotopic transplantation of the left lateral sector (LT) and biliary reconstruction via hepaticojejunostomy. 12 healthy children (7 boys) aged 10 ± 6 months and 12 healthy adults (5 men) aged 30 ± 6 year (related liver donors) served as comparison groups. Histopathologic analysis was performed using microscope. Fibrosis staging was scored according to METAVIR system. TGF-β1 concentration was determined in blood plasma samples by ELISA.
Results and Discussion: TGF-β1 blood level in children with ESLD was lower than in healthy children (6.3±5.0 vs 22.2±4.9 ng/mL, P=0.001), but depended on liver disease etiology. The cytokine levels in patients with BA, BH, Alagille syndrome, Byler's disease and other diseases (5.1±3.6, 7.1±8.6, 7.5±4.9, 10.4±4.6 and 6.5±5.6 ng/mL, resp.) were significantly lower than in healthy children (P=0.001, P=0.01, P=0.001, P=0.001 and P=0.01, resp), while at Caroli disease it did not differ from those in healthy children (14.9±6.0 ng/mL, P=0.11) (Fig.1). It could be explained by a negative effect of high bilirubin on TGF-β1 level at BA and other cholestatic diseases in contrary to Caroli disease, what leads to cholangitis. Histopathologic analysis of remover liver was performed in 73 pts, 6 (8%) of them had F1 (low fibrosis); 12 (16%) – F2 (mild fibrosis); 10 (14%) – F3 (severe fibrosis) and 45 (62%) – F4 (cirrhosis). There was weak correlation between fibrosis score and disease etiology (rs=0.39, p=0.003): 86% of pts with BA had F4. TGF-β1 level in pts with F1 was significantly lower than those with F2 and F3, but did not differ from those with F4 (3.1±1.7, 10.9±6.5, 8.7±5.2 and 5.2±4.7 ng/mL, P=0.02, P=0.04, P=0.33, resp.) (Fig.2). It suggests that TGF-β1 blood level reflects hepatic fibrosis process development: at F1 and F4, fibrosis process is small or is already over, while at F2 and F3 fibrosis is developing. In one month after LT TGF-β1 blood level increased in pediatric recipients (11.5±10.8 ng/mL, P=0.001) regardless of the initial diagnosis and severity of fibrosis. In adult liver donors, TGF-β1 blood level did not change in one month after liver resection (8.1±7.6 vs 12.9±11.7 ng/mL, P=0.20).
Conclusion: TGF-β1 blood level reflects liver disease etiology and hepatic fibrosis severity and can be regarded as a noninvasive biomarker of graft function in pediatric liver recipients.

Figure 1. TGF-β1 blood level in children with different etiology of ESLD



Middle line: median; Box: interquartile range (25-75%); Whisker: non-outliers range (minimum–maximum); * - P < 0.05 versus recipients with BA; BA - biliary atresia, BH - biliary hypoplasia

Figure 2. TGF-β1 blood level in children with different hepatic fibrosis severity



Middle line: median; Box: interquartile range (25-75%); Whisker: non-outliers range (minimum–maximum); * - P < 0.05 versus recipients with F1

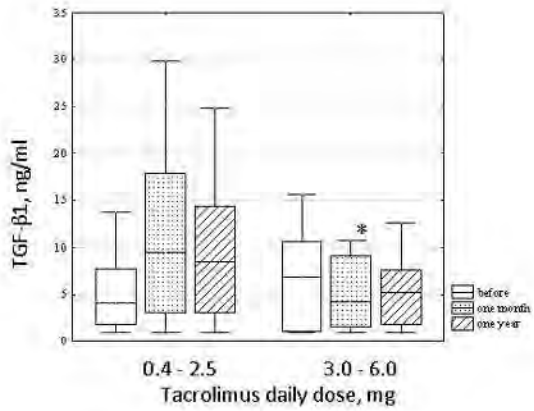
P.1226

Pediatric living donor liver transplantation: correlation plasma level of transforming growth factor beta -1 with tacrolimus dosage but not with its concentration

Sergey Gautier[2,4], Olga Tsirulnikova[2,4], Rivada Kurabekova[1], Irina Tsirulnikova[2,4], Olga Gichkun[3,4], Olga Shevchenko[1,4].
[1]Regulatory Mechanisms in Transplantology, V.I. Shumakov Federal Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation; [2]Pediatric Liver Transplantation, V.I. Shumakov Federal Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation; [3]Laboratory of Immunological Monitoring, V.I. Shumakov Federal Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation; [4]Department of Transplantology and Artificial Organs, I.M. Sechenov First Moscow State Medical University, Moscow, Russian Federation.

Introduction: It has been shown that plasma level of transforming growth factor-β1 (TGF-β1), a cytokine with immune suppressive activity, increased in animal models of spontaneous liver tolerance and decreased in rejection models within the first weeks after liver transplantation, and then it returned to some basal level. Thus, TGF-β1 level can serve as a biomarker of allograft immune tolerance. The aim of the study was analysis of relationship of TGF-β1 blood level with concentration and dosage of tacrolimus after living donor liver transplantation (LDLT) in pediatric recipients.
Materials and Methods: We studied 135 children (61 boys) aged from 3 to 73 (median: 8) months with end stage liver disease (ESLD) caused by biliary atresia (75 pts), biliary hypoplasia (12 pts), Byler's disease (10 pts), Alagille syndrome (9 pts), Caroli disease (8 pts) and 21 cases of other rare diseases. They underwent orthotopic transplantation of the left lateral sector and biliary reconstruction via hepaticojejunostomy. Three-component immune suppression therapy (tacrolimus, corticosteroids and mycophenolate mofetil) was initiated days 0 - 4 after LDLT. TGF-β1 concentration was determined by ELISA in blood plasma samples. Concentration of tacrolimus was measured by chemiluminescent microparticle immunoassay.
Results and Discussion: TGF-β1 plasma level of the children with ESLD was 4.6 (0.9–23.4) ng/mL. In first month after LDLT individual dynamics of TGF-β1 level in the recipients was multidirectional, but the median level increased to 7.0 (0.9–42.7) ng/mL (p=0.002). In one year after LDLT the median level of TGF-β1 was higher than before LDLT: 7.5 (0.9 – 37.4) ng/mL, p = 0.01, and did not differ from that in first month after LDLT (p = 0.98). There were no correlations of TGF-β1 level with tacrolimus concentration in blood. Analysis of TGF-β1 levels with tacrolimus daily dose has shown a significant negative correlation between TGF-β1 level in first month after LDLT and tacrolimus daily dose in one year after LDLT (rs = - 0.23). Recipients, who received low daily dose of tacrolimus in one year after LDLT (0.4 – 2.5 mg), had higher TGF-β1 plasma level in one month after LDLT than those, who received high (3.0 - 6.0 mg) dose: 9.4 (0.9 – 42.7) versus 4.2 (0.9 – 42.2) ng/mL, resp., p = 0.034 (Fig.).

Figure. TGF-β1 plasma level before and after LDLT in recipients with high and low tacrolimus daily dose



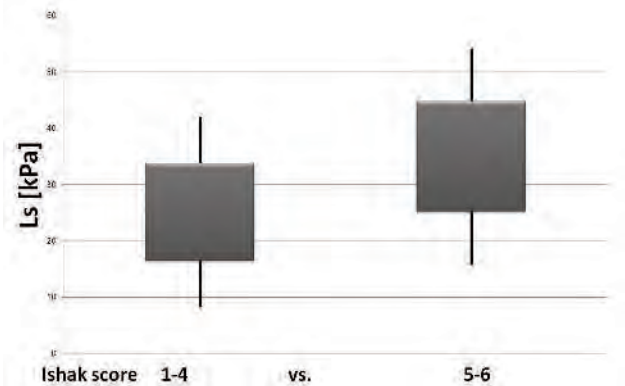
So, we suggest that eligible dose of tacrolimus associated with value of TGF-β1 level, to some extent. Assume that the measurement of level TGF-β1 may be useful in stratifying patients who need high or low dose of tacrolimus.
Conclusion: After LDLT there were no correlations of TGF-β1 level with tacrolimus concentration in blood. Plasma level of TGF-β1 associated with tacrolimus dosage, that it probably defines its relationship with immune tolerance in pediatric liver recipients.

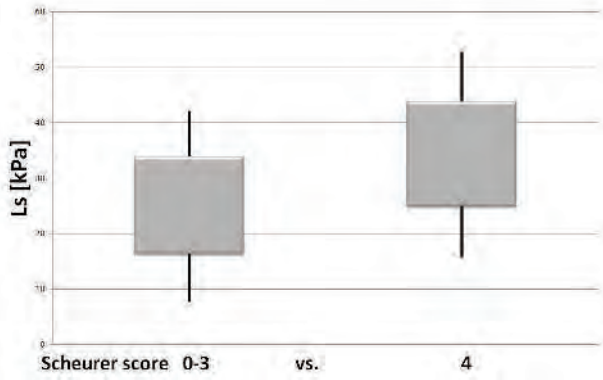
P.1227

First experience with liver shear wave imaging in patients with posttransplant hepatitis C recurrence

Tomasz Cieciura[1], Ewa Hryniewiecka[2], Agnieszka Perkowska-Ptasinska[3], Leszek Paczek[1].
[1]Department of Immunology, Transplant Medicine and Internal Diseases, Medical University of Warsaw, Warsaw, Poland; [2]Clinical Nursing Department, Medical University of Warsaw, Warsaw, Poland; [3] Department of Transplantation Medicine, Nephrology and Internal Medicine, Medical University of Warsaw, Warsaw, Poland.

The recurrence of hepatitis C (HCV) after liver transplantation leads to graft fibrosis and cirrhosis which can be life threatening condition. Liver biopsy remains the gold standard for both diagnosis and monitoring of the disease. The search for new and effective non-invasive diagnostic methods is still carried out.
Shear wave imaging is a new method that provides a measure of tissue stiffness. The data on the use of shear wave imaging to diagnose liver graft fibrosis are very scarce. Our objective was to evaluate the results of the shear wave imaging in patients with hepatitis C recurrence and compare them with liver graft histopathology.
The group of 40 liver transplant recipients with HCV recurrence (12 women [30%] and 28 men [70%]) was evaluated by graft biopsy and shear wave imaging. Median age of patients was 58.58 years, range: 25.71-70.15 years. The degree of fibrosis was assessed by measuring liver stiffness (Ls). In all patients liver biopsies were performed and processed using the same protocol. Liver graft fibrosis and cirrhosis were assessed with the use of the Ishak, Scheurer and Metavir scoring systems. The biopsies were performed for clinical indications.
Mean Ls was 27.39 kPa (standard deviation [SD] = 9.6), median alanine aminotransferase was 53.5 U/L (range: 11-257), aspartate aminotransferase was 44.5 U/L (range: 19-260) and MELD score 8.55 (range: 6.4-20.9). The degree of liver fibrosis by Ishak scoring system was: 1 (12.5%), 2 (10%), 3 (30%), 4 (22.5%), 5 (20%) and 6 (2.5%). The degree of fibrosis by Scheurer scoring system was: 0 (2.5%), 1 (20%), 2 (30%), 3 (20%) and 4 (25%). The degree of fibrosis by Metavir scoring system was: 0 (2.5%), 1 (22.5%), 2 (27.5%), 3 (17.5%) and 4 (27.5%). In order to differentiate between liver fibrosis and cirrhosis patients were divided into two subgroups: Ishak score fibrosis (If: 1-4 [75%]) and cirrhosis (Ic: 5-6 [22.5%]); Scheurer score fibrosis (Sf: 0-3 [72.5%]) and cirrhosis (Sc: 4 [25%]); Metavir score fibrosis (Mf: 0-3 [52.5%]) and cirrhosis (Mc: 4 [45%]).
We have observed statistically significant differences between liver fibrosis and liver cirrhosis groups by Ishak, Scheurer and Metavir: 25.14 kPa vs. 35 kPa (p=0.006); 25.03 kPa vs. 34.32 kPa (p=0.007); and 25.24 kPa vs. 32.94 kPa (p=0.2), respectively.





Our results indicate that shear wave imaging could be useful in differentiating patients with advanced cirrhosis from those with liver fibrosis and may be helpful in the non-invasive diagnosis of HCV recurrence after liver transplantation.

P.1228

HBIg discontinuation with maintenance oral anti-viral therapy: Failure of lamivudin but potency with entecavir or tenofovir

Zeki Karasu[1], ilker turan[1], Funda Yılmaz[2], Fulya Gunsar[1], Galip Ersoz[1], Ulus Akarca[1].
[1]Gastroenterology, Ege University, İamir, Turkey; [2]Pathology, Ege University, İzmir, Turkey.

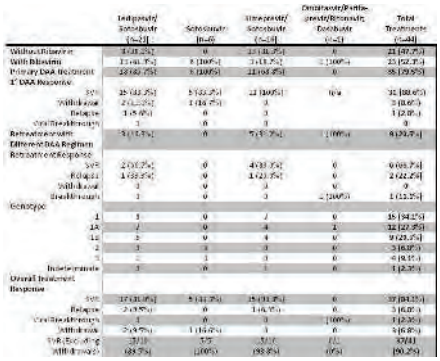
Prophylaxis with hepatitis B immune globulin (HBIG) plus an oral anti-viral (OAV) combination is widely accepted regimen for the prevention of recurrent hepatitis B virus (HBV) infection after liver transplantation. Because of high costs of HBIg and improving efficacy of new oral agents, there is increasing interest to investigate the safety and efficacy of HBIg discontinuation.
Methods: One-hundred and fifty (116 male, 34 female) patients were included study. All patients had HBIg+Lam prophylaxis and had no detectable serum virologic markers (HBsAg and/or HBV DNA) for recurrent disease during follow up of at least 3-years. Histologic examination is also performed in all cases. Presence of HBV DNA in liver tissue is investigated by PCR. If tissue HBVDNA was detectable, patients continued HBIg; if tissue HBVDNA was not detectable HBIg discontinued and patients contiuned to take an OAV (lamivudine or telbivudine, or entecavir, or tenofovir).
Results: Mean age was 47-years (18-64 years). Median duration of follow up was 5 (3-9) years after transplantation. Sixty-four patient had delta co-infection and 44 patients had HCC before transplantation. Of the patients, 108 (72%) had their grafts from living donors. The liver graft showed no hepatitis on histologic examination. After a median 25 months follow up no patients taking entecavir or tenofovir experienced HBV recurrence (reappearance of serum HBsAg); However %22 recurrence was observed in lamivudine and telbivudine group.
Conclusion: After combination prophylaxis with HBIg+OAV, HBIg discontinuation with maintenance oral anti-viral monotherapy with entecavir or tenofovir is safe and effective; But there is significant recurrence risk in case of lamivudine monoprophylaxis.

P.1229

Single center experience treating post-transplant Hepatitis C patients with direct acting antivirals (DAAs)

Gopal A.Ramaraju[1,2], M. Katherine Dokus[1], Lisa M.Cristoforo[3], Jennifer A.Iuppa[1,3], Menachem Weiss[1], Tiffany Gee[1], Jonathan Huang[1,2], Benedict Maliakkal[1,2], Mark S.Orloff[1], Randeep Kashyap[1].
[1]Center for Transplantation, University of Rochester Medical Center, Rochester, United States; [2]Gastroenterology and Hepatology, University of Rochester Medical Center, Rochester, United States; [3]Pharmacy, University of Rochester Medical Center, Rochester, United States.

Background: Recurrent hepatitis C virus (HCV) infection in the hepatic allograft is a major concern after successful liver transplantation (LTX). Left untreated, recurrent HCV can lead to graft failure and mortality. Given poor tolerability and limited efficacy of interferon-based therapies, transplant centers have transitioned to primary use of DAAs for the management of post-transplant HCV infection. DAAs not only have an improved tolerability profile, but are associated with high sustained virological response (SVR) rates. Herein we describe our single center, real world experience.
Methods: All patients with a positive HCV RNA after transplant and were treated with DAAs with at least 3 months of post-treatment follow-up were included. SVR was defined as undetectable HCV RNA 12 weeks or later after completion of therapy. Data was retrospectively collected from electronic medical records.
Results: Forty-three patients underwent a total of 44 treatments with the requisite follow up. The majority of included subjects were male (76.7%), were a mean age of 60.7 ±4.1 years, and were treated with ledipasvir/sofosbuvir (n=21) with (61.9%) or without (38.1%) ribavirin, 20.5% had undergone previous treatment with a DAA and interferon-containing regimen; treatment was initiated at a median of 7.6 (0.5-18.5) years post-transplant. Pre-treatment Ishak hepatitis activity index and fibrosis score were 4.0 (0-9) and 2.0 (0-6), respectively. Median HCV viral load prior to treatment was 2.38 x 106 IU/mL (568 - 2.94 x 107 IU/mL). Mean albumin, ALT, and tacrolimus levels pre-/post-treatment were 3.9 ± 0.4/3.9 ± 0.5; 56.4 ± 32.5/21.7 ± 15.3; and 4.6 ± 1.3/4.6 ± 2.1, respectively. Overall 37 (84.1%) patients achieved SVR, three (9.5%) relapsed, one (2.1%) suffered a viral breakthrough and three (9.5%) discontinued therapy prematurely. The three patients that discontinued early all received concomitant ribavirin and withdrew treatment for side effects attributable to this therapy (nausea, vomiting, fatigue, abdominal pain and itching); none required transfusion. Patients undergoing first DAA treatment experienced SVR at a higher rate than those being treated with a subsequent regimen (88.6% vs. 66.7%). Excluding treatment withdrawals, SVR was achieved in 90.2% of patients.



Conclusion: Our initial "real world" experience with interferon-free DAA based regimens provided high rates of SVR among patients that were both previously treated and treatment naïve. Future studies on patient and graft survival are warranted and likely to demonstrate good outcomes.

P.1230

Daclatasvir plus asunaprevir as interferon-free regimen for recurrent hepatitis C virus infection after liver transplantation: A preliminary study

Motofumi Tanaka, Takumi Fukumoto, Kaori Kuramitsu, Masahiro Kido, Hisoka Kinoshita, Shinichi So, Toshihiko Yoshida, Keisuke Arai, Sachio Terai, Taku Matsumoto, Tadahiro Goto, Sadaki Asari, Hirochika Toyama, Tetsuo Ajiki, Yonson Ku.
Hepato-Biliary-Pancreatic Surgery, Kobe University Graduate School of Medicine, Kobe, Japan.

Introduction: Recurrent infection with the hepatitis C virus (HCV) after liver transplantation (LT) is related to decreased graft and patient survival. Although Peg-interferon (IFN) plus ribavirin was a first choice for HCV reinfection, IFN-based therapy had limited efficacy and was poorly tolerated for LT recipients. In recent years, some clinical trials have shown revolutionary results in the treatment of HCV by use of direct-acting antiviral agents (DAA) with or without Peg-IFN. In Japan, the treatment of daclatasvir (DCV) plus asunaprevir (ASV) was firstly approved by national health insurance in July 2014 as IFN-free regimen. In this preliminary study, the efficiency and safety of DCV plus ASV for LT recipients with recurrent HCV infection were analyzed.
Materials and Methods: From November 2014 to August 2015, seven LT recipients with HCV reinfection were enrolled in this study. A standard dose of DCV (60mg once daily) and ASV (100mg twice daily) were orally administrated with 24-week course. The virological response, adverse effect, and tolerability were evaluated.
Results: At the time of LT, mean MELD score was 14.3 (7-26), one male received graft liver from cadaver donor and 6 females from living donor, and all did not undergo splenectomy. In immunosuppressive treatment, tacrolimus and steroid were used in all patients. Five patients previously received IFN plus ribavirin therapy. The mean duration from LT to DAA therapy was 2088 days (9-6958) and the mean HCV titer was 5.7 log IU/ml (4.1-6.8). One patient had a severe thrombocytopenia but treatment was completed in all patients. No severe liver damage was observed. Finally, four patients (57%) achieved sustained virological response (SVR). In three patients with non-SVR, two had the previous use of simeprevir in addition to IFN and one had a renal dysfunction requiring hemodialysis and longer use of low dose IFN for ten years. No resistant mutations of NS3 and NS5A were detected in non-responder.
Conclusions: Although severe thrombocytopenia was observed in one patient, the treatment of DCV plus ASV was well tolerated in all LT recipients in this study. The response rate was relatively low. A previous use of simeprevir and a longer time use of IFN were speculated to be associated with non-response to this therapy.

P.1250

Plasmapharesis on demand in ABO incompatible (ABOi) post kidney transplant management

Abdulnaser Alabadi, Ibtihal Alshaikh, Khalid B.Akkari, Khaled Hamawi, Amal Alamri, Ahmed Albahrani.
MOTC, King Fahd Specialist Hospital, Dammam, Saudi Arabia.

Introduction: ABOi Living kidney transplant (LKT) is a well established form of kidney transplant for ESRD patients. There is good evidence of comparable outcomes when compared to ABO compatible transplantation. Various conditioning protocols used trying to balance adequate immunosuppression while minimizing side effects and potential complications. Plasmapheresis (PLEX) on-demand in the immediate post-kidney transplant period can spare ABOi LKT recipients from undergoing unnecessary pre-planned PLEX. We analyzed the one year outcome of our first 12 ABOi LKT recipients, where our practice have shifted from pre-planned to on-demand post-transplant PLEX.
Materials and Method: We retrospectively reviewed all patients admitted to our unit for ABOi LKT between May 2012 and December 2014. All patients received the same pre-transplant desensitization protocol, consisting of one dose of Rituximab 375mg/m2 and a set number of PLEX with small dose IVIG (100mg/kg) based on baseline ABO iso-agglutinin titer. We targeted an immediate pre-transplant titer of ≤2 (IgG). Group A received at least 2 PLEX post-transplant to keep iso-agglutinin titer ≤ 4 (1st week) and ≤ 16 (2nd week). This practice was altered during the study in group B (PLEX on-demand), where patients had PLEX only if ABO iso-agglutinin titer increased >4 during the first week, or >16 during the second week. All patients received the same maintenance immunosuppression (Tacrolimus, MMF and prednisone).
Results: 12 patients underwent desensitization during the study period (mean age 34±11 years, 5 female). Eleven patients were transplanted, while desensitization was deemed unsuccessful in one patient. One case was excluded due to sepsis related mortality within 24-hours post-transplant. Five out of the remaining 10 patients had PLEX on-demand (group B). One patient in group B had graft nephrectomy 10 days post-transplant due to renal vein thrombosis, a subsequent histopathological examination showed severe AMR. Mean length of hospital stay was 18.6 ± 3 days(group A) vs 14.8±6 day (group B), while mean number of PLEX was 7.8±2.3 (group A) compared to 5.6±1.5 (group B). The mean one year serum creatinine was 82±27umol/l in group A vs 72±17umol/l in group B. One patient in group A had an episode of ACR (Banff Ib), and there was no AMR in group A. Neither of the two groups had BK viraemia or nephropathy, or CMV viraemia.
Conclusion: On-demand PLEX in the immediate post-operative period after ABOi LKT can be an effective and potentially a cost saving alternative approach with timely iso-agglutinin measurement and intervention.
References:
[1] Winters, J.L., Plasma exchange conditioning for ABO-incompatible renal transplantation. *J Clin Apher*. 2004. 19(2): p. 79-85.
[2] de Weerd A. Post-Transplantation Immunoadsorption Can Be Withheld in ABO-Incompatible Kidney Transplant Recipients. *Ther Apher Dial*. 2015
[3] Oettl T. ABO blood group-incompatible living donor kidney transplantation: a prospective, single-centre analysis including serial protocol biopsies. *Nephrol Dial Transplant*. 2009. 24(1): p. 298-303
[4] Wilpert J, On-demand strategy as an alternative to conventionally scheduled post-transplant immunoadsorptions after ABO-incompatible kidney transplantation. *Nephrol Dial Transplant* 2007. 22(10): p. 3048-51.

P.1251

Anti-HLA antibody-induced rejection in ABO-incompatible kidney transplant patients.

Jin Kong[1], Eun J.Whang[3], Joon H. Jeong[2], Won Y.Choi[3], Sung H.Son[1], Hyuk Y.Kwon[1].
[1]Nephrology, Hanseo Hospital, Busan, Korea; [2]Surgery, Hanseo Hospital, Busan, Korea; [3]Laboratory Medicine, Hanseo Hospital, Busan, Korea.

Antibody mediated rejection(AMR) in ABO-incompatible(ABOi) living donor kidney transplant(KT) patients can either be due to donor-specific anti-HLA antibody(DSA) or anti-blood group antibody(anti-ABO). The relative frequency and possible differential clinical features of these two types of AMR in ABOi KT patients is unknown.
Among 78 ABOi KT patients between 2007 and 2015 in our center, 9(11.5%) patients developed clinical (with an increase in serum creatinine) acute AMR. Since there is no histologic distinction between DSA- and anti-ABO-induced AMR, we assumed the causative antibody in each cases on the basis of anti-ABO level and DSA measured in serum collected at the time of AMR. DSA was determined by luminex panel reactive antibody (PRA) phenotype assay, and single antigen beads assay if PRA was positive.
Among the 9 cases of AMR, 5 with anti-ABO titer 16 or higher and undetectable DSA at the time of rejection were regarded as anti-ABO-induced AMR. Two cases of AMR with detectable DSA and low(4 and 8) anti-ABO during rejection were regarded as DSA-induced AMR. In another 2 cases, anti-ABO titer was low(2), and DSA also was not detectable. We considered these two cases as DSA-AMR, since this low level of anti-ABO is unlikely to cause rejection, and in ABO-compatible KTs, cases of AMR with DSA not detectable in serum, probably due to the adsorption of antibody on the graft, are not infrequently observed. The day of onset of AMR was within 2 weeks in all cases and comparable between two types of AMR; median(range) 7(1-11) days in ABO-AMR and 4.5(1-7) days in DSA-AMR. Initial anti-ABO titer was also not statistically different; median(range) 128(64-4096) in ABO-AMR and 160(16-256) in DSA-AMR. All the 5 patients with anti-ABO-AMR had negative PRA before KT, whereas 3 of 4 patients with DSA-AMR had positive PRA before KT, and one DSA-AMR patient had persistent DSA before KT and at the time of AMR. All the AMR were recovered by treatment with steroid pulse and plasmapheresis, with ATG and/or additional rituximab in some patients.
We conclude that a significant proportion of AMR in ABOi KT are caused by DSA, and clinical features and possible differential therapeutic approach of these 2 types of AMR needs to be explored by further studies.

P.1252

Role of plasmapheresis in management of ABO incompatible renal transplantation: A single centre experience

Kamal Kanodia[1], Aruna Vanikar[1], Amit Prajapati[1], Rashmi Patel[1], Kamlesh Prajapati[1], Lovelesh Nigam[1], Sheetal Chandak[1], Himanshu Patel[2].
[1]Department of Pathology, Laboratory Medicine, Transfusion Services and Immunohematology, Institute of Kidney Diseases & Research Centre and Institute of Transplantation Sciences, Ahmedabad, India; [2]Department of Nephrology and Transplantation Medicine, Institute of Kidney Diseases & Research Centre and Institute of Transplantation Sciences, Ahmedabad, India.

Background: ABO-incompatibility(ABOi) was an absolute contraindication for renal transplantation (RT) till recently. However now ABOiRT are well-accepted. Plasmapheresis/ immunoadsorption help in reducing antibody titres in recipients. We carried out this study from December'14 to November'15 to evaluate the role of plasmapheresis in management of ABOi RT.
Material and Methods: We analyzed demographic data including ABO-antibody titres before and after RT. Graft function was measured in terms of serum creatinine (SCr). Plasmapheresis was performed on alternate days pre-transplant to achieve nadir ABO-antibody titres of ≤ 1:4, and repeated twice weekly for 1st week, weekly for 1st month, monthly for 6 months and 6 monthly thereafter.
Result: Plasmapheresis was safe and effective. The following table depicts the outcome of all patients.

Table-1: Patients demography and outcome

Patient	Age/Sex	Donor recipient blood group	Baseline anti-body titre	Plasmapheresis sessions Pre transplant	Pre-Transplant Anti-body titre	Post-Transplant antibody titre	Plasmapheresis sessions Post transplant	Follow up (in months)	Current Anti-body titres	SCr (mg/dl)	Graft loss
1	[illegible]	[illegible]	1:128	7	[illegible]	[illegible]	[illegible]	22	-	-	Yes
2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
6	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
7	[illegible]	[illegible]	[illegible]	[illegible]	NEG	NEG	[illegible]	[illegible]	NEG	[illegible]	[illegible]

Discussion: All patients had successful transplantation except one patient with mean SCr of 1.66 mg/dL at discharge. Short-term outcome of ABO-I transplantation was similar to those of ABO-compatible RT. Prevention of immune reactions due to anti-A/B antibodies is crucial for transplant success. ABO antigens are expressed not only on red blood cells but also in various cells such as the endothelial cells of grafted organs. ABO-I graft implantation often results in immediate hyperacute rejection, involving failure of graft reperfusion due to thrombosis of the microvasculature.Titers of the anti-A/B IgG antibodies are considered to be more important than titers of IgM. Gloor et al reported that initial anti-A/B IgG titer of > 1:256 is significantly associated with the incidence of antibody-mediated rejection (AMR). In our study baseline titres of all patients were below 1:256. Further Toki et al reported that an anti-A/B IgG titer > 1:32 at the time of transplantation is an independent risk factor for AMR. In our study ABO-antibody titres at the time of transplantation were <1:32 in all patients. In our study, 2 out of the 8 patients underwent post-transplantation plasmapheresis for treatment of AMR. However one patient (no.1) underwent graft nephrectomy due to fungal infections associated with immunosuppression. Second patient has recovered and is doing well.
Conclusion: Plasmapheresis helps in safe minimization of ABO-antibody load for performing ABO-I RT thus helping in increasing the donor pool. Short term outcome is acceptable.
Keywords: ABOi, Renal transplant, TPE
References:
[1] ABO Blood Type–Incompatible Kidney Transplantation and Access to Organs Gerald S. Lipshutz, MD; Suzanne McGuire, RN; Qing Zhu, RN; Alyssa Ziman, MD; ARCH SURG/ VOL 146 (NO. 4),

APR 2011 WWW.ARCHSURG.COM
[2] In: McLeod BC, Szczepiorkowski ZM, Weinstein R, Winters JL, eds. Apheresis: Principles and Practice, 3rd edition page no.373
[3] Mollison's Blood Transfusion in Clinical Medicine,11th edition
[4] Rossi's Principles of Transfusion Medicine, 4th Edition
[5] Gloor JM, Lager DJ, Fidler ME, Grande JP, Moore SB, Winters JL, et al. A Comparison of splenectomy versus intensive posttransplant antidonor blood group antibody monitoring without splenectomy in ABO-incompatible kidney transplantation. Transplantation. 2005;80:1572–1577.
[6] Toki D, Ishida H, Setoguchi K, Shimizu T, Omoto K, Shirakawa H, et al. Acute antibody-mediated rejection in living ABO-incompatible kidney transplantation: long-term impact and risk factors. Am J Transplant. 2009;9:567–577

P.1253

De novo focal and segmental glomerulosclerosis in renal allograft: a single center five year experience

Kamal Kanodia[1], Aruna Vanikar[1], Pakesh Baishya[1], Rashmi Patel[1], Kamlesh Suthar[1], Lovelesh Nigam[1], Himanshu Patel[2], Vivek Kute[2]. [1]Department of Pathology, Laboratory Medicine, Transfusion Services and Immunohematology, Institute of Kidney Diseases & Research Centre and Institute of Transplantation Sciences, Ahmedabad, India; [2]Department of Nephrology and Transplantation Medicine, Institute of Kidney Diseases & Research Centre and Institute of Transplantation Sciences, Ahmedabad, India.

Background: De Novo Focal Segmental Glomerulosclerosis (FSGS) often presents as nephrotic syndrome (NS) post-transplant due to nephron loss/ glomerular hyperfiltration / calcineurin inhibitor(CNI) induced toxicity. We carried out this study to evaluate the natural history of de novo FSGS in renal allograft biopsies, and to evaluate their impact on graft function.
Materials and Methods: This was a retrospective single center study performed between January,'11 and October,'15. Renal allograft biopsies were evaluated by standard techniques and diagnosed as per modified Banff 2013 guidelines. Associated urinary proteins, serum creatinine (SCr) and immunosuppression were studied.
Results: Out of 1586 biopsies, 36(2.26%) revealed de novo FSGS, at mean 1.73 ±1.70 years post-transplant with mean SCr of 2.22 ± 1.09 mg/dl. Mean patient age was 30.17 ± 8.25 years, males (91.6%) outnumbering females; and majority were living donor transplants (91.6%) on CNI based immunosuppression. Mean 24 hours urinary proteins were 3.84 ±2.90 gms. Histopathology revealed classical (47.2%), collapsing (44.4%), cellular (5.5%) and perihilar (2.7%) variants. Five and 10 year patient survival was 57.6%. Five year graft survival was 76.2%, all grafts were lost at 10 years. Mean SCr (mg/dl) at 5 and 9 years was 1.86 ± 0.40 and 1.93 ±0.40, with 0.18 ± 0.26 gms /24 hour urinary protein loss at 9 years.
Discussion: De novo FSGS is one of the common causes of NS post-transplant. It is associated with higher rate of graft loss which was found in our study also. Chronic CNI toxicity causing hyaline arteriosclerosis aggravates ischemic changes in the graft and development of collapsing glomerulopathy. So efforts should be made to identify the factors responsible for FSGS and take appropriate therapeutic measures to prevent its development after transplantation, such as minimization or avoidance of CNI and obesity.
Conclusion: Renal allograft recipients on CNI based immunosuppression are prone to develop de novo FSGS, commonly presenting with proteinuria at mean of 1.7 years post-transplant with common histopathological findings of classical or collapsing variants. It may occur in recipient of any age, and in living or deceased donor transplants and can cause long term graft/ patient loss.
Keywords: De Novo FSGS, kidney transplantation, renal allograft biopsy, calcineurin inhibitor

P.1254

Successful transplantation in ABO- and HLA-incompatible living kidney-transplant patients: a report on 12 cases

Lionel Rostaing[1,3,5], Béatrice Karam[1], Nicolas Congy-Jolivet[5,6], Valérie Hage[1], Federico Sallusto[2], Laure Esposito[1], Nicolas Doumerc[2], Bénédicte Debiol[8], Céline Guilbeau-Frugier[7], Xavier Game[2,5], Asma Allal[1], Nassim Kamar[1,4,5].
[1]Néphrologie, Dialyse et Transplantation d'Organes, CHU Rangueil, Toulouse, France; [2]Urologie, Andrologie et Transplantation d'Organes, CHU Rangueil, Toulouse, France; [3]INSERM U563 - IFR -BMT, CHU Purpan, Toulouse, France; [4]INSERM U1043 - IFR-BMT, CHU Purpan, Toulouse, France; [5]Université Toulouse III - Paul Sabatier, Faculté de Médecine de Rangueil, Toulouse, France; [6]Laboratoire d'Immunologie, CHU Rangueil, Toulouse, France; [7]Laboratoire d'Histopathologie, CHU Rangueil, Toulouse, France; [8]Etablissement Français du Sang de Midi-Pyrénées, CHU Purpan, Toulouse, France.

Background: Few studies have assessed the outcomes of ABOi/HLAi living-kidney transplantation. We report a single-center experience of 12 ABOi/HLAi living-kidney recipients.
Method: Twenty-seven donor-specific alloantibodies (DSAs) were found (1–6 per patient) with fluorescence intensities of 1,500–15,000. Desensitization was based on IV-Ig, two doses of rituximab (375 mg/m2), tacrolimus-based (0.2 mg/kg) immunosuppression (started on day-10 pretransplant), and 11 (6–27) pretransplant apheresis sessions (plasmapheresis, specific or semi-specific immunoadsorption).
Results: By day 0, 17 of the 27 DSAs had become undetectable. After 19 (3–51) months, patient- and graft-survival rates were 100% and 91.6%, respectively. One patient had an acute humoral rejection whereas three had a chronic antibody-mediated rejection (CAMR). At the last follow-up, kidney biopsies were nearly normal in seven cases (58.3%) and renal function was excellent except for the three cases of CAMR. Four patients had a BKV infection.
Conclusion: ABOi/HLAi living-kidney transplantation is a reasonable option for highly sensitized patients.

P.1255

NODAT development is associated with Tacrolimus blood level in relatively more elderly patients with pre-transplant impaired fasting glucose

Fatih Gokhan Akbay[1], Gokhan Seker[2], Nilay Orak Akbay[3], Suheyla Apaydin[1], Selcuk Sahin[2].
[1]Nephrology, Bakirkoy Dr Sadi Konuk Egitim Arastirma Hastanesi, Istanbul, Turkey; [2]Urology, Bakirkoy Dr Sadi Konuk Egitim Arastirma Hastanesi, Istanbul, Turkey; [3]Pulmonary Medicine, Istanbul Medical Faculty, Istanbul, Turkey.

Objective: New-onset diabetes after transplantation (NODAT) is a metabolic disease that occurs due to various reasons, characterized by varying degrees of insulin resistance. Its incidence has been reported between 4% and 25%.The aim of our study is the evaluation of risk factors that can be foreseen for NODAT incidence and NODAT development in patients who are non-diabetic and who received renal transplantation in our hospital.
Materials and Methods: Between the years 2010 through 2012, 72 patients who had kidney transplant operation, over 18 years of age, who had no diagnosis of diabetes previously, and who had at least 1-year follow-up were included in the study. Age, gender, height, weight, body mass index values, primary disease, history dialysis, HLA tissue compatibility, the age of the kidney donor, gender, the receiver's history of hepatitis B or C, thyroid hormones, lipid profiles, magnesium values, systolic blood pressures, fasting and postprandial blood sugar and HbA1c values, postoperative insulin needs, creatinine values, immunosuppressive drug types and blood levels, pulsed steroid history, rejection history, CMV infection history, pyelonephritis history and the treatment given to patients for NODAT were recorded fort he patients who had or did not have diagnosis of NODAT, and those who use similar immunosuppressive medication protocols. Risk factors for development of NODAT were assessed by using Chi-square, independent T test, Mann Whitney U test andlogistic regression analysis.
Results: 33 of the 72 patients who were included in this study (46 %) were males, and 39 of them were females (54 %). The average age of the patients was 40,6±12,6. While NODAT developed in 17 patients (23,6 %), NODAT was not detected in 55 patients (76,4 %) NODAT. During the analysis with a single variable ((in univariate analyses, the fact that the age is over 45 ($p<0,0001$), the body mass index being over 30 before the transplant and in the 1st month ($p=0,009$, $p=0,002$), postoperative insulin needs ($p<0,0001$), impaired fasting blood glucose values before the transplant and in the 2nd, 3rd and 4th week ($p<0,0001$, $p=0,001$, $p= 0,004$, $p<0,0001$), impaired glucose tolerance before the transplant ($p=0,009$) and Tacrolimus blood level being over 10 ($p=0,006$) were detected as significant risk factors for NODAT development. As a result of multivariate analysis, however, the age being over 45 ($p=0,01$), impaired fasting blood glucose before the transplant ($p=0,001$), postoperative insulin needs ($p=0,01$) and Tacrolimus blood level in the 1st month ($p=0,04$) were detected as significant risk factors for NODAT development.
Conclusion: Impaired fasting blood glucose before the transplantation, postoperative insulin needs, the age being 45 and over, Tacrolimus blood level being high were detected as significant risk factors that can be foreseen for NODAT. NODAT development can be reduced by keeping Tacrolimus blood levels in relatively older patients who have impaired fasting glucose before kidney transplant at minimum level that will not give rise to rejection.

P.1256

Peak panel reactive antibody related states of patients awaiting for deceased kidney transplantation

Süheyla Apaydin[1], Fatih Gokhan Akbay[1], Murvet Yilmaz[1], Reyhan Calayoglu-Salcıav[1], Fatma Sibel Kocak-Yucel[1], Aysegul Kudu[1], Emel Yanik-Gezer[1], Selcuk Sahin[1,2].
[1]Nephrology, Bakirkoy Dr.Sadi Konuk Research and Training Hospital, Istanbul, Turkey; [2]Urology, Bakirkoy Dr. Sadi Konuk Research and Training Hospital, Istanbul, Turkey.

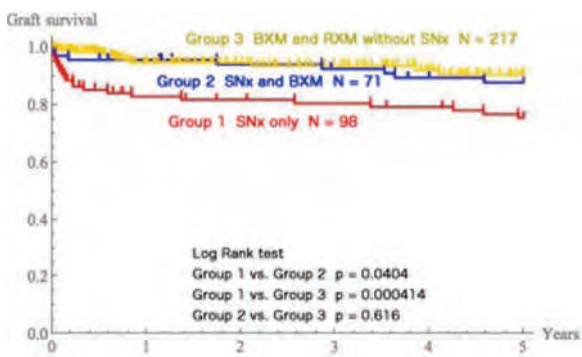
Anti HLA antibodies are used in monitoring sensitization status of patients who wait for deceased donor kidney transplantation. Antibodies directed against class 1 and class 2 HLA antigens can cause hyperacute or accelerated acute graft rejection.
Patients on waiting list for deceased renal transplantation from 2010-2015 at Our Hospital were recruited in this retrospective study.119 patients awaiting for last 5 years on list were accepted for this study. Regular PRA measurement for every 6 months was done with ELISA between 2010-2012. After 2012, LUMINEX was used. Peak PRA level higher than 15 % was accepted as positive. Possible risk factors for high PRA levels for each classes such as age, sex, primary disease, vintage of dialysis, awaiting time, Charlson's co-morbidity index score, BMI, pregnancy, blood transfusion, hepatitis status were evaluated in all patients. The results were analysed with an IBM SPSS 20.0 for MAC statistical package. T-test, Fisher's exact, Mann-Whitney U tests and multivariate analysis were used for evaluation of high PRA related factors. The results were considered statistically significant for values of P< 0.05.
Mean awaiting time was of 34±26 months and dialysis vintage was of 56±42. Percentage 55 of them was male. Mean age was of the patients were 49 ± 12 years. Mean BMI was of 25±6. Peak class 1 PRA was consistently positive in 20 % of patients and class 2 was positive in 19 % patients, and also both class 1 and class 2 were positive in %13. Female sex (27% vs. 11%; p:0.038), dialysis vintage (71 mo. vs. 52 mo., p: 0.05), and blood transfusion (30% vs. 10%; p:0.007) were significantly related with positive peak class 1 PRA level. Dialysis vintage (72 mo. vs. 52 mo., p: 0.0043), awaiting time (45 mo. vs. 32 mo., p:0.05), and also blood transfusion (28% vs. 12 %,p:0.042) were significantly related with positive peak class 2 PRA level. In patients had both class 1-2 positivity when compared with negative corresponding groups with multivariate analysis, blood transfusion were found only risk factor (OD: 3 CI: 1.24-7.36, p:0. 015). Other factors such as age, primary diseases, co-morbidity index score, pregnancy and hepatitis status were not seen related with positive PRA levels.
As a conclusion, monitoring sensitization status of patients is necessary because sudden transplantation of sensitized patients with deceased kidneys may cause undesirable consequences In our study, blood transfusion, vintage of dialysis and awaiting time were found the causes of sensitization of patients awaiting for deceased kidney transplantation.

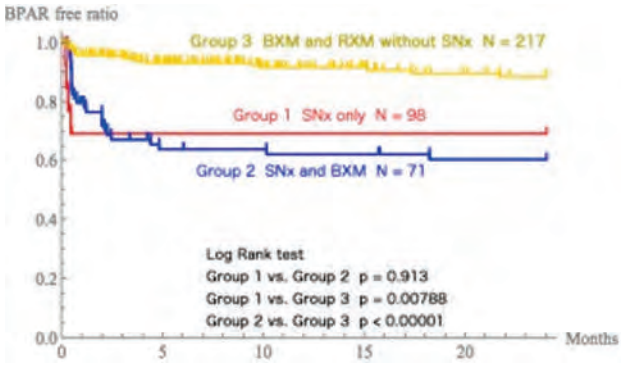
P.1257

Rituximab induction significantly reduced acute rejection in ABO-incompatible kidney transplantation: A cohort study of 405 patients over 25 years

Akihito Sannomiya, Shohei Fuchinoue, Ichiro Nakajima, Kazuhiro Iwadoh, Kumiko Kitajima, Ichiro Koyama, Toru Murakami, Kotaro Kai.
Department of Surgery Kidney Center, Tokyo Women's Medical University, Tokyo, Japan.

Introduction: Ever since ABO-incompatible kidney transplantation (ABOi-KTx) was introduced in Japan in 1989, it has become a major source of organ procurement for KTx nationally, and today it is practiced worldwide. Reviewing our 25 years of experience in ABOi-KTx, we present the effectiveness and importance of induction therapies upon the clinical outcomes in ABOi-KTx.
Materials and Methods: Between 1989 and 2015, we have performed 405 cases of live ABOi-KTx. They were divided into three groups based on induction therapy: Group 1; splenectomy (SNx) only (G1; n=98), Group 2; SNx and basiliximab (BXM) (G2; n=71) and Group 3; BXM and rituximab (RXM) without SNx (G3; n=217). Plasmapheresis was performed in all cases before surgery. Graft and patient survival rates, biopsy-proven acute rejection (BPAR)-free ratios and renal function were compared. The induction therapy in G1 (SNx only) was used until 2002, that of G2 (SNx and BXM) was used between 2002 and 2007, and that of G3 (BXM and RXM) was used after 2007.
Results and Discussions: There was significant difference in the average donor and recipient ages between the three groups (G1 vs. G2 vs. G3) (p<0.001). Mean donor age was 53.1±11.4 vs. 58.6±10.0 vs. 58.6±10.4, respectively. Mean recipient age was 35.1±12.3 vs. 46.7±12.6 vs. 50.6±13.0, respectively. Spousal donors comprised 18.6% vs. 52.9% vs. 59.5% of all donors in each group (p<0.001). There was no significant difference in the 5-year patient survival rates of each group, with the survival rate being 92.4% vs. 94.2% vs. 95.5%. 5-year graft survival rates were 75.5% vs. 87.9% vs. 90.5%, and the graft survival rate in G1 was significantly lower than the other two groups (p<0.05) [Figure 1]. 5-year BPAR-free ratios were 69.2% vs. 60.5% vs. 88.6%, and G3 had a significantly higher BPAR-free ratio compared to the other two groups (p<0.01) [Figure 2]. Average creatinine levels (mg/dl) for 5 years were 2.19±1.14 vs. 1.91±1.15 vs. 1.66±0.89, with significant difference between the three groups (p<0.05). Average eGFR levels (ml/min) for 5 years were 35.3±17.4 vs. 38.9±13.7 vs. 43.7±13.6, and these were also significantly different between the three groups (p<0.001).
Conclusion: Difference of induction strategies in ABOi-KTx resulted in significant differences in BPAR-free ratios, graft survival rates and renal function. The introduction of RXM considerably improved the BPAR-free ratio in the few months following KTx, and this has probably led to improved graft survival and renal function. The introduction of BXM was also shown to have improved graft survival rates.





P.1258

Gastrointestinal perforation after renal transplantation

Ji Yoon Choi, Joo Hee Jung, Sung SHIN, Young Hoon Kim, Duck Jong Han.
Department of Surgery, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea.

Backgrounds: Gastrointestinal (GI) perforation rate varied between 1% and 12% in renal transplant recipients. Half of them occurred within the first year. GI perforations can cause serious complications leading to graft loss and patient death in recipients with old age, DGF and PDKD, especially. In this study, we reviewed post-transplantation GI perforation in our center.
Materials and Methods: Between January 1990 and January 2016, 4300 renal transplantation were performed in our center. We analyzed the incidence of gastrointestinal perforation and the clinical characteristics.
Results: Nineteen cases (0.45%) developed GI perforation. Mean age of recipients was 45.1 years old. Thirteen were male. The median time from transplant to perforation was 16 days (1-6173). Thirteen cases occurred within 1 post-transplant year. Perforations occurred in the small bowel (n=14), colon (n=3), stomach (n=1), and duodenum (n=1). One (5%) lost his graft function and died as a direct result of his GI perforation due to transmural infarction of whole small bowel. The causes of perforation were fungal(1) and EBV(1) infection, transmural infarction due to kayexalate crystal(2), known malignanies(2), and the others had no apparent underlying cause. For the follow up periods, graft functions were maintained in 16 cases.
Conclusion: GI perforation can be occurred by various reasons. Especially, during the first post transplantation year can be accompanied serious complications effecting on patient survival. The follow-up after renal transplantation should include a careful evaluation for early diagnosis and timely treatment.

P.1259

Clinical outcomes of DSA positive alone in kidney transplantation

Sung Shin[1], Young Hoon Kim[1], Joo Hee Jung[1], Ji Yoon Choi[1], Heung-Bum Oh[2], Seog -Woon Kwon[2], Su-Kil Park[3], Duck Jong Han[1]. [1]General Surgery, Asan Medical Center, Seoul, Korea; [2]Laboratory Medicine, Asan Medical Center, Seoul, Korea; [3]Nephrology, Asan Medical Center, Seoul, Korea.

Background: Cell based assay (CDC or FCXM) methods, can identify antibodies that can mediated acute humoral rejection. Furthermore recent new solid phase assay, by single bead PRA (Luminex®) is more sensitive method for detection of anti-donor antibody. However the need and benefit of desensitization for DSA positive but FCXM negative patients is controversial.
Methods: From Aug 2012 to Jan 2016 we had performed 802 Living donor kidney transplantations. Among them 50 patients were DSA positive alone patients (FCXM negative). Six patients were excluded because they were ABO incompatible kidney transplant recipients who need preoperative desensitization. In these patients induction followed by triple immunosuppressant was used without pre operative desensitization.
Results: Mean FCXM MFI value was 1.1 (reference value; positive ≥2.0). Mean PRA class I and II was 34±28% and 48±29% respectively. Peak DSA MFI value was 2413±1384 in Class I and 3477±4194 in class II HLA antigen.
One year patient and graft survival were 95.4% each (2 case of mortality from infection). During mean 581±380 days of follow up, 6 rejection episodes were developed (13.7% 1 year rejection rate). Among whom, 3 case of antibody mediated rejection, 2 case of cellular rejection and one case of mixed rejection were developed. Interestingly rejections were developed during relatively early postoperative period (Median 18 days) and relatively severe rejection (Banff IB, IIA and IIB), but all this severe rejection reversed by plasmapheresis, rituximab and IVIG. In the analysis of the risk factor for rejection, we could not detect any factors affecting rejection, such as the intensity of DSA or value of cPRA.
Conclusions: Although all the rejections were was reversible by rescue therapy and majority of patients does not developed acute rejection, DSA positive alone patients had relative higher rejection rate than no DSA recipient in our center(7.8%). Careful follow up and monitoring may be mandatory in this category patients, and desensitization might be needed in some patients according to further analysis.

P.1260

Living donor renal transplantation in patients with antiphospholipid syndrome

Ji Yoon Choi, Joo Hee Jung, Sung Shin, Young Hoon Kim, Duck Jong Han.
Department of Surgery, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea.

Background: Antiphospholipid syndrome(APS) is a disorder in which autoantibodies directed against phospholipid-binding proteins are associated with vascular thrombosis. In renal transplantation, the presence of APS can be a risk of early graft loss from arterial or venous thromvosis, or thrombotic microangiopathy(TMA). While perioperative anticoagulation for reducing the risk of graft thrombosis, it can result in bleeding as a postoperative complication and the efficacy is uncertain. Here, we reported the two cases of renal transplantation with APS.
Case Presentation: A 53 year old man with ESRD due to FSGS was scheduled to do a living donor renal transplantation from his sun. In his Past history, he first experienced deep venous thrombosis in bilateral lower extremity 16 years ago. He started anticoagulation with warfarin. However, he frequently experienced recurrent DVT despite taking anticoagulation therapy. Before the surgery, he confirmed APS by positive finding of lupus anticoagulant.
A 42 year old woman with ESRD was diagnosed APS 7 years ago. She experienced leg swelling, paresthesia and coldness in left leg. So she checked the CT angiography, and popliteal artery occlusion was founded. Serologically, lupus anticoagulant and anti-B2-glycoprotein 1 were present. Anticoagulation therapy was started after then.
They stopped taking warfarin 5days prior to surgery and started intravenous heparin therapy. And during the surgery, we used the heparin injection(3000unit, bolus) for preventing arterial or venous thrombosis by clamping. After the surgery, heparin was maintained and warfarin was restarted after postoperative 5 days. The woman developed clinical rejection by increasing serum creatinine and received steroid pulse therapy and plasmapheresis. After rejection treatment, she recovered her graft function within normal range. In both patients, renal graft function was good during the admission and maintained in outpatient clinic.
Conclusion: Living donor renal transplantation can be successful in a patient with APS following a perioperative anticoagulation therapy. However, because they still have a higher risk of TMA in early postoperative periods, the close monitoring about hypercoagulability and continuous anticoagulation should be maintained for maintain their graft function.

P.1261

Clinical significance of ABO incompatible kidney transplantation from spousal donors

Chul Woo Yang, Ji Hyun Yu, Byung Ha Chung.
Transplantation research center Division of Nephrology, Department of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea., Seoul, Korea.

Kidney transplantation (KT) from spousal donor (SD) has been performed more widely because the ABO incompatible (ABOi)-KT became feasible. We evaluated the effect of the introduction of ABOi-KT on the increase of SD-KT and investigated the clinical outcomes of ABOi-SD-KT compared to ABO compatible (ABOc)-SD-KT and living related donor (LRD)-KT in Korea.
Using the Korean Network for Organ Sharing database, we compared the proportion of SD-KT or ABOi-KT among the total LD-KT between before and after the introduction of ABOi-KT in 2007, Korea and compared the proportion of ABOi-KT between SD-KT and LRD-KT since 2007. In addition, we compared the development of acute rejection, allograft and patient survival rate across ABOi-SD-KT, ABOc-SD-KT and LRD-K.
Both the ABOi-KT and SD-KT have increased significantly since 2007, the first year of ABOi-KT implementation in Korea (p for trend < .001, respectively). The proportion of ABOi-KT in SD-KT has been significantly higher compared to LRD-KT between 2007 and 2014 (p < .05 for each year). Biopsy-proven acute rejection (BPAR) occurred more frequently in ABOi-SD-KT (23.9%) compared to ABOc-SD-KT (15.8%, p = 0.081) or LRD-KT (11%, p< .001), and ABO incompatibility was an independent risk factor for BPAR (hazard ratio[HR], 1.499; 95% confidence interval [CI], 1.001-2.245; p = 0.05). However, in the matched analysis between ABOi-SD-KT and ABOc-SD-KT, no significant difference was found in the acute rejection, death-censored graft survival and patient survival rates (p > .05, respectively). The allograft and patient survival rates at 3-year post-transplantation showed no significant differences across the three groups (p > .05, respectively), and neither ABO incompatibility nor SD associated independently to allograft failure (for ABO incompatibility, HR, 1.417; 95% CI, 0.697-2.879; p = 0.336; for SD, HR, 1.445; 95% CI, 0.833-2.507; p = 0.196).
In conclusion, the introduction of ABOi-KT has contributed to an increase of SD-KT with comparable clinical outcomes to ABOc-SD-KT or LRD-KT. Therefore, ABOi-SD-KT can be recommended for KT candidates whose only potential donor is an ABO mismatched spouse.
Keywords: ABO-incompatibility; spousal donor; kidney transplantation

P.1262

Outcomes of ABO incompatible kidney transplantation without antibody remove: Multicenter experiences from China

Wang Yi[1,2], Liu Logen[2], Shi Bingyi [3], Lin Tao[4], Chen Gang[5], Liu Hongtao[6], Wen Jiqiu[7], Changxi Wang[8], Luo Zhigang[1], Gui Peigeng[1], Jiang Hongtao[1], Zhou Hao[1], Qin Guoqing[1], Zhang Yunsheng[1], Zou Yizhou[9].
[1]Organ Transplantation Unit/Department of Urology, The Second Affiliated Hospital, University of South China, Hengyang, People's Republic of China; [2]Clinical Research Center, The Second Affiliated Hospital, University of South China, Hengyang, People's Republic of China; [3]Institute of Organ Transplantation, The 309th Hospital of Chinese People's Liberation Army, Beijing, People's Republic of China; [4]Department of Urology /Organ Transplantation Center, West China Hospital, Sichuan University, Chengdu, People's Republic of China; [5]Institute of Organ Transplantation, Tongji Hospital, Huazhong University of Science and Technology, Wuhan, People's Republic of China; [6]The Organ Transplantation Center, Anhui Provincial Hospital, Hefei, People's Republic of China; [7]National Clinical Research Center of Kidney Disease, Jinling Hospital, Nanjing University School of Medicine, Nanjing, People's Republic of China; [8]Organ Transplantation Center, The First Affiliated Hospital of Sun Yat-sen University, guangzhou, People's Republic of China; [9]Department of Immunology, Xiangya Medical School, Central South University, Changsha, People's Republic of China.

Objective: To determine whether low titer of ABO-incompatible (ABOi) kidney transplantation can be performed safely and result in acceptable posttransplantation outcomes.
Materials and Methods: In this study, 13 patients from 4 centers of kidney transplantation in China, with renal failure and an living kidney donor were included, 6 patients underwent ABO incompatible transplantation and 7 patients underwent ABO compatible transplantation. Patient and allograft survival; 1-, 3-, and 6-month and 1-year renal function; and incidence of rejection are the Main Outcome Measures
For 6 patients of ABOi the titers of them are 1:1 to 1:4, only 3 of ABOi receptions received anti-CD20 antibody pre-operation, All kidneys were transplanted heterotopically, and all patients received induction immunosuppression followed by a combination of prednisone, mycophenolate mofetil, and tacrolimus. Isoagglutinin titers were monitored.
Results: For 6 patients of ABOi, the donor to recipient transplantation was A to O in 2 cases, B to A in 1, B to O in 1, AB to A in 1 and AB to B in 1. For 7 Patients of ABOc, the donor to recipient transplantation was O to O in 4 cases, A to A in 1, O to B in 1 and A to AB in 1.
All patients with allograft survival was 100%.Mean (SD) creatinine levels, a measure of graft function, in ABOi group were 96.3 (11.2) umol/L at discharge.127.6 (10.4) umol/L at 1 month, 119.0 (13.3) umol/L at 3 months, 92.5(7.3) umol/L at 6 months, and 112 (6.9) umol/L at 1 year; in ABOc group were 87.9 (17.3) umol/L at discharge.118.2 (13.5) umol/L at 1 month, 99.1 (14.0) umol/L at 3 months, 94.5(6.3) umol/L at 6 months, and 103 (7.7) umol/L at 1 year. No statistically significant difference was observed. One episode of cellular rejection occurred in ABOi group.
Conclusions: These short-term results suggest that without antibody remove simplify preconditioning regimen in ABOi kidney transplant compare with the ABOc, it can be obtained the same 1-year patient and graft survival rates in China.

P.1263

Pilot conversion study from mycophenolate mofetil to everolimus in stable ABO-incompatible kidney transplant recipients

Junjji Uchida[1], Tomoaki Iwai[1], Nobuyuki Kuwabara[1], Kazuya Kabei[1], Shunji NIshide[1], Toshihide Naganuma[1], Norihiko Kumada[2], Yoshiaki Takemoto[1], Tatsuya Nakatani[1].
[1]Urology, Osaka City University Graduate School of Medicine, Osaka, Japan; [2]Urology, Suita Municipal Hospital, Suita, Japan.

Background: ABO-incompatible kidney transplantation has been developed with excellent outcomes, although it is an immunologically high-risk procedure. Our previous study demonstrated that conversion from mycophenolate mofetil (MMF) plus standard-dose of calcineurin inhibitors (CNIs) to everolimus plus low-exposure CNIs in stable ABO-incompatible kidney transplantation was safe and effective during a 3 month observation period. We here report our 1 year follow-up data of stable ABO-incompatible kidney transplant recipients converted from MMF to everolimus.
Patients and Methods: An open-label design was used to examine the efficacy and safety of conversion of stable ABO-incompatible kidney transplant recipients from MMF with standard exposure CNIs to everolimus with very low exposure CNIs. A total of 17 recipients of ABO-incompatible kidney transplantation at our institution were enrolled. Patients without acute rejection by graft biopsy were switched from MMF to everolimus with dose adjustments to target an everolimus trough level of 3 to 8 ng/ml. The CNI dose was reduced to a target trough level of 2-4 ng/ml (tacrolimus) or 25-50 ng/ml (cyclosporin). At 3 and 12 months after conversion, graft biopsies were performed to check for acute rejection and C4d deposition, even though everolimus was continued or discontinued. At baseline and at 1, 2, 3, 6, and 12 months after conversion, the peripheral CD19+ cells and CD20+ cells were monitored.
Results: Treatment with everolimus was stopped due to adverse events in 8 patients (47.1%). Median time from conversion to discontinuation was 124.5 days, with a range between 109 and 273 days. Five patients with general fatigue, 1 with interstitial pneumonia, 1 with peripheral edema, and 1 with menoxenia were led to discontinuation of everolimus and reconversion to MMF. Conversion to everolimus with CNI minimization did not induce acute rejection and C4d deposition at 3 and 12 months after conversion. There were no significant changes in CD19+ cells and CD20+ cells during the study period. The patients in whom everolimus was stopped were significantly older than those in whom everolimus was maintained (61.5±15.2 vs 50.3±12.3 years old).
Discussion: Our study showed that conversion to everolimus with CNI minimization in ABO-incompatible kidney transplantation recipients induced discontinuation of everolimus due to adverse events in 47.1% of the recipients enrolled in this study. However, everolimus elicited no acute rejection and suppressed B cell function and activation even though everolimus was maintained or stopped within 1 year after conversion. Furthermore, late conversion to everolimus and CNI minimization in elderly ABO-incompatible kidney transplant recipients may be associated with more frequent adverse events and discontinuations.

P.1264

Acute cellular rejection in ABO-incompatible kidney transplant recipients receiving rituximab is associated with late-onset neutropenia

Junjji Uchida[1], Tomoaki Iwai[1], Nobuyuki Kuwabara[1], Shunji Nishide[1], Kazuya Kabei[1], Takeshi Yamasaki[1], Toshihide Naganuma[1], Norihiko Kumada[2], Yoshiaki Takemoto[1], Tatsuya Nakatani[1].
[1]Urology, Osaka City University Graduate School of Medicine, Osaka, Japan; [2]Urology, Suita Municipal Hospital, Suita, Japan.

Background: Due to the severe shortage of deceased donors in Japan, ABO-incompatible kidney transplantation accounts for up to 30% of living kidney transplantation in Japan. A new avenue in the management of ABO-incompatible kidney transplantation has been demonstrated by the introduction of rituximab. Rituximab induces long-lasting B cell depletion in the peripheral blood, and it has been suggested that proinflammatory cytokine release associated with regulatory B cell depletion might prime antigen-presenting cells. However, patients receiving rituximab were reported to have a rate of acute cellular rejection (ACR) that was higher than the rate previously observed among patients who had not received induction therapy. Moreover, a recent study showed a marked elevation in the levels of serum B cell activating factor, a B cell-related cytokine, in ABO-incompatible kidney transplant recipients with late-onset neutropenia (LON). This study was conducted to investigate the relationship between ACR and LON in ABO-incompatible kidney transplant recipients receiving rituximab.
Patients and Methods: Between June 2006 and July 2015, 47 patients with end-stage renal disease underwent ABO-incompatible transplantation with rituximab induction at Osaka City University Hospital. All 47 patients who received rituximab underwent successful transplantation with a median current serum creatinine of 1.17 mg/dl (0.52-3.21 mg/dl), and patient and graft survival rates were 100%. Rejection episodes were confirmed by histological diagnosis. LON was defined as grade III to IV neutropenia occurring at least 4 weeks after the last administration of rituximab in the absence of any alternative reason to explain its occurrence.
Results: Fourteen recipients (48%) experienced ACR (ACR(+) group), while 33 patients did not develop ACR (ACR(-) group). LON developed in 19 out of the 47 patients (40.4%) receiving rituximab. Nine of the 14 patients (64.3%) who developed ACR had LON, as compared with 10 of the 33 patients (30.3%) who did not develop ACR. The frequency of LON was higher in the ACR (+) group than in the ACR (-) group (p=0.0657). Multivariate logistic regression analysis revealed that the frequency of ACR correlated significantly with the prevalence of LON.
Discussion: These results indicated that ACR in ABO-incompatible kidney transplant recipients receiving rituximab was associated with LON, and that B cell-related cytokines may play a role in the development of ACR and/or LON in these recipients.

P.1265

Impact of low BMI on renal graft outcome

Nusrat Akber[1], Kehkashan Hossein[1], Fanda Ahmed[1], Rubina Naqvi[2], Anwar Naqvi[3], Tahir Aziz[2].
[1]Nutrition, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [2]Nephrology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [3]Urology and Transplantation, Sindh Institute of Urology and Transplantation, Karachi, Pakistan.

Background: Generally, patients with ESKD on maintenance hemodialysis have some degree of malnutrition. Various studies have shown adverse impact of pre and post transplant obesity on post transplant graft outcomes however few studies have related low BMI with poor graft outcomes. None of the studies have related severity of thinness.
The aim of this study is to observe the prevalence of low BMI in renal transplant recipients and its effect on graft outcome.
Methods: We retrospectively analyzed graft outcome of 815 recipients transplanted between May 2002-Jun 2008 with follow up time of 7-13yrs. From the available pre-transplant data BMI was calculated. WHO international classification was used to categorize BMI. Missing records, pediatrics, cadaver, second transplant recipients and recipients with immediate graft failure were excluded. Recipients were followed from transplant date till graft loss, death or the study end date. Data was analyzed using SPSS version 22.
Results: The mean age of the total population was 31.0 ± 8.6 years (78% male, 21% female). Mean BMI was 19.66 with min and max BMI of 12.30 - 34.34 kg/m2. According to categories of BMI <16.00, 16.00-16.99, 17.00-18.49, 18.50-22.99 and ≥23.00 the percentages were 9.1, 9.6, 21.5, 46.6 and 13.2 respectively. In BMI <18.5, 18.5 – 22.9 and ≥23, the percentage graft loss 35, 36 and 34 respectively was not statistically significant.
64.8% recipients were alive with functioning graft, 19.8% had graft rejection, and 15.4% died. No significant relationship was found between low BMI and graft survival. Similar results were found for patient survival.
Conclusion: Our findings do not suggest a negative impact of low BMI on graft outcome. Whether severe thinness is appropriate or not at time of renal transplant needs further investigation.

P.1266

Dialysis vintage: A dominating factor affecting growth and final height after pediatric renal Transplantation: A single center experience

Sabeen Siddiqui[1], Kehkashan Hossein[1], Tabassum Zehra[1], Tayyaba Quadri[1], Anwar Naqvi[2], Tahir Aziz[2].
[1]Nutrition, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [2]Urology and Transplantation, Sindh Institute of Urology and Transplantation, Karachi, Pakistan.

Introduction: Growth retardation is a common occurrence in children with chronic kidney disease, and majority of the children have a short stature at the time of transplant. For many children catch-up growth after transplant remains sub optimal.
Our aim was to observe change in height from time of transplant to final adult height (FAH) and the effect of dialysis duration on growth and FAH
Methodology: Height change and duration of dialysis of 454 RTX children transplanted between Feb 1986 to Feb 2014 were analyzed retrospectively. Children aged less than 17years at time of transplant with st RTX growth was analyzed by height Z score at RTX, till date or when the child achieved FAH (age=19 years). SPSS22 version was used for statistical analysis.
Result: We analyzed data of 344 children with male female ratio 2:1. The mean duration of dialysis prior to regular follow up for one year were included while recipients with incomplete data were excluded. Po RTX was 10.29±15.34 years. Mean baseline age at the time of RTX was 13.53±2.6 years with Ht_Z -2.33±1.44. The mean change in Ht_Z despite improvement at 1st yr, 3rd yr and 5th yr did not achieve statistical significance (0.11±0.60, 0.08±1.42 and -0.01±0.41 respectively). Children < 12 years showed a significant increase in Ht_Z in first 4 yrs of transplant (P<0.001, 0.01, 0.005, 0.001) but at 2ndyr only in children >12 years (P<0.01). Mean FAH was -2.37±1.29 and showed positive association with HDX duration (P<0.01).
Conclusion: Our pediatric population showed an increase in height post RTX but height deficit did not show significant change. Dialysis duration is an important factor affecting growth and FAH post RTX.

P.1267

Post transplant infections: A single centre experience

Tahir Aziz[1], S Adibul Hasan Rizvi[2].
[1]Nephrology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [2]Urology and Transplantation, Sindh Institute of Urology and Transplantation, Karachi, Pakistan.

Introduction: Infections are one of the major causes of morbidity and mortality in the developing countries. Managing infections in transplant recipients is a challenge. Diagnosis may get confounded by atypical presentations; also treatment regimens may be complicated by drug-drug interaction and a need to maintain immunosuppression.
Methods: It was a retrospective study done at SIUT over 26 years post transplant follow-up period. We included 4000 live related transplants from 1985 to 2013.
Results: At SIUT, the cause of death in the first year post-transplant is infections in 70% of the patients. In a follow-up of 26 years, infections as the cause of graft loss are seen in 28% of the patients. Graft pyelonephritis is the most common infection with recurrent pyelonephritis a major problem. Pneumonias are the second most common infections in which 14% of the patients had Streptococcus pneumoniae. Among viral infections, Cytomegalovirus infections (60%) are most common. We see fungal skin and soft tissue infections more than invasive fungal infections. After INH prophylaxis, the onset of tuberculosis seems to get delayed and there is no significant change in the pattern of INH resistance. We also face infections like rhodococcus pneumonia, dematiaceous fungal brain abscess, adenovirus pneumonia and malakoplakia which are challenging and difficult to treat.
Conclusion: SIUT has a rich experience in diagnosing and managing infections in renal transplant recipients. With a robust team work of nephrologists, infectious diseases experts, microbiologists and urologists we work hard and face the challenge of infections head on.

P.1268

The safety using intraoperative ureteric double J stent for prevention of urological complications in kidney transplant recipients

Nam Kyu Choi.
Hepatobiliary & Transplantation, Chosun University Hospital, Gwangjo, Korea.

Objective: To evaluate the impact of the use of the double J stent in in kidney transplant recipients with or without ureteric double J stent. To examine the benefits and harms of routine ureteric double J stenting to prevent urological complications in kidney transplant recipients.
Materials and Methods: Between March 2011 and February 2012, 80 adult recipients underwent renal transplant at a single center and one surgeon. A retrospective study was conducted on two groups of patients: Group A undergo Double-J stent and group B no-stent ureterovesical anastomosis. We reviewed the urological complications: fistula, ureteral obstruction, urinary leakage and urinary tract infection. The patients were scheduled for stent removal after 2 weeks.
Results: A total of 80 patients were randomized to a stent (20) and a no-stent (60) group. Group A: No ureteral obstruction and urinary leakage were developed in this group. 3 patients (20%) had a positive urinary culture. Group B: Two patients (13%) developed ureteral obstruction and another four (26.7%) developed urinary leakage. Four patients (26.7%) had a positive urinary culture.
Conclusion: The routine insertion of a double J stent in kidney transplants reduces the number of early complications urinary fistula and ureteral obstructions.
Using a ureteral stent at renal transplantation significantly decreases the early urinary complications of urine leakage and obstruction. However, stent removal within 4 weeks of insertion appears advisable.

P.1269

ABO incompatible liver transplantation using plasma exchange and Anti-CD20 monoclonal antibody

Nam Kyu Choi.
Hepatobiliary & Transplantation, Chosun University Hospital, Gwangjo, Korea.

Background: ABO incompatible liver transplantation (ABOi LT) has been recommended only for emergency case because of the risk of antibody-mediated rejection (AMR). However, various managements for prevention of AMR have improved the complication rate and survival outcomes. Herein, we introduce our first ABOi LT case although the patient has been into nadir.
Methods: At June 2013, first ABOi LDLT was performed in our center and more two case was done. Our institution protocol for ABOi LDLT was planned using rituximab at preoperative 2 weeks, several plasma exchanges (PE, target level: isoagglutinin titer ≤1:8), basiliximab during operation and postoperative 4 day, and routine intravenous immunoglobulin (IVIG) at postoperative 1 to 5 days with splenectomy.
Results: The 53-yr old and 30-year & 43- year old male patients were admitted due to liver cirrhosis with viral hepatitis B (HBV). ABO blood type of the recipient was Rh+ O (O+). All patients received the right lobe of liver from a related young male donor with Rh+ B (B+) and Rh+ A (A+) blood type. One patient initial isoagglutinin titier were 1:32 and two patient initial isoagglutinin titier were 1:128. Recipient received rituximab at 2 weeks before LDLT, followed by total 6 times of PE. The recipient received the modified Rt lobe (MRL) transplantation with the splenectomy. First ABOi LDLT patient was a postoperative hemorrhage with coagulopathy and a re-exploration for hemostasis of it and other patients were no other complication.
Conclusions: The new ABOi-LDLT protocol using rituximab, PE, basiliximab, and IVIG is the pivotal and safe strategies. However, the frequent PE for predetermined low ABO titer level is considered to result in the peri- and post-operative hemorrhage following the coagulopathy in our first case.

P.1270

Age-dependent impact of acute rejection on graft survival in young and old kidney transplant recipients

Christian Denecke[1,2], Matthias Biebl[1,2], Josef Fritz[3], Tomasz Dziodzio[1,2], Felix Aigner[1,2], Claudia Bösmüller[1], Johann Pratschke[1,2], Robert Öllinger[1,2].
[1]Department of Visceral, Transplantation and Thoracic Surgery, Medical University Innsbruck, Innsbruck, Austria; [2]Department of General, Visceral and Transplantation Surgery, Charite Universitätsmedizin, Campus Virchow Clinic, Berlin, Germany; [3]Department for Medical Statistics, Informatics and Health Economics, Medical University Innsbruck, Innsbruck, Austria.

Background: The proportion of marginal grafts and elderly recipients awaiting kidney transplantation is ever increasing. The correlation of age with DGF and acute rejection determines longterm graft outcome.
Methods: Data from patients >65years (n=193) and patients <65years (n=1054) transplanted between 2000 and 2010, were retrospectively analyzed regarding the age-dependent impact of delayed graft function (DGF) and biopsy-proven acute rejection (BxAR).
Results: Overall 10 year survival was lower in patients >65 years ($p<0.0005$) while death-censored graft survival was comparable to patients <65 years. DGF rates, HLA-MM and retransplantation rates were higher in elderly patients. However, patterns of acute rejection were significantly different in patients >65 years; Boderline and Banff Ia rejections were more frequent while more severe BxAR were significantly decreased. Ordinal regression analysis showed that the risk of BxAR declines with increasing age ($p<0.0005$).
First, risk factors influencing graft survival were analyzed: donor age, DGF, BxAR and induction therapy impacted on graft outcome in young patients while donor age, DGF and induction therapy were risk factors in old patients. In this context, BxAR significantly impaired graft survival in recipients <65years but not in older patients ($p=0.055$). Interestingly, this correlation was independent of the type of graft (ECD/DCD vs SCD).
Next, age-dependent risk factors for BxAR were identified; DGF, anastomosis time (AT) and recipient age were independently associated with BxAR in young patients while only DGF was a risk factor in old patients. Further analysis showed that DGF had a significant impact on BxAR in elderly recipients of ECD but not SCD grafts, underlining the importance of DGF rates in "old-for-old" transplantation.
Conclusion: Our study demonstrated a significant, age-dependent correlation of DGF and BxAR with graft survival. Reduction of DGF rates may help to decrease BxAR particularly in younger patients, thereby improving graft survival.

P.1271

Outcomes of kidney transplantation for elderly aged more than 70 years

Akira Kurosawa, Takaaki Kimura, Toshihiro Shimizu, Atsushi Miki, Shigeru Nakamura, Koji Nanmoku, Yasunaru Sakuma, Takashi Yagisawa.
Surgical Branch, Institute of Kidney Diseases, Jichi Medical University Hospital, Tochigi, Japan.

Introduction and Aims: The number of elderly dialysis patients and kidney transplants has increased in Japan. We analyzed the clinical characteristics and outcomes of kidney transplants for aged 70 and older at the time of transplantation at a single center in Japan.
Methods: Of 255 adults who underwent kidney transplantation between April 2003 and October 2015 (235 living donor transplants and 20 deceased donor transplants), nine adults (3.5%) were older than 70 years. The backgrounds and outcomes of these transplants were retrospectively investigated.
Results: All cases were living donor transplants. The mean age at transplantation was 72.5 ± 2.13 (70-77). The cases consisted of 7 males and 2 females. Seven cases received the grafts from their spouses and 1 from her son and 1 from his daughter. One case was preemptive transplant and the mean duration of dialysis therapy was 34.6±29.1 (0-99.7) months. The follow-up period after transplantation was 630.4±746.0 (44-1962) days. All grafts have functioned well and the latest mean serum Cr level was 1.12±0.20mg/dL. Urination disorder appeared in 2 patients including 1 male with prostatic hypertrophy and 1 female with neurogenic bladder. In addition, pneumonia, lung cancer, and acute appendicitis were complicated in 1 patient each. These complications were treated with success conservatively or surgically. The post-transplant courses were favorable in all cases.
Conclusions: Kidney transplantation is an effective replacement therapy for the elderly, aged 70 and older, with end stage renal disease.

P.1272

Outcomes in kidney transplantation from older donors: A single center experience

Kyung Hee Lee, Min Young Seo, Kyu Yeun Kim, Minjung Kim, Wonseok Do, Youngae Yang, Taehoon Yim, Inryang Hwang, Sukyung Lee, Hee-Yeon Jung, Ji-Young Choi, Jang-Hee Cho, Sun-Hee Park, Yong-Lim Kim, Chan-Duck Kim.
Department of Internal Medicine, Kyungpook National University Hospital, Daegu, Korea.

Background: Kidney transplantation (KT) from older kidney donors has been considered as a solution of organ shortage. We performed detailed analyses about the clinical outcomes of KT from older kidney donors.
Methods: The present study was a retrospective cohort study including patients over 18 years who underwent KT between January 2000 and December 2013 at the Kyungpook National University Hospital. Donors and recipients were categorized by donor type and age. Death-censored graft survival, patient survival and estimated glomerular filtration rate (eGFR) were compared among the groups during the 5 year follow up.
Results: A total of 463 patients, 318 were living donors with 20 older than 60 years and 145 were deceased donor with 110 standard criteria donors (SCD) and 35 extended criteria donors (ECD). Recipients from living donors older than 60 years (90.0%) showed equivalent graft survival compared to recipients from SCD (90.0%), and higher graft survival than recipients from ECD (82.9%). However, the graft survival of recipients from older living donors was lower than that of recipients from younger living donors (91.6%). There was no significant difference in patient survival among the groups. Regardless of donor type, graft survival of older recipients than 60 years from older donors was not significantly different compared to the other patients. Older recipients from older donors revealed the lowest eGFR during the follow-up period.
Conclusions: KT from older living donors showed better or similar graft survival compared to deceased donor KT, although worse than KT from young living donors. Increased donor age could not affect the graft survival after KT. These findings support that older living donors might be an appropriate alternative for the patients waiting deceased donors.

References:

[1] Brian R. Englum. Outcomes in Kidney Transplant Recipients From. *Transplantation*. 2015; 99: 39-315
[2] Jagbir Gill. Outcomes of Kidney Transplantation From Older Living Donors. *American Journal of Kidney Diseases*. 2008; 52: 541-552
[3] Jonathan C. Berger. Living Kidney Donors Ages 70 and Older: Recipient and Donor Outcomes. *Clinical Journal of the American Society of Nephrology*. 2011; 6: 2887-2893
[4] Ruth Sapir-Pichhadze. Living donor age and kidney transplant outcomes:an assessment of risk across the age continuum. *Transplant International*. 2013; 26: 493-501
[5] K.D. Yoo. Clinical Outcomes in Asian Elderly Kidney Transplant Recipients: A Multicenter Cohort Study. *Transplantation Proceedings*. 2015; 47: 600-607
[6] Radomir Naumovic. Effect of donor age on the outcome of living-related kidney transplantation. *Transplant International*. 2005; 18: 1266-1274
[7] Paolo Ferrari. Effect of donor–recipient age difference on graft function and survival in live-donor kidney transplantation. *Nephrol Dial Transplant*. 2011; 26: 702-708
[8] Miklos Z. Molnar. Age and the Associations of Living Donor and Expanded Criteria Donor Kidneys With Kidney Transplant Outcomes. *Am J Kidney Dis*. 2012; 59: 841-848
[9] Takuzo Fujiwara. Old-for-Old Age Matching in Living Donor Kidney Transplantation: A Single-Center Experience. *J Transplant Technol Res* 2014: 4:1-3
[10] Y. Iordanousa. Recipient Outcomes for Expanded Criteria Living Kidney Donors: The Disconnect Between Current Evidence and Practice. *American Journal of Transplantation*. 2009; 9: 1558-1573

P.1273

The new Kidney Allocation System hits the 305

Adriana Dejman[1], Franco Cabeza[2], Aldo Torres[3], Jeffrey Gaynor[2], Analucia Schneegans[2], David Roth[2], Warren Kupin[2], George Burke[2], Gaetano Ciancio[2], Linda Chen[2], Adela Mattiazzi[2], Rodrigo Vianna[2], Giselle Guerra[2].
[1]Nephrology and Hypertension, University of Miami, Miami, United States; [2]Kidney Transplant, Miami Transplant Institute, Miami, United States; [3]Internal Medicine, Hospital San Jose Tec de Monterrey, Monterrey, Mexico.

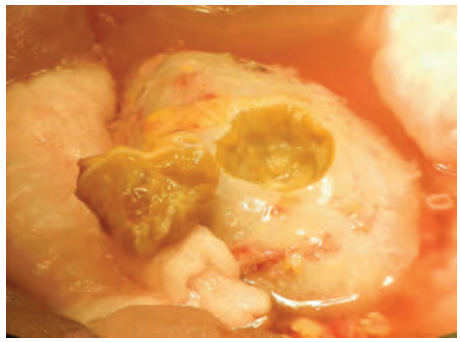
The new national Kidney Allocation System (KAS), effective 12/4/14, was designed to improve limitations of the prior system: higher discard rates of kidneys, inequity of access to transplants by blood type, sensitization level, geographic location and high rates of unrealized graft years and re-transplantation due to long potential longevity kidneys allocated to candidates with shorter potential longevity and vice versa. We evaluated the impact of the KAS in patient characteristics and 6-month clinical outcomes of deceased donor kidney transplants in our center.
We performed a retrospective chart review of patients transplanted from 12/2/2014 to 4/31/2015 (N=79).
Patient demographics: Mean recipient age was 52.6 years, with 48% in the 50-64 years range. African-Americans represented 70.9% (56/79) of the population followed by Hispanics at 17.7% (14/79). No significant gender difference noted. Pre-transplant diabetes was 25% (20/79). Patients with an Estimated Post Transplant Survival (EPTS) of <20% represented 19% (15/79), and Kidney Donor Profile Index (KDPI) <20% were used in 15.2% (12/79) of them. 6.3% (5/79) had a calculated panel of reactive antibodies of ≥99%. 67.1% (50/79) were locally procured kidneys and 32.9% (29/79) were imported. Cold Ischemia Time >24 hours was 53.2% (61/79). Mean time on dialysis was 120 months, with no preemptive transplantation. 40.5% of our patients were on dialysis for ≥120 months.
Clinical outcomes: Delayed graft function rate was 33% (26/79) and slow graft function 12.7%. 60% (47/79%) of patients required at least one hospitalization for rejection and infections with 19.0% (15/79) and 25.3% (20/79) respectively.
Based on this analysis KAS is achieving its goals. However, in our center, there is 3 times the increase of patients with prolonged dialysis compared to national rates, increase in imported kidneys and no preemptive transplantation occurred. This is reflected in higher DGF and re-hospitalization rates.

P.1275

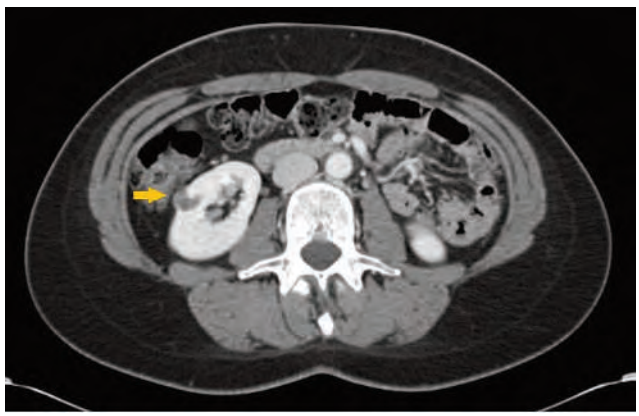
It is safe to transplant kidneys with renal masses

Mahmoud Alameddine[1], Zhobin Moghadamyeghaneh[1], George Burke[1], Rodrigo Vianna[1], Vincent Chia[1], Mohammed Osman[1], Mahmoud Morsi[1], Gaetano Ciancio[1], Linda Chen[1].
[1]Transplant Surgery, Miami Transplant Institute, Miami, United States.

Introduction: Transplantation of kidneys with suspicious renal masses is still debatable. We reviewed our cohort who received kidneys with cystic renal masses after ex-vivo partial nephrectomy.
Method: six patients were identified. All tumors were resected totally at the back-table preparation with negative margins.



The kidneys were obtained from living donors who were asymptomatic prior to surgery and were found incidentally to have renal masses during the preoperative work up.



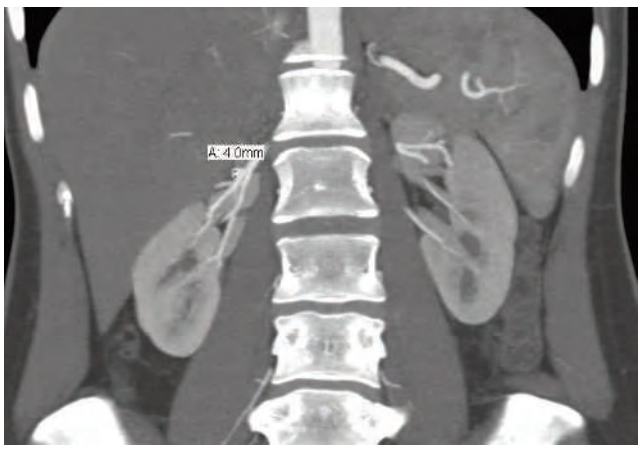
The donor surgery was laparoscopic except in one patient was open. Recipients and donors had follow up renal ultrasound (US) at 6 and 12 months, then every year.
Results: Mean tumor size was 11.7 mm (range 2.5 to 30). Histopathological characteristics were Renal Cell Carcinoma in four patients including Clear Cell type (n=2, Furhman grade I/II), Papillary (n=1, Furhman grade I) to Multilocular cystic RCC (n=1, Furhman grade 1) and the other two were Angiomyolipoma and simple renal cyst. All patients had immediate graft function with mean creatinine at discharge (2 mg/dL ± 0.5). One patient lost his kidney at 3 years after T-cell mediated rejection (Non-compliance). The other five patients maintained a stable allograft renal function (mean creatinine at three years 1.4 mg/dL ± 0.3). Recipients and donors had no tumor recurrence after 2 years of follow up.
Conclusion: Transplanting kidneys with small and incidentally discovered renal masses after complete resection is safe yet it needs to be discussed with both recipients and donors for long term follow up. Also, it can play an important method to expand the donor pool.

P.1276

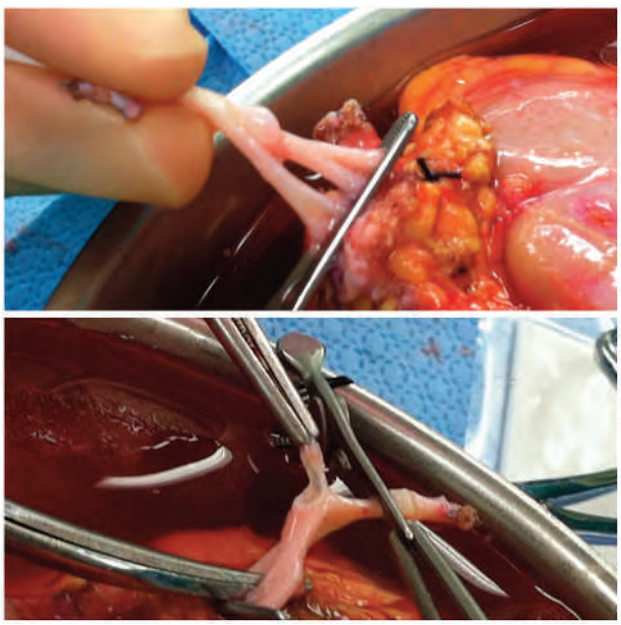
Transplantation of kidneys from donors with renal artery aneurysm

Mahmoud Alameddine, Zhobin Moghadamyeghaneh, Mahmoud Morsi, Mohammed Osman, Rodrigo Vianna, Vincent Chia, Linda Chen, Gaetano Ciancio.
Transplant Surgery, Miami Transplant Institute, Miami, United States.

Objective: Renal artery aneurysm (RAA) holds a potential risk for donors such as dissection and rupture. We report our experience in transplanting kidneys with RAA after back table reconstruction.
Method: Four patients had kidneys from living donors with RAA.



All aneurysms were repaired at the back table before transplantation. The repair was excision of the RAA with ostial closure and in one of them ovarian vein patch was used. We reviewed the safety and outcomes of this procedure. All donors were asymptomatic before the surgery and were diagnosed incidentally during the pre-transplant evaluation.



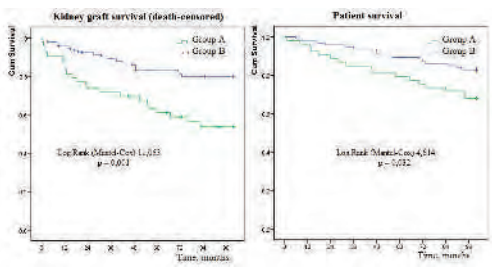
All donors underwent laparoscopic nephrectomy. All recipients had follow up ultrasound duplex (USD) of renal artery at 6 and 12 months then annually.
Results: Mean age of the recipients is 28.7 years (range from 3 to 45). Mean size of the aneurysm (maximal diameter is 6.3 mm ±3). All patients had immediate graft function with serum creatinine at discharge was (1.9 mg/dL ± 1.5). Average length of hospital stay was (6.25 days ± 2.6). Also, they maintained a good renal function (1 mg/dL ± 0.4) and patent vessels at one year. No deaths have been recorded. One patient had simultaneous benign renal cyst which was resected at the back table. Three kidneys were right side and one left. Mean cold ischemia time was (86 min ± 18).
Conclusion: Transplanting kidneys with RAA after ex-vivo repair is safe and the outcomes are encouraging. Also, it plays an important role in expanding the donor pool in the face of current organ shortage.

P.1277

Long-term outcomes after kidney transplantation in patients with the history of hemodialysis vascular access thromboses

Janis Jushinskis[1,2], Vadims Suhorukovs[1,2], Ieva Ziedina[1,2], Aleksandrs Malcevs[1,2], Inese Jurkane[2], Viktors Shevelevs[1], Rafail Rozental[1,2].
[1]Riga Stradins University, Transplant laboratory, Riga, Latvia;
[2]Latvian Transplantation Center, P. Stradins University Hospital, Riga, Latvia.

Introduction: Thrombosis of hemodialysis arterio-venous fistulas (AVF) are frequently associated with co-morbid conditions that may impact also post-transplant outcomes in those patients. The aim of this study was to analyse the rate of post-transplant complications and outcomes depending on the results of AVF surgery.
Materials and Methods: This study included all patients who passed AVF surgery and then deceased donor renal transplantations performed in a single centre between January 1, 2004 and December 31, 2007, and where patients were available for 8-year follow-up. All cases were divided into two groups according to AVF surgery outcomes: AVF thrombosis group (group A, n = 53) and group with primarily functioning AVF (control, group B, n = 94). Groups were compared for frequency of post-transplant complications and outcomes (surgical complications, development of delayed graft function, acute rejections, chronic graft dysfunction, graft losses and patient deaths).
Results and Discussion: AVF thrombosis was observed in 36% of cases. Analysis of association with demographical and clinical features showed association only with female gender (66% vs. 44% in group B, $p < 0.05$).
Analysis of post-transplant complications showed similar rate of vascular and urological complications in group A and B (18.9 vs. 18.1% and 5.7 vs. 7.4%, respectively, p = NS). Patients in group A had higher rate of chronic graft dysfunction (63,3 vs. 34.8%, $p < 0.05$) and relatively higher rates of delayed graft function and acute rejections (26.4 vs. 16.0% and 37.7 vs. 29.8%, respectively, p = NS), significantly higher rates of graft losses and patient deaths (43.4 vs 19.1%, and 32.1 vs. 17.0%, $p < 0.05$).
Kaplan-Meyer survival analysis showed that patients in group A had lower graft survival and patient survival ($p < 0.05$ for both) [Figure 1].
Analysis in groups revealed different factors associated with graft losses: in group A graft losses were associated with diabetes mellitus diagnosis (26.1 vs. 3.3%) and younger patient age (35.3 + 14.1 vs. 44.7 + 10.8 yy) ($p < 0.05$ for both); and in group B – with development of urological complications after transplantation (22.2 vs. 3.9%) and higher serum creatinine level at discharge after transplantation (162 + 109 vs. 118 + 27 mkmol/l) ($p < 0.05$ for both), as also relatively higher rate of graft losses in cases when there was need for kidney graft vascular reconstruction at "back-table" (83.3 vs. 60.8%, p = 0.060) and allocation "female donor – male recipient" (38.9 vs. 19.7%, p = 0.082).
Conclusion: Results show that pre-transplant complications associated with AVF surgery may indicate higher risk of development of complications also after kidney transplantation, including graft losses and patient deaths. These patients may need more prolonged anticoagulant therapy and coagulation control after transplantation, especially diabetic and young patients with history of multiple hemodialysis vascular access thromboses.



P.1278

Quantitative measurement of elasticity of the donor kidney using shear wave elastography to evaluate donor kidney status – pilot study

Sung Hoon Kim[2], Jae Won Yang[1], Jhii-Hyun Ahn[3], Jae Seok Kim[1], Byoung Geun Han[1], Seung Oak Choi[1].
[1]Departments of Internal Medicine, Yonsei University Wonju College of Medicine, Wonju Severance Christian Hospital, Wonju, Korea;
[2]Departments of Surgery, Yonsei University Wonju College of Medicine, Wonju Severance Christian Hospital, Wonju, Korea;
[3]Departments of Radiology, Yonsei University Wonju College of Medicine, Wonju Severance Christian Hospital, Wonju, Korea.

Backgrounds: The utilization of expanded criteria donors (ECD) has increased to overcome donor shortage. Unfortunately, the discard rate has also increased, especially in ECDs with acute kidney injury (AKI). There is no consensus about method to evaluate the donor kidney status. We measured the elasticity of the donor kidney and analyzed the transplantation outcomes.
Methods: We reviewed the medical records of patients who underwent living or cadaveric kidney transplantation. Single radiologist measured the elasticity of donor kidney by using shear wave elastography. We analyzed transplantation outcomes. We used AKI definition that was published by the Kidney Disease: Improving Global Outcomes group.
Results: 22 patients underwent kidney transplantation from living donor (n=6) or cadaveric donor (n=16). Five donors showed more grade 2 acute kidney injury. Six patients experienced delayed graft function (DGF) and all patients received the kidney from donor with grade 2 or 3 AKI. The median elasticity of living donor was 2kPa and cadaveric donor was 3.35kPa (p=0.005). Initial creatinine (Cr) and estimated glomerular filtration rate (eGFR) of donor were significantly low in living donor group. However, final Cr and eGFR of all recipients were similar. The median elasticity of recipient with DGF was 4.8kPa and recipient without DGF was 3.2kPa. However, it did not show statistical difference.
Conclusions: Many surgeons hesitate to encounter the donor with AKI because there is no consensus about criteria to discard or use the kidney in patients with AKI. Our results is pilot study and did not show statistical significance. However, if we will collect more large data, we think that the elasticity of kidney may be one tool to evaluate the kidney status and predict the outcomes of transplantation.

P.1279

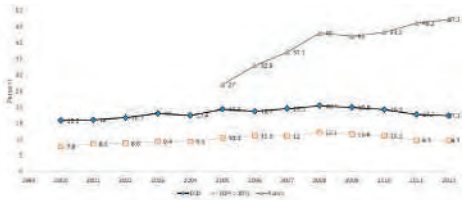
Kidney donor profile index trend analyses

Aparna Rege[1], Anthony Castleberry[2], Kadiyala Ravindra[1], Scott Sanoff[4], Uptal Patel[4], William Irish[3], Debra Sudan[1], Deepak Vikraman[1].
[1]Surgery, Div of Abdominal Transplant, Duke University Medical Center, Durham, United States; [2]Surgery, Duke University Medical Center, Durham, United States; [3]Health Outcomes Research & Biostatistics, CTI Clinical Trial and Consulting, Durham, United States; [4]Transplant Nephrology, Duke University Medical Center, Durham, United States.

Introduction: In spite of improved outcomes associated with the usage of extended criteria donor (ECD) kidneys, the number of kidney transplants (KT) using this donor population (DP) have declined over time. Kidney donor profile index (KDPI) is a numerical measure (0 to 100%) that combines ten dimensions of information about a donor, including clinical parameters and demographics, to express the quality of the donor kidneys relative to other donors. KDPI is now used for organ allocation with a higher KDPI value associated with lower donor quality. A Donor KDPI >85% (DK85) is thought to be equivalent to an ECD kidney.
Objective: Analyze the trends in usage and outcomes of KT associated with the KDPI.
Methods: All adult recipients (>18) with deceased donor KTs between 2000 and 2012 were included in the study. The study period was chosen to reflect changes in allocation policy- ECD introduction in 2002 and CMS program evaluation started in 2007. We retrospectively calculated the KDPI for this DP. The trend analysis of the kidney transplant recipients based on KDPI was then carried out.
Results: 92,935 KTs were performed during the study period. DK85 had increased incidence of diabetes, hypertension, higher BMI and DCD status.

	2000-2002		2003-2006		2007-2012	
Recipient Characteristics	KDPI ≤ 85%	KDPI > 85%	KDPI ≤ 85%	KDPI>85%	KDPI ≤ 85%	KDPI > 85%
Mean Age (SD)	49.0 (12.8)	55.6 (12.3)	50.8 (12.9)	59.3 (10.6)	52.5 (12.9)	60.9 (10.3)
% Age >60	20.1	39.3	24.7	50.4	30.0	56.9
% Female Gender	39.3	41.1	38.5	36.8	38.9	35.8
% Black	30.5	35.6	31.5	36.5	34.7	37.9
% Diabetes	39.0	34.3	32.9	42.2	37.2	47.8
Mean BMI (SD) kg/m^2	27.0 (5.4)	26.9 (5.2)	27.6 (5.4)	27.4 (5.1)	28.4 (5.5)	28.1 (5.1)
% BMI ≥35 kg/m^2	8.1	7.0	9.7	8.0	12.9	10.3

Even though the KDPI was relatively stable from 2000-2012, the % of delayed graft function was lower probably representing improved donor and recipient selection. The % of kidneys with machine perfusion has increased with time. Induction with lymphocyte depleting agents increased from 23.5% to 63.4%, with decreasing utilization of IL2 receptor antagonists (38.3% to23.6%) and corticosteroids (89% to 62.7%). The proportion of KDPI >85% transplants increased from 7.8 percent age in 2000 to 12.1% in 2008 and declined thereafter to 9.7% in 2012.



The 5 year graft survival also improved from for 49.8% to 56.1% over the study period.
Conclusions: Although kidney transplant outcomes with DK85 have improved over time, utilization of DK85 shows a downward trend. Further studies will help to assess the impact of improved organ selection (by kidney biopsy findings and machine perfusion) and increased use of lymphocyte depletion agents.

P.1280

Is Weight Gain a Barrier to Transplant Listing for Peritoneal Dialysis Patients?

Denise Fracchia, Sarah Terril, Heather Jordan.
Nephrology, Western Health, Melbourne, Australia.

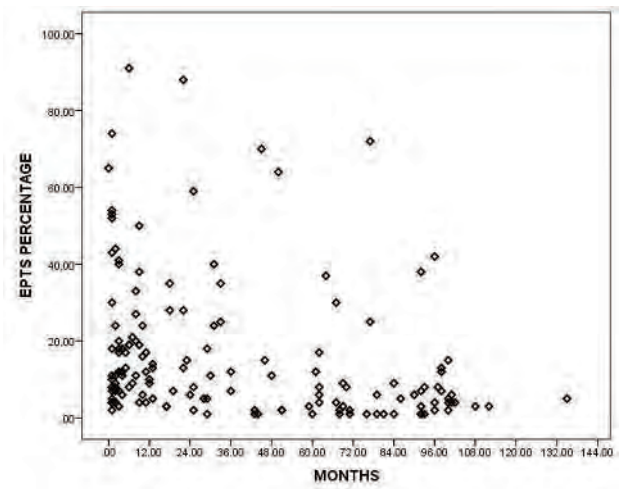
Background: Renal transplantation is the optimal form of renal replacement therapy for those who are deemed suitable[1] Peritoneal dialysis is thought to be the best form of treatment to maintain residual renal function[2],[3] However, one consistent side effect is weight gain through absorption of glucose in the dialysate[4],[5] Obesity often prevents patients access to renal transplantation because of poor post-transplant outcomes[6]
Aim: To review files from our peritoneal dialysis patient cohort who are deemed suitable for renal transplantation or listed for deceased donor kidney transplantation, and to evaluate degree of weight gain since commencement of dialysis We also plan to investigate social, medical, biochemical and nutritional parameters in the file review
Results: In an initial review we discovered that 68% of our patients had increased body weight within twelve months of commencing peritoneal dialysis
Conclusion: This result is representative of other studies that show weight gain with peritoneal dialysis. We need to closely monitor the degree of weight gain in this cohort of patients to assist in preventing access to renal transplantation.
References:
[1] Tonelli, M et al. American Journal of Transplantation. 2011; 11(10): 2093 -2109
[2] Teruel-Briones, JL et al. Nefrologia. 2013. Volume 33(5): 640 – 649
[3] Wang, AY-M & Ly, K-N. Kidney International. 2006. 69: pp 1726 – 1732
[4] Jolly, S et al. Journal of Artificial Organs. 2001. April 24(4) pp 197 -202
[5] Kim, J-Y et al. PLoS ONE 10(9): e0139033. doi: 10.1371/journal.pone.0139033
[6] Gill, JS et al. American Journal of Transplantation. 2013. 13: pp 2083 - 2090

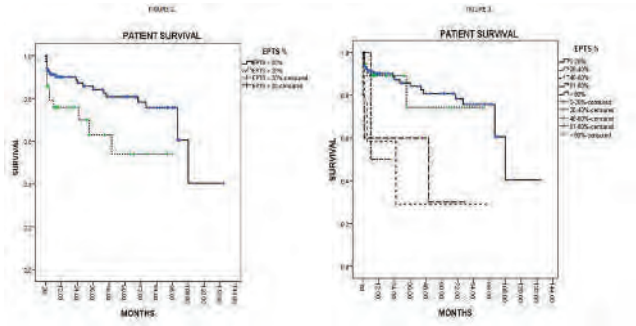
P.1281

Validation In Veracruz Mexico of the estimated posttransplant survival score for allocation of deceased donor kidney in the United States

Gustavo Martinez-Mier[1], Ernesto Soto-Miranda[1], Marco Mendez-Lopez[1], Luis Budar-Fernandez[1], Sandro Avila-Pardo[2], Edgar Aguilar-Sandoval[1], Stefan Zilli-Hernandez[3], Francisco Lajud-Barquin[3].
[1]Organ Transplantation, IMSS - Adolfo Ruiz Cortines, Veracruz, Mexico; [2]Organ Transplantation, HAEV - Virgilio Uribe, Veracruz, Mexico; [3]Research, HAEV - Virgilio Uribe, Veracruz, Mexico.

Background: The US kidney allocation system adopted in 2013 allocates the best 20% of deceased donor kidneys (based on kidney donor profile index/kidney donor risk index) to the 20% of the waiting list patients with the highest estimated posttransplant survival (EPTS)[1-4]. Validation of EPTS outside US is scant. We examined EPTS using data from our local transplant registry.
Methods: Retrospective study of deceased donor kidney transplant recipients in two transplant centers in Veracruz Mexico. EPTS was calculated at www.optn.transplant.hrsa.gov/resources/allocation-calculators/epts-calculator.com. Spearman correlation was calculated between patient survival (months) and EPTS percentage. Kaplan-Meier (log rank) and Cox regression patient survival were compared between EPTS <20% and EPTS 20% increments. P < 0.05 was considered statistically significant. SPSS version 21 was utilized.
Results: We included 152 adult deceased donor kidney transplant from 2003 to October 2015. Mean age was 35.16 ± 10.98 years, 48.7% were male and 51.3% female, 4.6% were diabetic; mean years on dialysis was 5.29 ± 3.92 and 3.9% (n = 6) has a previous transplant. Mean EPTS was 16.09 ± 18.37 % (1-91%, range). Spearman coefficient correlation was -0.365 (p=0.0001) (figure 1) Kaplan-Meier mean patient survival time in patients with EPTS < 20% was 98.5 ± 6.8 months (85.1-112.02 95% CI) against patients with EPTS > 20% (60.6 ± 8.5 months (43.8-77.3 95% CI) (p=0.014 by log rank) (Figure 2). For every 20% increase of EPTS patient survival had statistically significant decrease (p=0.004) (Figure 3) (Table 1). Cox regression for EPTS < 20% was 0.926 (0.225-3.813 95%CI) (p=0.9) and EPTS 20% increments was 1.667 (0.961-2.893 95%CI) (p=0.06).
Conclusion: There was a negative correlation between EPTS percentage and patient survival. Patients with EPTS > 20% had worse survival after kidney transplantation in our population.





		Odds Ratio (95%CI)	
EPTS (%)	Mean±STD	Lower Limit	Upper Limit
0-20	98.567±6.867	85.108	112.025
20-40	73.483±9.886	54.105	92.860
40-60	36.097±18.635	0.000	72.623
60-80	38.300±14.944	9.009	67.591
> 80	14.000±5.657	2.913	25.087

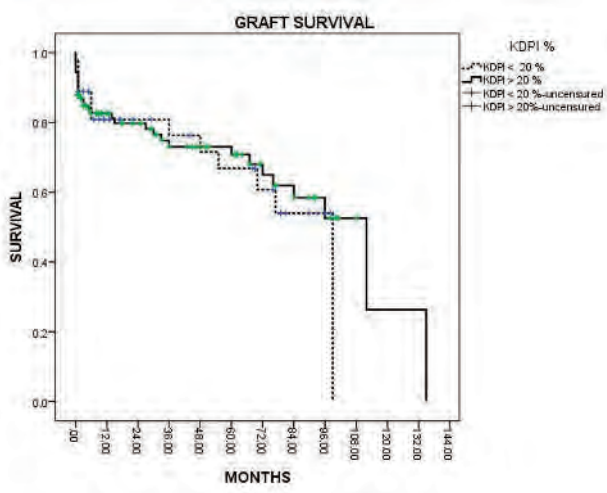
References:
[1] Clayton PA, McDonald SP, Snyder JJ, Salkowski N, Chadban SJ. External validation of the estimated posttransplant survival score for allocation of deceased donor kidneys in the United States. Am J Transplant. 2014 Aug;14(8):1922-6. doi: 10.1111/ajt.12761. Epub 2014 Jun 5.
[2] Ramanathan R, Gupta G, Kim J, Quinn K, Behnke M, Kang L, Sharma A. Retroactive application of the new kidney allocation system to renal transplants performed in the ECD/SCD era. Clin Transplant. 2015 Oct 5. doi: 10.1111/ctr.12642.
[3] www.optn.transplant.hrsa.gov/resources/allocation-calculators/epts-calculator.com.
[4] Schold JD, Buccini LD, Reese PP, Poggio ED, Goldfarb DA. Effect of dialysis initiation for preemptively listed candidates in the revised kidney allocation policy. Am J Transplant. 2014 Dec;14(12):2855-60. doi: 10.1111/ajt.12957. Epub 2014 Oct 2

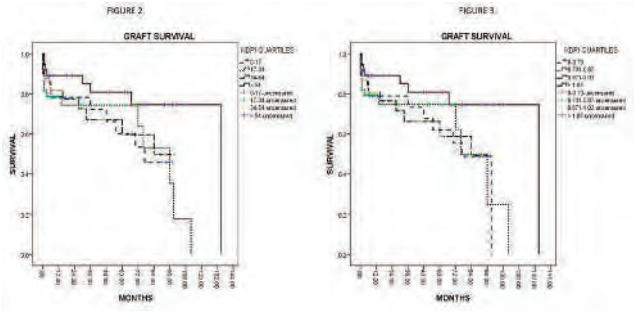
P.1282

Validation in Veracruz Mexico of kidney donor profile index for allocation of deceased donor kidney in the United States

Gustavo Martinez-Mier[1], Ernesto Soto-Miranda[1], Marco Mendez-Lopez[1], Luis Budar-Fernandez[1], Sandro Avila-Pardo[2], Edgar Aguilar-Sandoval[1], Stefan Zilli-Hernandez[3], Francisco Lajud-Barquin[3].
[1]Organ Transplantation, IMSS - Adolfo Ruiz Cortines, Veracruz, Mexico; [2]Organ Transplantation, HAEV - Virgilio Uribe, Veracruz, Mexico; [3]Research, IMSS - Adolfo Ruiz Cortines, Veracruz, Mexico.

Background: The US kidney allocation system adopted in 2013 allocates the best 20% of deceased donor kidneys (based on kidney donor profile index/kidney donor risk index) (KDPI/KDRI) to the 20% of the waiting list patients with the highest estimated post transplant survival[1]-[3]. Validation of KDPI/KDRI outside US is scant. We examined KDPI/KDRI using data from our local transplant registry.
Methods: Retrospective study of deceased donor kidney transplant recipients in two transplant centers in Veracruz Mexico. KDPI/KDRI was calculated at www.optn.transplant.hrsa.gov/resources/allocation-calculators/epts-calculator.com[4]. Spearman correlation was calculated between graft survival (months) and KDPI/KDRI percentage. Kaplan-Meier (log rank) graft survival were compared between KDPI/KDRI quartiles. $P < 0.05$ was considered statistically significant. SPSS version 21 was utilized.
Results: We included 152 adult deceased donor kidney transplant from 2003 to October 2015. Mean age was 35.16 ± 10.98 years, 48.7% were male and 51.3% female, 4.6% were diabetic; mean years on dialysis was 5.29 ± 3.92 and 3.9% (n = 6) has a previous transplant. Mean KDPI was 37.3 ± 23.2 % (2-94%, range). Mean KDRI was 0.9 ± 0.23 % (0.59-1.72%, range). Spearman coefficient correlation between KDPI and graft survival was 0.112 (p=0.171). Spearman coefficient correlation between KDRI and graft survival was 0.112 (p=0.168). Kaplan-Meier mean uncensored graft survival time in patients with KDPI < 20% was 69.9 ± 6.7 months (56.6-83.2 95% CI) against patients with KDPI > 20% (83.9 ± 7.3 months (69.5-98.3 95% CI) (p=0.713 by log rank) (Figure 1). There was no statistical significant differences in uncensored graft survival between KDPI quartiles (0-17; 17-34; 34-54 and > 54) (p=0.172) (Figure 2). There was no statistical significant differences in uncensored graft survival between KDRI quartiles (0-0.73; 0.73-0.87; 0.87-1.03 and > 1.03) (p=0.177) (Figure 3).
Conclusion: There was no correlation between KDPI/KDRI and patient survival in our population. Other factors following transplantation might be consider influencing our graft survival.





References:
[1] Rao PS, Schaubel DE, Guidinger MK, Andreoni KA, Wolfe RA, Merion RM, Port FK, Sung RS. A comprehensive risk quantification score for deceased donor kidneys: the kidney donor risk index. Transplantation. 2009 Jul 27;88(2):231-6. doi: 10.1097/TP.0b013e3181ac620b.
[2] Ramanathan R, Gupta G, Kim J, Quinn K, Behnke M, Kang L, Sharma A. Retroactive application of the new kidney allocation system to renal transplants performed in the ECD/SCD era. Clin Transplant. 2015 Oct 5. doi: 10.1111/ctr.12642.
[3] Massie AB, Luo X, Chow EK, Alejo JL, Desai NM, Segev DL. Survival benefit of primary deceased donor transplantation with high-KDPI kidneys. Am J Transplant. 2014 Oct;14(10):2310-6. doi: 10.1111/ajt.12830. Epub 2014 Aug 19. Erratum in: Am J Transplant. 2015 Jul;15(7):2013.
[4] www.optn.transplant.hrsa.gov/resources/allocation-calculators/epts-calculator.com.

P.1283

Successful short-term kidney transplantation outcomes after desensitization with plasmapheresis, intravenous immunoglobulin and rituximab in highly sensitized living donor kidney transplant patients: Mexican single-center experience

Gustavo Martinez-Mier[1], Ernesto Soto-Miranda[1], Marco T.Méndez-López[1], Luis F.Budar-Fernández[1], Stefan Zilli-Hernández[2], Lhisty M.Vázquez-Ramírez [2].
[1]Organ Transplantation, Instituto Mexicano del Seguro Social (UMAE) 189 - Adolfo Ruiz Cortines, Veracruz, Mexico; [2]Research, Instituto Mexicano del Seguro Social (UMAE) 189 - Adolfo Ruiz Cortines, Veracruz, Mexico.

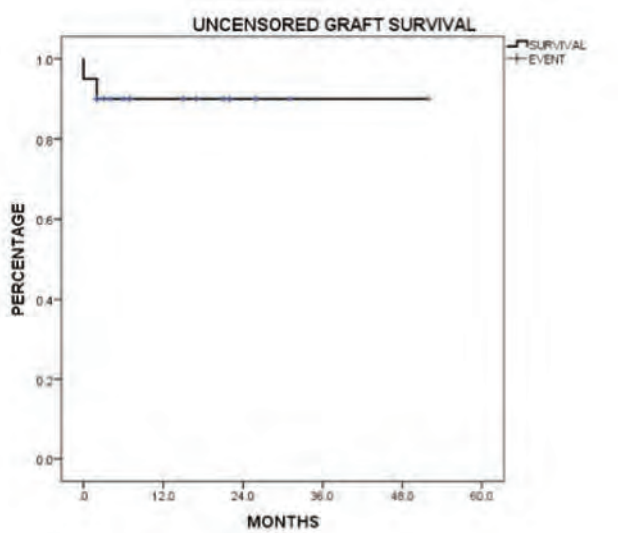
Background: Highly sensitized kidney transplant candidates have few transplant opportunities. In order to increase their chances, desensitization protocols have been implemented to allow kidney transplant in positive cross-match/high sensitized transplant candidates. Herein, we describe our living donor kidney transplant experience in highly sensitized patients[1]-[4].
Methods: Eleven highly sensitized living kidney transplant patients between September 2012 and October 2015 were analyzed. All patients underwent desensitization using pre-transplant plasmapheresis (three cycles as minimum), (PP) and low-dose intravenous immunoglobulin (IVIG; 100 mg/kg) with rituximab (five patients). Immunosuppression consisted of thymoglobulin, tacrolimus, mycophenolate mofetil and steroids. Demographics, immunologic characteristics of patients, acute rejection (AR) episodes, survival, allograft function and adverse events were evaluated.
Results: Eleven patient were included (9 female), mean age 32 ± 6.9 years, mean weight 58.9 ± 18 kg and 84.6 ± 63.7 months on dialysis. Four patients were re-transplant (1 third transplant), mean HLA match was 2.7 ± 1.9 and mean PRA class I was 51.3 ± 29.4% and class II 58.2 ± 34.3% respectively. Three had one positive cross-match before desensitization. Mean Rituximab dose was 204.5 ± 245.4 mg and thymoglobulin dose 4.5 ± 0.78 mg/kg. AR rejection rate was 18.1%, Patient and graft survival are 100% with 19.2 ± 14.2 survival months. Glomerular filtration rate a 6 and 12 months was 75.2 ± 22.2 and 61 ± 20.3 ml/min/1.73m2BSA respectively. CMV infection rate was 18.1% (n=2). Other infective episodes were 1.6 ± 0.9 events (n=18) UTI´s mostly (88.8%). No neoplasia have occurred during follow-up.
Conclusion: Good short-term kidney transplant outcomes can be achieved with PP/IVIG with or without rituximab as desensitization regimen for positive cross-match and/or highly sensitized living donor renal transplant patients. Longer follow is warranted to evaluate further efficacy and safety of this protocol.
References:
[1] Mattiazzi AD, Centeno A, Amador A, Fernandez-Bango C, Scowby CD, O'Rourke M, Hill-Matthie T, Patel R, Barrios F, Ruiz P, Chen L, Saghesima J, Ciancio G, Burke GW, Goldstein M, Chandar J, Contreras G, Roth D, Kupin W, Guerra G, Vianna R. Highly Sensitized Patients: Miami Transplant Institute Experience. Clin Transpl. 2014: 171-8.
[2] Yuan XP1, Wang CX, Gao W, Fu Q, He XS. Kidney transplant in highly sensitized patients after desensitization with plasmapheresis and low-dose intravenous immunoglobulin. Clin Transpl. 2010 Jun;8(2): 130-5.
[3] Jin MK1, Cho JH, Kwon O, Hong KD, Choi JY, Yoon SH, Park SH, Kim YL, Kim CD. Successful kidney transplantation after desensitization using plasmapheresis, low-dose intravenous immunoglobulin, and rituximab in highly sensitized patients: a single-center experience. Transplant Proc. 2012 Jan;44(1):200-3.
[4] Stegall MD1, Gloor J, Winters JL, Moore SB, Degoey S. A comparison of plasmapheresis versus high-dose IVIG desensitization in renal allograft recipients with high levels of donor specific alloantibody. Am J Transplant. 2006 Feb;6(2):346-51

P.1284

Thymoglobulin induction in living donor renal transplant recipients: Southern Mexican center experience

Gustavo Martinez-Mier[1], Ernesto Soto-Miranda[1], Marco T.Méndez-López[1], Luis Budar-Fernández[1], Edgar Aguilar-Sandoval[1], Stefan Zilli-Hernández[2], Francsico A.Lajud-Barquin[2], Lhisty Vázquez-Ramírez[2].
[1]Organ Transplantation, Instituto Mexicano del Seguro Social (UMAE) 189 - Adolfo Ruiz Cortines, Veracruz, Mexico; [2]Research, Instituto Mexicano del Seguro Social (UMAE) 189 - Adolfo Ruiz Cortines, Veracruz, Mexico.

Background: Thymoglobulin antibody immunosuppression is associated with improved outcomes following renal transplantation. There is limited clinical experience in thymoglobulin induction in living donor kidney transplantation in Mexican population. Herein, we describe our results[1]-[3].
Methods: Twenty living donor kidney transplant recipients between 09/12 and 1215 were analyzed. Demographics, immunologic characteristics of patients, acute rejection (AR) episodes, allograft function, adverse events patient and graft survival were evaluated. P < 0.05 was considered statistically significant
Results: Fourteen (70%) female receptor were included, mean age 60 ± 34.6 years, mean BMI 24.8 ± 4.7 kg/m2 and 59.5 ± 56.2 months on dialysis. Five (25%) patients were re-transplant (1 third transplant), mean HLA match was 2.6 ± 1.9 matches. Mean PRA class I was 37.3 ± 32.6% and class II 40.6 ± 36.4% respectively. Fourteen (70%) underwent desensitization treatment (IVIG/plasmapheresis/rituximab). Mean donor age was 34.8 ± 10 years and mean GRF was 112.6 ± 13.6 ml/min/1.73m2BSA. Mean thymoglobulin dose 4.7 ± 0.62 mg/kg (total dose 265 ± 33.2 mg). AR rejection rate was 20%. One and five-year patient and graft survival was 90% with 46.9 ± 3.4 survival months (40.1-53.6 95%CI). Glomerular filtration rate a 6 and 12 months was 70.8 ± 22.5 and 58.2 ± 20.3 ml/min/1.73m2BSA respectively. CMV infection rate was 10% (n=2). There was 23 other infective episodes, UTI´s mostly (86.9%, n=20). No neoplasia have occurred during follow-up.
Conclusion: Good kidney transplant outcomes can be achieved using thymoglobulin induction in living donor kidney transplant patients. Longer follow-up is warranted to evaluate further efficacy and safety outcomes.



References:
[1] A. Osama Gaber, Arthur J. Matas, Mitchell L. Henry, Daniel C. Brennan, R. Brian Stevens, Sandip Kapurm Jillian N. Ilsley, Kristen D. Kistler, A. Benedict Cosimi. Antithymocite Globulin induction in living donor renal transplant recipients: Final report of the TAILOR registry. Transplantation. Volume 94, Number 4, August 27, 2012.
[2] Brennan DC, Flavin K, Lowell JA, et al. A randomized, double-blinded comparison of Thymoglobulin versus Atgam for induction immu- nosuppressive therapy in adult renal transplant recipients. Trans- plantation 1999; 67: 1011.
[3] Woodle E, Peddi V, Tomlanovich S, et al. A prospective, randomized, multicenter study evaluating early corticosteroid withdrawal with Thymoglobulin in living-donor kidney transplantation. Clin Trans- plant 2010; 24: 73.

P.1285

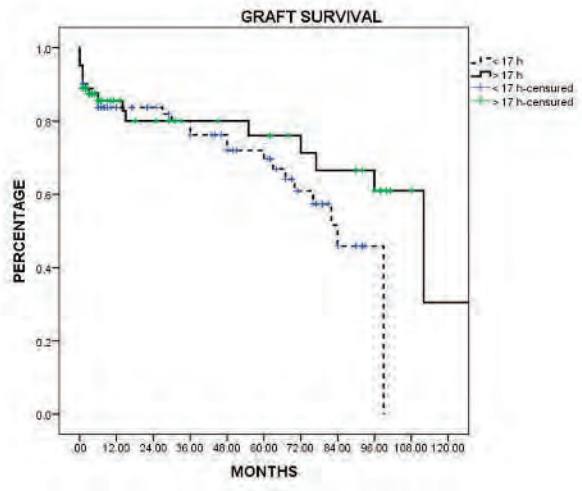
Impact of brain death duration on kidney transplant outcomes in Veracruz, Mexico

Gustavo Martinez-Mier[1], Ernesto Soto-Miranda[1], Marco T.Méndez-López[1], Luis Budar-Fernández[1], Andrés Gómez-Díaz [2], Antonio Rodríguez-Fernández[3], Stefan Zilli-Hernández[4], Francsico A.Lajud-Barquin[4].
[1]Organ Transplantation, Instituto Mexicano del Seguro Social (UMAE) 189 - Adolfo Ruiz Cortines, Veracruz, Mexico; [2]Organ/Tissue Procurement, Instituto Mexicano del Seguro Social (UMAE) 189 - Adolfo Ruiz Cortines, Veracruz, Mexico; [3]Organ/Tissue Procurement, Hospital de Alta Especialidad de Veracruz – Virgilio Uribe, Veracruz, Mexico; [4]Research, Instituto Mexicano del Seguro Social (UMAE) 189 - Adolfo Ruiz Cortines, Veracruz, Mexico.

Background: In kidney transplantation, deceased donor graft survival is lower than living donors. However, little is known about the effect of the duration of brain death (BDdur) on outcome after kidney transplantation[1]-[3].
Methods: Organ procurement and transplant network retrospective analysis using kidney donor and recipient data from 2003 to 2015. BDdur was calculated as the period between brain death declaration and aortic cross clamp. Effects of BDdur on delayed graft function (DGF), acute rejection (ARE), hospitalizations during first transplant year and graft survival were calculated using T-student test and Kaplan-Meier. $P < 0.05$ was considered statistically significant.
Results: 163 adult deceased donor kidney patients were included. Donor and recipient demographic characteristics are displayed in table 1. Mean BDdur was 16.57 ± 8.48 hours. There was no difference in BDdur hours in patients with or without DGF (16.1 ± 8.4 vs 16.7 ± 8.5, p=0.7) Patients who had an ARE had less BDdur hours (13.8 ± 7.0) than patient who did not suffer an ARE (17.2 ± 8.7) (p = 0.03). BDdur did not have significant differences in 1-year post transplant hospitalization rate (15.8 ± 8.3 vs 17.1 ± 8.6, p=0.3). Patient who had longer BDdur (>17 hours) had better graft survival than patients with lower BDdur (< 17 h) although this difference did not reach statistical significance. (Figure 1)
Conclusion: BDdur had a significant impact in ARE during first year. BDdur does not have a significant impact on other transplant outcomes such as DGF and graft survival in our population.

Donor demographics (n = 163)	
Donor age (year)*	33.5 ± 14.0
Female donor (%)	41.7
Male donor (%)	58.3
ECD donor (%)	7.4
Traumatic cause of death (%)	63.2
Donor history of hypertension (%)	12.9
Donor history of Diabetes mellitus (%)	2.5
Donor Creatinine (mg/dl)*	1.08 ± 0.51
Donor KDPI (%)*	36.6 ± 23.6
Recipient demographics	
Recipient age (year)*	33.8 ± 11.6
Female recipient (%)	50.3
Male recipient (%)	49.7
Dialysis time (year)*	5.1 ± 3.9
Previous transplant (% ≥ 1)	4.9
EPTS (%)*	15.2 ± 18.0
DGF (%)	25.8
Rejection during first year (%)	20.9
Graft survival at 1 year (%)(Kaplan-Meier)	84.3
Graft survival at 3 year (%)(Kaplan-Meier)	77.4
Graft survival at 5 year (%)(Kaplan-Meier)	71.7
Graft related factors	
Cold ischemia time (h)*	14.4 ± 5.06

*Mean ± STD



References:
[1] Willemijn N. Nijboer, Cyril Moers, Henri G. D. Leuvenink, Rutger J. Ploeg; How important is the duration of the brain death period for the outcome in kidney transplantation?. Transpl Int 2010; 24: 14-20.
[2] Kristina Kunert, Sascha WeiB, Katja Kotsch, Johann Pratschke. Prolonged brain death - does it improve graft quality?. Transpl Int 2010; 24: 12-13
[3] Ulrich Kunzendorf, Bernd Hohensteinm Martin Oberbarnscheid, Erika Müller, Lutz Renders, Günther Eugen Schott, Gerd Offermann. Duration of Donor Brain Death and its Influence on Kidney Graft Function. Am J Transplant 2002; 2: 292-294.

P.1286

Relationship between dialysis duration and prevalence of cardiovascular diseases in kidney transplantation recipients

Keisuke Hata[1], Kohei Unagami[2], Masayoshi Okumi[1], Toshihito Hirai[1], Yasuhiro Okabe[3], Kosuke Masutani[4], Tomokazu Shimizu[1], Kazuya Omoto[1], Masashi Inui[1], Hideki Ishida[1], Takanari Kitazono[4], Kosaku Nitta[2], Kazunari Tanabe[1].
[1]Urology, Tokyo Women's Medical University, Tokyo, Japan; [2]Nephrology, Tokyo Women's Medical University, Tokyo, Japan; [3]Surgery, Kyushu University, Fukuoka, Japan; [4]Medicine and Clinical Science, Kyushu University, Fukuoka, Japan.

Background: Cardiovascular disease (CVD) accounts for approximately 40%-50% of all causes of deaths in patients with end-stage renal disease (ESRD) in Japan. Among kidney transplantation (KTx) recipients, the prevalence of CVD is also high; thus, CVD is a very important complication of KTx. However, to our knowledge, the relationship between the prevalence of CVD in KTx recipients and the hemodialysis duration before KTx has not yet been evaluated. Moreover, although we previously reported that there was no significant relationship between patient or graft survival and dialysis duration, we did not evaluate the relationship with prevalence of CVD. In this study, we evaluated the influence of dialysis duration on the outcome of KTx.
Methods: Between 2000 and 2013, we performed 1050 KTx from living related donors. We divided the patients into three groups according to dialysis duration: group A: ≦16 months (N=350), group B: 17-47 months (N=351), group C: ≧48 months (N=349).
Results: Data were statistically analyzed using the log-rank test for trend. The mean duration of dialysis across all groups was 28 months (7, 28, 86 months in groups A, B, and C, respectively; $p<0.001$). Seventy-four patients in Group A underwent preemptive KTx. Age of recipients, follow-up period, type of related donors, and kinds of calcineurin inhibitors administered for immunosuppresion were significantly different (both $p <0.001$). The prevalence of CVD increased significantly with the increase in hemodialysis duration ($p=0.026$). However, no significant differences were observed in patient or graft survival among the three groups ($p=0.942$, $p=0.581$, respectively). Serum creatinine levels, estimated GFR levels, and biopsy proven rejection rates after KTx showed no significant differences in both groups.
Conclusions: Despite no significant differences in patient or graft survival, the prevalence of CVD significantly increased with the increase in hemodialysis duration.
Katsunori Shimada, PhD. (STATZ Institute, Inc, Tokyo, Japan); Pharma Inc. (Tokyo, Japan)

P.1287

Development of protocol for deceased kidney transplantation recipients while waiting for kidney transplantation

Hyun Jung Kim[1], Anes Kim[1], Myoung Soo Kim [2], Jae Geun Lee[2], Beom Seok Kim[2], Jayoung Yoo[1], Soon Il Kim [2], Kyung Ock Jeon[1], Sunyoung Son[3].
[1]The Research Institute for Transplantation, Yonsei University, Seoul, Korea; [2]Department of Surgery, Yonsei University College of Medicine, Seoul, Korea; [3]Department of Surgery, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea.

Background: According to the statistics of Korean Network for Organ Sharing(KONOS), the number of patients waiting for kidney transplantation has been growing on an annual basis. In particular, the number went up to 14000 in 2015. However, the number of kidney transplant recipients from brain death was 674 in 2013, and 808 in 2014. The average waiting time for kidney transplantation is about 5-6 years in Korea. Brain death usually occurs at random, which is why it is very difficult for physicians to make the adequate assessments of patients before kidney transplantation. It indicates that the patients and physicians have to be confronted with unnecessary risks. In this regard, we suggest that physicians need systematic management programs reducing complications for patients after kidney transplant. Moreover, it could improve the life quality of recipients. Therefore, we propose in this study, to develop protocol which patients use for systematic administration while waiting for kidney transplantation.
Method: This study was developed by six coordinators and five physicians from two university hospitals in Seoul. We register and manage the patients who have been waiting for kidney transplantation. We had regular meetings three times for this study. We reviewed literature at the first meeting, and developed a draft of kidney transplant waiting list at the second meeting. At the last meeting, we evaluated and revised the kidney transplant waiting list through validity and applicability.
Result: The kidney transplant waiting list has 3 contents for the patients waiting for kidney transplantation from brain death.
1. Laboratory exams and progress schedules for all patients
2. Laboratory exams and progress schedules for the patients who had underlying disease like diabetes mellitus, cardiovascular disease, and infectious disease
3. Cancer screen tests and progress schedules while waiting time
We developed Electronic Medical Record(EMR) to record the kidney transplant waiting list in order to develop applicability and appropriateness in working places.
Conclusion: According to this study, we expect the protocol would be used in medical working places where there was no systematic program for patients waiting kidney transplant from the death. The kidney transplant waiting list can help physicians who assess patient before emergency kidney transplantation surgery from brain death. Furthermore, even though the patient had emergency kidney transplantation surgery, they would witness their improvement of their life quality through the eased risks of the operation.
References:
[1] Gaston RS, Danovitch GM, Adams PL, Wynn JJ, Merion RM, Deierhoi MH, et al. The report of a national conference on the wait list for kidney transplantation. *Am J Transplant*. 2003;3:775-85.
[2] Meier-Kriesche HU, Port FK, Ojo AO, Rudich SM, Hanson JA, Cibrik DM, et al. Effect of waiting time on renal transplant outcome. *Kidney Int*. 2000;58:1311-7.
[3] Kauffman HM, Cherikh WS, McBride MA, Cheng Y Hanto DW. Post-transplant de novo malignancies in renal transplant recipients: the past and present. *Transpl Int*. 2006;19:607-20.
[4] Korean Network for Organ Sharing (KONOS) [Internet].Seoul: KONOS; 2014. Available from: *http://www.konos.go.kr*.
[5] Merion RM, Ashby VB, Wolfe RA, Distant DA, Hulbert-Shearon TE, Metzger RA, et al. Deceased-donor characteristics and the survival benefit of kidney transplantation. *JAMA* 2005;294:2726-33.

P.1288

Anti-diuretic hormone response related to kidney transplantation in dialysis patients

Jin Soo Kim[1], Sihyung Park[2], Bong Soo Park[2], Yang Wook Kim[2].
[1]Surgery, Inje University Haeundae Paik Hospital, Busan, Korea; [2]Internal medicine, Inje University Haeundae Paik Hospital, Busan, Korea.

Introduction: Antidiuretic hormone (ADH) has vasoconstrictive and antidiuretic effects. The most powerful stimuli for ADH release are plasma osmolality and volume status. However, ADH is inappropriately increased in the majority of dialysis patients despite of normal osmolality and volume overload. On the other hands, researchers revealed that proadrenomedullin (proADM), which has vasodilatative and natriuretic effects, could reflect excessive volume status and cardiac dysfunction in dialysis patients. We assessed mechanism of ADH response during kidney transplantation with proADM as a marker of volume and cardiac dysfunctions.
Materials and Methods: As a single center study, five hemodialysis and five peritoneal dialysis patients' plasma ADH and proADM with other parameters were analyzed at four times before and after kidney transplantation (pre-operative, post-operative 48 hours, 120 hours, 168 hours). Pre-operative echocardiography was done in all patients. Mann-Whitney and Wilcoxon signed rank test used as statistical methods.
Results and Discussion: All patients showed elevated plasma osmolality (309 ± 6.06 mOsm/Kg·H2O). Three hemodialysis and two peritoneal dialysis patients' levels of pre-operative ADH was increase two folds more than normal upper limits (34.7 ± 26.1 vs 5.52 ± 2.8 pg/mL), and that group's level of pre-operative proADM was higher than the other group's one (32155 ± 29899 vs 9175.9 ± 8724.1 pmol/L, $p = 0.05$). ProADM had positive correlation with ADH levels pre-operatively ($r = 0.75$, $p = 0.01$) and negative correlation with ratio of diastolic and systolic left ventricular internal diameter ($r = -0.64$, $p = 0.04$). ADH and serum osmolality were decreased significantly within post-operative 120hours ($p = 0.04$, 0.02). Post-operative proADM did not decreased until post-operative 168hours ($p = 0.3$). It seemed like pre-operative ADH response was handle by baroreceptors which related with effective circulating volume and cardiac dysfunctions. However, post-operative ADH response was related to osmoreceptors. Unchanged or even increased post-operative proADM might be related to fluid therapy after transplantation and unrestored cardiac functions.
Conclusion: The cause of inadequately elevated ADH in dialysis patients might be related to effective circulating volume status. However, Additional large and longtime follow up study are needed for defining the causes of elevated proADM in kidney transplanted patients.

P.1289

Comparison of barriers to wait listing for kidney transplantation between hemodialysis center

Keun-Myoung Park[1], Kee Chun Hong[1], Junho Song[2].
[1]Surgery Department, Inha University Hospital, Incheon, Korea;
[2]Nephrology Department, Inha University Hospital, Incheon, Korea.

Background and Objectives: Kidney transplantation is the preferred modality of treatment for patients with end-stage renal disease (ESRD). Many factors have been associated with ESRD patient placement on the waiting list of kidney transplantation. But, objectives of our study is to evaluate factors associated with rejection of placing on waiting listing of Kidney transplantation and to compare the difference between hemodialysis center of primary hospital and tertiary hospital.
Method: We examined patients in hemodialysis center of 1 tertiary hospital and 4 primary hospitals. Total 198 patients were enrolled. We excluded the old patients (over 65) and patients placed on waiting list. We questioned the patients about barrier of kidney transplantation. Uni- and multivariable logistic models were used to assess factors associated with waiting list placement, comparison between hemodialysis center.
Result: The patients placed on the waiting list in tertiary hospital(21%,18 in 85 patients) are more than primary hospital(9.7%,10 in 103 patients). Total 48 patients(56%) in tertiary hospital and 59 patients (57%)in primary hospital were enrolled and completed questionaries. There were no difference in patients characteristics between two groups. Barriers of placement on waiting list were scare of surgery(37.5%, 18), low possibility of selection(35.4%,17), economic problem(20.8%,10) and lack of knowledge about transplantation(4.1%,2). But, economic problem(42%,25), lack of knowledge about transplantation(30.5%,18), low possibility of selection(22.0%,13) and scare of surgery(3.3%,2).
Conclusion: The ratio of patients placed on the waiting list is still low. The factors of barrier to placement to the waiting list were variable. Especially, in hemodialysis center of primary hospital economic support and education about transplantation are necessary comparing tertiary hospital.

P.1290

Mean fluorescence intensity by Luminex single antigen bead and one year graft outcome in DSA positive kidney transplantation

Leslie Yap, Glenda Eleanor Pamugas, Romina Danguilan.
Nephrology, National Kidney and Transplant Institute, Quezon City, Philippines.

Introduction: Luminex single antigen bead (SAB) is an important test to detect levels of pre-transplant donor specific antibodies (DSA), which may predict the risk of kidney rejection and graft loss. Our study aims to determine the one year graft outcome of DSA positive kidney transplantation at the National Kidney Transplant and Institute (NKTI), to correlate pre-transplant mean fluorescence intensity (MFI) levels with graft survival and estimated glomerular filtration rate (eGFR) and to recommend a cut off MFI level before transplantation.
Materials and Methods: Thirty-one kidney transplant recipients with pre-transplant DSA were retrospectively analyzed from January 2011 to December 2014, and followed to a year. Levels of DSA expressed as MFI was obtained by Luminex SAB.
Results and Discussion: Among the 31 recipients, we have 90% patient survival (n=28) and 87% graft survival (n=27) in a year. Recipients with graft failure had a mean highest pre-transplant MFI of 4996, compared to mean of 2394 with graft survival. A significant inverse association between the highest pre-transplant MFI level and one-year eGFR was observed (r=-0.365, p=0.044). There was also a significantly lower eGFR (< 60 ml/min/1.73 m^2) among those with high MFI who did not undergo desensitization (p=0.029).
Conclusion: Recipients with graft failure had a higher mean MFI. High pre-transplant MFI showed a significantly lower graft function in a year, and desensitization therapy may decrease this risk. Based on our population, MFI cut-off level of <1000 had the best sensitivity to predict a better renal function in a year (AUC=0.717, 95% CI: 0.521-0.915).
References:
[1] Class FHJ: Clinical relevance of circulating donor-specific HLA antibodies. Curr Opin Organ Transplant 2010; 15: 462-466.
[2] Gebel, H.M., Bray, R.A. and Nickerson, P. Pre-Transplant Assessment of Donor-Reactive, HLA-Specific Antibodies in Renal Transplantation: Contraindication vs Risk. Am J Transplant 2003; 3: 1488-1500.
[3] Tait BD, Hudson F, Cantwell L, et al. Review article: Luminex technology for HLA antibody detection in organ transplantation. Nephrology 2009; 14: 247-254.
[4] Lee PC, Ozawa M, Hung CJ, et al. Reappraisal of HLA antibody analysis and crossmatching in kidney transplantation. Transplant Proc 2009, 41:95-98.
[5] Lefaucheur C, Loupy A, Hill GS, Andrade J, Nochy D, An- toine C, Gautreau C, Charron D, Glotz D, Suberbielle-Boissel C. Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation. J Am Soc Nephrol 2010; 21: 1398–1406.
[6] Caro-Oleas JL, Gonzalez-Escribano MF, Gonzalez-Roncero FM, et al. Clinical relevance of HLA donor-specific antibodies detected by single antigen assay in kidney transplantation. Nephrol Dial Transplant. 2012; 27: 1231.
[7] Park WD, Larson TS, Griffin MD, Stegall MD. Identification and characterization of kidney transplants with good glomerular filtration rate at one year but subsequent progressive loss of renal function. Transplantation. 2012 Nov 15;94(9):931.

P.1291

Evolution of renal transplantation - experience from a single center

Viktor Denisov, Vadym Zakharov, Nikolay Oleshchenko, Eugene Onishchenko, Alexander Sidorkin, Tatyana Golubova, Sergey Kichatyi, Olga Zakharova.
Transplant Center, Regional Hospital, Donetsk, Ukraine.

Introduction: Our Center performed 697 kidney transplants (KTx) between April 1986 and January 2016. We analyzed the trends and reviewed the influence of these trends on outcomes of KTx for further evaluation of its therapeutic potential and better understanding the tasks for professionals.
Materials and Methods: The median recipient age was 42.6 years with M:F ratio of 1.6:1. Most of them were on hemodialysis (96%), 28 patients received pre-emptive transplant, 515 (73.9%) transplants were from deceased donors, 182 (26.1%) were from living donors.
Results and Discussion: Patients in the start and more late stage of transplant program had the following significant differences: the range of their age has been changed from 14-62 to 6-71 years. Transplantations from deceased donors decreased from 99.7% to 41.6% of cases. Last decades, more and more patients who were not considered as transplant candidates earlier have been referred to kidney transplantation. Amount of high risk factors such as diabetes, systemic lupus erythematosus, amyloidosis and other increased dramatically. Miniinvasive and other modern surgery technology were introduced.
Immunosuppression at the beginning consisted of cyclosporine, azathioprine and steroids. Later in all cases of induction anti-CD-25 monoclonal or other depleting antibodies were used. Immunosuppression maintenance spectrum was added by tacrolimus, prolonged-release tacrolimus, mycophenolate mofetil, mycophenolate sodium or everolimus. Over the last decade steroid-free protocols were used in about 30% of all primary KTx to avoid the long-term side effects of steroid use. Immunosupression-free protocol in one recipient with normal graft function is using last 3 years.
Our data showed that in spite of extension of indications, the number of primary functioning kidney transplant increased from 71.2 % to 78.3 %. It makes the best start for long-term rehabilitation of recipients. One-year survival of high risk recipients was 95,7 % with a good quality of life. One-year graft and patient survival in standard conditions riches 100%. The maximum term of satisfactory function of the kidney transplanted at our Center to 44-year-old patient with chronic glomerulonephritis, is more than 26 years. Monitoring continues. Long-term follow-up revealed a large and sometimes dominant influence of the social aspects on graft and patient's survival, as well as their quality of life.
Conclusions: The results of renal transplantation indicate the possibility of prolonged rehabilitation including high risk patients. The later improvement is related to expanding of transplant activities through education and multidisciplinary strategies.

P.1292

Surgical and technical aspects of re-operative (>/=3) Kidney transplantation

Tarunjeet Klair, Raphael Rosen, James Guarrera, Kasi McCune, Rodrigo Sandoval, Lloyd Ratner.
Abdominal Organ Transplantation, Columbia University Medical Center, New York, United States.

Introduction and Objectives: Description of technical approaches and surgical complications involved in a 3rd or greater kidney transplant. Limited reports are available in literature regarding this group of kidney transplant recipients.
Materials and Methods: All 3rd or greater transplants performed between January 2005-June 2015 at a single center were examined.
Results and Discussion: 58 cases of 3rd or greater transplants were identified. Median follow-up was 4.2 years. Mean age at transplant was 43 years. 46 were 3rd and 12 were 4th transplants. 26 were living donor transplants. Glomerular etiologies of renal failure were the commonest cause of renal failure accounting for 19(32.7%) cases. Mean Panel Reactive antibody level was 53.4%. Desensitization was performed in 9 cases. In 37(63.7%) cases a left allograft was implanted on recipient's right side. Midline incision was used in 11 cases. Allograft was placed intraperitoneally in 27(46.5%) cases. Previous allograft was present in-situ on the same side in 32(55%) cases. Simultaneous transplant nephrectomy was performed in 10(17%) cases. Vascular anastomoses were performed to the External Iliac vein and artery in 27(46.5%) cases. Various combinations of inflow and outflow vessels were used for the remaining cases.

Inflow and outflow vessels	External Iliac artery	Common Iliac artery	Contralateral Common Iliac artery	Aorta	Previous Aorto-Iliac bypass graft	Old allograft renal artery
External Iliac vein	27	1				
Inferior Vena Cava		16	1	1	1	
Common Iliac vein	4	6				
Old allograft renal vein						1

Combinations of venous and arterial anastomotic sites

Ureteroneocystostomy was performed in all cases. Ureteral stents were used in 47(81%) cases. Intraoperative blood transfusion was needed in 10 cases. Ureteral complications requiring intervention were seen in 5(8.6%) cases. Allograft artery stenosis requiring interventions were seen in 3(5%) cases. 3 and 5-year allograft survival were 70% and 41% respectively. Chronic rejection and recurrence of primary disease accounted for 66% of allograft losses.
Conclusions: Technical flexibility and creativity allows 3rd or greater kidney transplant to be performed with reasonable outcomes. Patient selection, incision placement, laterality and landing sites are key considerations. Operative technical hurdles are usually not the limiting factors when considering a 3rd or greater transplant. Rejection and recurrent disease are the major limitations.

P.1293

Renal transplant for patients on long-term dialysis (more than 20 years) at our center

Koichi Kozaki[1], Kenji Yuzawa[2].
[1]Department of Surgery and Transplantation Surgery, NHO Mito Medical Center, Ibaraki, Japan; [2]Department of Transplantation Surgery, NHO Mito Medical Center, Ibaraki, Japan.

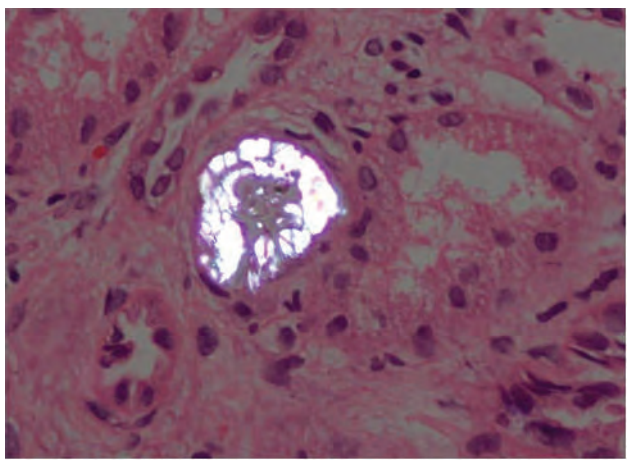
The patients on dialysis(HD) of our country increased every year and became 320,000 people at the end of 2014. The patient on long-term dialysis (more than 20 years:LHD) becomes approximately 25,000 people, and it becomes 7.7% of whole. Whereas the number of the renal transplant(RTx) of our country was 1,586 cases at the end of 2013, and the contents were 1,431 living RTx(LR), cadaveric RTx(CR) 155. The CR did not increase, but the CR enrolled patients reached approximately 13,000 people. Thus, for the CR enrolled patients, it is the present status of our country that there is extremely little number of RTx. Furthermore, a mean waiting period of CR is long with 12.9 years and many RTx enrolled patients have LHD. The patient on LHD has many complications, and, as for the atrophic bladder of the patients, it becomes a major problem in performing RTx. In this time, we mentioned results of RTx to the LHD in our hospital.
Patients: 83 cases underwent renal transplantation (LR:72, CR:11) from May 2006 to January 2016 in our hospital. All patients received quadruple sequential immunosuppression, such as tacrolimus or cyclosporine, mycophenolate mofetil, methylprednisolone, and basiliximab. Mean age 46.1(y), male 55, and female 28. 11 patients were treated with LHD before receiving a RTx(LR:2, CR:9).
Results: Mean age was, 53.3 in LHD, and 44.6 in other. Significantly elderly in LHD($p<0.005$). Mean serum creatinine level (S-Cr:mg/dl) was, 1.32 in LHD, and 1.55 in other. There was no significant difference in both. 1) HCV patients was, 5/11 (45.5%) in LHD, and 4/72 (5.6%) in other, 2) the fatal case was, 2/11(18.2%) in LHD, and 3/72(4.2%) in other, 3) HD reintroduction case was, 6/11(54.5%) in LHD, and 13/72(18.1%) in other, it significantly occurred a lot in LHD($p<0.005$). And six cases in LHD group rebuilt urinary tract by ureteroureterostomy without having ureterocystostomy for atrophic bladder.
Conclusions: As for S-Cr, no significant difference was between both groups, but the prognosis was poor in patient on LHD. And, many HCV patients were in LHD. It was thought that transfusion was considered to be it as for the reason when erythropoietin preparation was not commonly used for renal anemia. When we performed RTx on LHD, severe surveillance is necessary.

P.1294

Need for more than just renal replacement - Analysis of a special cohort

Gomathy Narasimhan, Chandrasekar venkatraman, Naresh Shanmugam, Mettu S.Reddy, Ilankumaran Kaliamorrthy, Venugopal Kota, Mohamed Rela.
Institute of Liver and Renal Transplantation, Global Hospital, Chennai, India.

Introduction: Patients with primary hyperoxaluria 1 often present with varying degrees of renal insufficiency to the nephrologists. This cohort of patients needs more than just renal replacement in most instances and in some, with the right intervention, renal replacement can be avoided. The aim of the study is to analyse our management of patients who presented with primary hyperoxaluria.
Materials and Methods: Retrospective analysis of a prospectively maintained database of patients who presented to our centre with a diagnosis of primary hyperoxaluria 1 was performed.
Results and Discussion: Seventeen patients (11 pediatric, 6 adults) with a diagnosis of primary hyperoxaluria have been evaluated at our centre. Fourteen of them presented with CKD V and three with CKD II-III. Among the CKD V patients, 9 (3 adults, 6 pediatrics) underwent combined liver and kidney transplantation (CLKT), two are on the waitlist for liver and kidney transplantation, three died on the waitlist. Of the 9 CLKT, one had earlier undergone renal transplant alone elsewhere and had allograft failure from recurrent oxalate nephropathy (Figure). Of the two on the waitlist, one was under follow up for recurrent renal calculi but was not diagnosed to have PH1 until he developed CKD V when he was referred to our centre. Two of the patients who died on the waitlist presented with very poor ejection fraction secondary to the high oxalate load and died within 2 weeks of initiation of aggressive dialysis at to our centre. 3 patients (all pediatrics) were diagnosed with CKD II to III, 2 received pre-emptive liver transplant alone with stable renal function subsequently, one patient is awaiting a liver transplant. All three patients were diagnosed early based on family history and confirmed by genetic analysis.
Conclusion: It is important to recognize this special cohort of patients with primary hyperoxaluria in order to prevent progression of renal failure, salvage the kidney with pre emptive liver transplant alone, prevent allograft loss by avoiding isolated renal transplant, optimize the patient with aggressive hemodialysis and perform CLKT when ESRD has set it.



P.1295

Decremental change in perioperative hemoglobin concentration is associated with poor early graft function in kidney transplant patients: a retrospective study

Arpa Chutipongtanate[1], Somchai Chutipongtanate[2], Arpakorn Kantain[1], Atiporn Inksathit[3,4], Surasak Kantachuves[3], Vasant Sumethkul[3], Siriwan Jirasiritham[1], Sopon Jirasiritham[5].
[1]Anesthesiology, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; [2]Central Laboratory, Department of Pediatrics, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; [3]Division of Nephrology, Department of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; [4]Section for Clinical Epidemiology and Biostatistics, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; [5]Division of Vascular and Organ Transplant Surgery, Department of Surgery, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.

Background: Previous studies have shown that even small decrement in perioperative hemoglobin level may be associated with acute kidney injury in patients who underwent non-cardiac surgery. In kidney transplant patients, however, the effect of perioperative hemoglobin change on early graft function has not been investigated.
Methodology: A retrospective study was conducted at Ramathibodi Hospital, Thailand. Patients who underwent kidney transplant during January 2011 to December 2013 were included. Change in perioperative hemoglobin concentrations (cHb) was compared between patients with poor early graft function (PEGF) and immediate graft function. The strength of association was determined by univariate and multivariate analysis.
Results: A total of 269 kidney transplant patients were enrolled, of whom 98 (36.4%) developed PEGF. Comparing the two study groups, patients with PEGF had a greater decremental change of perioperative hemoglobin concentration (-1.60 [-2.38, -0.83] vs. -0.70 [-1.35, 0.20] g/dL, respectively; p<0.001). An optimal cut-point of perioperative cHb of -1.35 g/dL was obtained from receiver operating characteristic curve (AUC 0.648, sensitivity 54.1%, specificity 75.4%; p<0.001). Multivariate analysis showed that decremental change in perioperative cHb greater than 1.35 g/dL was an independent risk of PEGF (adjusted OR 2.91, 95%CI 1.31–6.47; p=0.009).
Conclusion: This study suggests that decremental change in perioperative cHb greater than 1.35 g/dL might be a potential modifiable factor of PEGF.

References:

[1] Perico N, Cattaneo D, Sayegh MH, Remuzzi G. Delayed graft function in kidney trans-plantation. Lancet. 2004;364(9447):1814-27
[2] Walsh M, Garg AX, Devereaux PJ, Argalious M, Honar H, Sessler DI. The association between perioperative hemoglobin and acute kidney injury in patients having noncardi-ac surgery. Anesth Analg. 2013;117(4):924-31.
[3] Abou-Jaoude MM, Nawfal N, Najm R, Honeidi M, Shaheen J, Almawi WY. Effect of pretransplantation hemoglobin blood concentration on renal allograft survival and func-tion. Transplant Proc. 2010;42(3):760-2.
[4] Hellegering J, Visser J, Kloke HJ, D'Ancona FC, Hoitsma AJ, van der Vliet JA, et al. Poor early graft function impairs long-term outcome in living donor kidney transplanta-tion. World J Urol. 2013;31(4):901-6.

P.1296

Rate of dysregulation of serum immunoglobulins is higher in acute allograft rejection in renal transplant recipients

Ravi Dhital[1], Mukut Minz[1], Ranjana Minz[2], Ashish Sharma[1], Ritambhara Nada[3], Sarbpreet Singh[1], Deepesh Kenwer[1].
[1]Renal Transplant Surgery, Post Graduate Institution of Medical Education and Research, Chandigarh, India; [2]Immunopathology, Post Graduate Institution of Medical Education and Research, Chandigarh, India; [3]Histopathology, Post Graduate Institute of Medical Education and Research, Chandigarh, India.

Background: Serum immunoglobulins are dysregulated in recipients with renal transplantation and are severely impaired during graft rejection. However, the degree of change of immunoglobulins during rejection has not been well described. Therefore we aimed to study the rate of change of immunoglobulins level in the serum at the time of acute rejections.
Methodology: Immunoglobulin (Ig)G, IgA, IgM, IgG1, IgG2, IgG3 and IgG4 levels were compared among 38 sera obtained during acute renal graft rejection, 40 sera obtained from recipients with stable graft function at corresponding time points and 40 sera obtained from healthy controls. Immunoglobulins were quantified by Nephleometry. Post transplant rate of change of immunoglobulin levels in serum was calculated as (Post transplant level- pre transplant level)/ Post operative days of sample collection. Similarly, the rate of percentage change of serum immunoglobulins during rejection was calculated as [{(Level at rejection - pre transplant level)/ Pre transplant level}*100]/ post operative days of sample collection. All the values were expressed as Mean ± Standard error of mean.
Result: Post- transplantation, serum IgA and IgG level were found to decrease significantly at the time of rejection as compared to that of stable graft function (Table 1). The rate of decrease of serum IgG during rejection episodes (0.514 ± 0.159 units/day) was significantly higher than that of recipients with stable graft function (0.086 ± 0.02772 units/ day) (P= 0.0083). Similarly, the rate of decrease of serum IgG1 (202.5 ± 65.48 units/day), IgG2 (83.47 ± 26.67 units/ day) and IgG3 (42.75 ± 8.692 units/day) during rejection were statistically higher than that of recipients with stable graft function (38.78 ± 10.80, 28.43 ± 7.121, 9.584 ± 4.424 respectively (P= 0.0135, P= 0.0449 and P= 0.0009 respectively).
Conclusion: During rejection, serum immunoglobulins are significantly depleted from peripheral circulation. Assuming that absorption of immunoglobulins by rejecting grafts is the possible reason of their diminution from peripheral circulation, the hierarchy of rate of percentage absorption of serum immunoglobulin by graft is IgA> IgG> IgM and IgG3>IgG4>IgG1>IgG2.

P.1297

Paricalcitol pretreatment attenuates apoptosis and inflammation in renal ischemia-reperfusion injury via EP4 pathway

Hyeon Seok Hwang, Yu Ah Hong, Yoon Kyung Chang, Suk Young Kim.
Division of Nephrology, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Daejeon, Korea.

Objective: We investigated whether paricalcitol attenuates apoptosis and inflammation in renal ischemia reperfusion (IR) injury through the prostaglandin E2 receptor (PGE2) EP4.
Design: Randomized laboratory animal study.
Settings: Animal research laboratory.
Subjects: Human proximal tubular (HK-2) cells, male C57BL/6J mice.
Interventions: HK-2 cells were exposed to ischemia and lipopolysaccharide (LPS) treatment. Male C57BL/6 mice were subjected to bilateral kidney ischemia for 23 min and reperfusion for 24 h. The effects of paricalcitol pretreatment with and without EP4 blockade were investigated.
Measurements and Main Results: Paricalcitol upregulated the expression of cyclooxygenase-2, PGE2, and EP4 in HK-2 cells. Cellular membrane expression of EP4 was increased in paricalciol-treated cells with and without IR exposure. Paricalcitol pretreatment prevented cell death induced by IR and LPS exposure, and EP4 antagonist co-treatment offset these protective effects. Paricalcitol increased the phosphorylation of Akt and cyclic AMP-responsive element binding protein (CREB) in IR-exposed cells and suppressed nuclear factor-κB (NF-κB) activation in LPS-exposed cells. EP4 antagonist and small interfering RNA against EP4 blunted these cell survival signals and inhibited the suppressive effects of paricalcitol on nuclear translocation of p65 NF-κB. In vivo studies showed that paricalcitol pretreatment improved renal dysfunction and tubular necrosis after IR injury, and co-treatment with EP4 antagonist inhibited the protective effects of paricalcitol. Phosphorylation of Akt increased and nuclear translocation of p65 NF-κB decreased in paricalcitol-treated mice kidney with IR injury. Paricalcitol pretreatment decreased terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeled (TUNEL)-positive cells, increased protein expression of B cell leukemia/lymphoma 2 (Bcl-2), and decreased expression of BCL-2-associated X (Bax), and attenuated the infiltration of inflammatory cells and production of proinflammatory cytokines after IR injury. Co-treatment with EP4 antagonist abolished all of these anti-apoptotic and anti-inflammatory effects.
Conclusions: EP4 plays a pivotal role in the anti-apoptotic and anti-inflammatory effects of paricalcitol pretreatment in renal IR injury.
This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2015R1A1A1A05001599)

P.1298

Pretreatment with cilastatin attenuates renal ischemia-reperfusion injury

Hyeon Seok Hwang[1], Yu Ah Hong[1], Yoon Kyung Chang[1], Chul Woo Yang[2], Suk Young Kim[1].
[1]Division of Nephrology, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Daejeon, Korea; [2]Division of Nephrology, Department of Internal Medicine,, College of Medicine, The Catholic University of Korea, Seoul, Korea.

Backgorund: Cilastatin is a specific inhibitor of renal dehydrodipeptidase. We investigated whether cilastatin attenuates apoptosis and inflammation in renal ischemia-reperfusion (IR) injury.
Methods: Human proximal tubular cell line (HK-2) was exposed to LPS and ischemia by mineral oil monolayer. Male C57BL/6 mice were subjected to 23 min of bilateral kidney ischemia and 24 h reperfusion. The effects cilastatin pretreatment was investigated in both in vitro and in vivo models.
Results: Cilastatin pretreatment prevented the HK-2 cell death induced by IR and LPS exposure. In mice with renal IR injury, cilastatin pretreatment decreased serum creatinine level. Compared to mice kidney with IR injury alone, tubular necrosis and TUNEL-positive cells were decreased in cilastatin-treated mice kidney with IR injury. The cilastatin pretreatment decreased the Bax level and increased the expression of Bcl-2 in mice kidney with IR injury. The mRNA expression of cIAP2 mRNA level was increased in cilastatin-treated mice kidney with IRI injury. The cilastatin attenuated the production of proinflammatory cytokines (RANTES and interleukin-1β).
Conclusions: Cilastatin attenuates apoptosis and inflammation in renal IR injury.
This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2015R1A1A1A05001599)

P.1299

Intra-abdominal cooling system prevents ischemia-reperfusion injury during robotic-assisted renal transplantation

Raphael Meier, Monika Hagen, Vincent Piller, Charles Joliat, Pamela Sun, Jean-Bernard Buchs, Antonio Nastasi, Raphael Ruttimann, François Lazeyras, Philippe Morel, Leo Bühler.
Visceral and Transplant Surgery, Geneva University Hospital and Medical School, Geneva, Switzerland.

Background and Objective: Robotic renal transplantation allows a minimally invasive approach yet it lengths operative time in which long warm ischemia is a concern. We developed a novel intra-abdominal cooling system has been designed to intra-operatively cool the donor kidney. Here we investigate the efficacy of this new intra-abdominal cooling system in the robotic-assisted versus standard open renal transplantation in a porcine model.
Methods: All porcine kidneys were procured by standard procurement technique via laparotomy. Donor kidneys were flushed with preservation fluid IGL-1 at a cooling temperature of 4°C prior to being auto-transplanted. The experimental group of the intra-abdominal cooling system used in the robotic-assisted technique (n=11) was compared to a control group of 4°C saline solution in a conventional open technique (n=6) and a control group of robotic-assisted technique without cooling system (n=6). Temperature was measured using a cortical thermal probe prior to transplantation. For functional outcome, a MRI gadolinium perfusion study at 6h post-transplantation was performed to measure the reperfusion of the transplanted kidneys; whilst histology was used to quantify the degree of ischemia reperfusion injury.
Results: Robotic renal transplantation required a longer operative time, with and without cooling system, compared to open approach (70.4±17.7 min and 74.0±21.5 min vs. 49.4±12.4 min, p-values<0.050). The temperature was higher in the robotic group, compared to the open approach group (28.7±3.3°C vs 22.5±6.5°C; p=0.133) and compared to the cooling system group (6.5±3.1°C; p=0.002). Functional outcomes from the MRI gadolinium perfusion study were equivocal in all groups. Ischemia reperfusion injuries were moderate in the robotic group without cooling system whilst they were mild in the open technique with 4°C saline and mild in the robotic group with the cooling system.
Conclusion: Robotic renal transplantation prolongs warm ischemia time of the donor kidney. We developed a new intra-abdominal cooling system that allowed to minimize the warming of donor kidneys during transplantation and prevented ischemia-reperfusion injuries.

P.1300

Increased gene expression of BAFF-R, TACI and BCMA is associated with acute rejection in kidney transplantation

Ranjana Minz[2], Mukut Minz[1], Ravi Dhital[1], Ashish Sharma[1], Ritambhara Nada[3], Sarbpreet Singh[1], Deepesh Kenwer[1].
[1]Renal Transplant surgery, Post Graduate institute of Medical Education and Research, Chandigarh, India; [2]Immunopathology, Post Graduate institute of Medical Education and Research, Chandigarh, India; [3]Histopathology, Post Graduate institute of Medical Education and Research, Chandigarh, India.

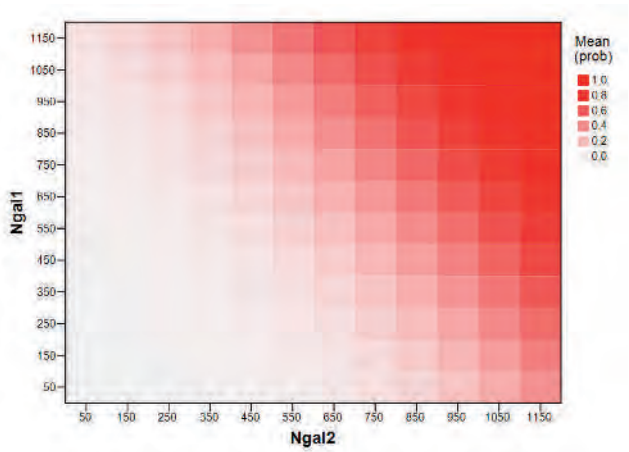
Background: B- Cell activation factor (BAFF) and a proliferation inducting ligand (APRIL) are well known for their B- cell survival and growth stimulator functions respectively.
BAFF and APRIL share two receptors: transmembrane activator and cyclophilin ligand interactor (TACI; also known as TNFRSF13B) and B cell maturation antigen (BCMA; also known as TNFRSF17). Additionally, BAFF has strong affinity to BAFF receptor (BAFF-R; also known as TNFRSF13C).
We intend to analyze the mRNA expression of BAFFR, TACI and BCMA in peripheral mononuclear cells of kidney transplant recipients during acute rejection.
Methods: Peripheral venous blood was collected from all the patients before transplantation. Patients were observed longitudinally for the evidence of rejection within the first 6 months of transplantation. Blood samples were collected periodically at 1 month, 3 months and 6 months of transplantation as well as at the time of rejection before initiation of anti- rejection therapy.
mRNA was isolated from PBMCs, converted to cDNA and stored at -80°C until further use. These cDNA were used to quantify mRNA expression level of BAFF-R, TACI and BCMA by Real time PCR (Light Cycler 480, Roche Diagnostics, Germany) using RT2 Profiler PCR Array (CAPH12966F, QIAGEN). The expression level was calculated as fold change using the formula, 2-ΔΔCT. Also, the % CD3-CD20+ B- cells in peripheral circulation was calculated by flow cytometry. All the values are expressed as the mean ± standard error of mean (SEM). Difference of values was taken significant only when P value is less than 0.05.
Result: Of 211 total kidney transplantation, this study includes 76 first kidney transplant recipients who were divided into two groups: Rejection group (N=36) and Non Rejection group (NR, N=40).
Normalizing the pre- transplant gene expression level as 1, we found a significant rise in the gene expression level of BAFF-R (15.43 ± 4.469, P= 0.0027), TACI (5.425 ± 1.457, P= 0.0045) and BCMA (2.621 ± 0.7485, P= 0.0372) at the time of rejection. At the same time, we could also demonstrate a significant rise in peripheral % of CD3-CD20+ B-cells during the rejection. Unlike rejection, we could not demonstrate significant changes in mRNA expression level of these genes in patients with stable graft function at post- 1 month and 3rd month of transplantation.
Conclusion: During acute rejection, the mRNA expression of BAFFR, TACI and BCMA increases resulting in higher concentration of B- cells in peripheral blood. Thus these factors could serve as important prognostic markers in the prediction of acute rejection.

P.1301

Plasma neutophil gelatinase-associated lipocalin (NGAL) - biomarker in kidney injury. Is it a useful tool to predict the renal function after transplantation?

Marta Sekowska[1], Maciej Glyda[1], Anna Masajtis-Zagajewska[2], Violetta Wink[1], Iwona Radko[3].
[1]Department of Transplantology General Surgery with subdepartment of Urology, Provincial Hospital Poznan Poland, Poznan, Poland; [2]Department of Nephrology, Hypertension and Kidney Transplantation, Medical University of Lodz, Lodz, Poland; [3]Laboratory Department, Provincial Hospital Poznan, Poznan, Poland.

Neutrophil gelatinase-associated lipocalin (NGAL) is a protein belonging to the lipocalin superfamily found initially in activated neutrophils[1]. NGAL level may predict the future appearance of acute kidney injury after treatment potentially detrimental or as a result of ischemia-reperfusion[2]. The key-advantage of NGAL is that the plasma level grows earlier than others renal function biomarkers. The aim of the study was to assess whether donor and recipient NGAL concentration in kidney transplantation(KTx) can be helpful tool to evaluate the quality of the organ, useful for prediction renal function after KTx and for differentiation delayed graft function (DGF) and acute rejection (AR)[3]-[5].
Study population comprised 56 kidney recipients, mean age 51±10 years (27 females, 29 male) and 43 donors. The biochemical parameters such as plasma NGAL and serum creatinine in both: donors (dNGAL, dCr) before kidney harvesting and recipients (rNGAL, rCr) were obtained. All parameters were assessed: before transplantation (day 0) and repeated after KTx 1, 2, 7, 14, 30 days and 3,6,12 months thereafter in recipients. The kidney weight were also measured. We also assessed number of DGF and AR.
A Spearman's correlation was computed to assess dNGAL vs rNGAL with each day of study and dNGAL vs rCr, both correlations were not significant (p>0.119 and p>0.3 respectively). A Spearman's correlation was also computed to assess rNGAL 1,2 and7 day vs rCr 3,6 and12 month relations. There was significant positive relations for rNGAL on 1day and rCr in 6 month, rNGAL in 7 day and rCr in 3 month and in 6 month. As can be seen from the ANOVA table, the value of dNGAL can not significantly predicts DGF (p>0 .238). Also nonpatametric version of ANOVA test (Welch) shows that dNGAL can not significantly predicts DGF (p>0.108). Nonparametric version of ANOVA test (Welch) shows that the model of rNGAL (0 to 1 year) can not significantly predicts AR (p for all rNGAL are >0 .15). Spearman analysis also showed that there's no statistically significant correlation between the kidney weight and NGAL.



Possibility of DGF vs NGAL1 and 2. The more reddish the higher possibility of DGF.
rNGAL obtained on days 1 and 7 seems to be reliable biomarker to predict the graft function. dNGAL concentration does not predict short or long term outcomes of transplanted kidney. rNGAL is not useful in assessing acute rejection. There is no correlation between kidney weight and dNGAL and rNGAL.

References:

[1] Mahdavi-Mazdeh M, Amerian M, Abdollahi A, Hatmi ZN, Khatami MR. Comparison of Serum Neutrophil Gelatinase-associated Lipocalin (NGAL) with Serum Creatinine in Prediction of Kidney Recovery after Renal Transplantation. Int J Organ Transplant Med. 2012;3(4):176-82
[2] Hollmen ME, Kyllönen LE, Merenmies J, Salmela KT. Serum neutrophil gelatinase-associated lipocalin and recovery of kidney graft function after transplantation. BMC Nephrol. 2014 Jul 28;15:123
[3] Buemi A, Musuamba F, Frederic S, Douhet A, De Meyer M, De Pauw L, Darius T, Kanaan N, Wallemacq P, Mourad M. Is plasma and urine neutrophil gelatinase-associated lipocalin (NGAL) determination in donors and recipients predictive of renal function after kidney transplantation? Clin Biochem. 2014 Oct;47(15):68-72
[4] Bataille A, Abbas S, Semoun O, Bourgeois É, Marie O, Bonnet F, Resche-Rigon M, Abboud I, Losser MR, Jacob L Plasma neutrophil gelatinase-associated lipocalin in kidney transplantation and early renal function prediction. Transplantation. 2011 Nov 15;92(9):1024-30
[5] Van den Akker EK, Hesselink DA, Manintveld OC, IJzermans JN, de Bruijn RW, Dor FJ. Neutrophil Gelatinase-Associated Lipocalin, but Not Kidney Injury Marker 1, Correlates with Duration of Delayed Graft Function. Eur Surg Res. 2015 Dec;55(4):319-327

P.1302

When does delayed graft function not make a difference in deceased donor kidney transplant outcomes?

Robert Stratta[1], Muhammad Khan[1], Hany El-Hennawy[1], Alan Farney[1], Giuseppe Orlando[1], Amber Reeves-Daniel[2], Amudha Palanisamy[2], Michael Gautreaux[3], Samy Iskandar[3], William Doares[4], Scott Kaczmorski[4], Jeffrey Rogers[1].
[1]Surgery, Wake Forest, Winston-Salem, NC, United States; [2]Internal Medicine, Wake Forest, Winston-Salem, NC, United States; [3]Pathology, Wake Forest, Winston-Salem, NC, United States; [4]Pharmacy, Wake Forest, Winston-Salem, NC, United States.

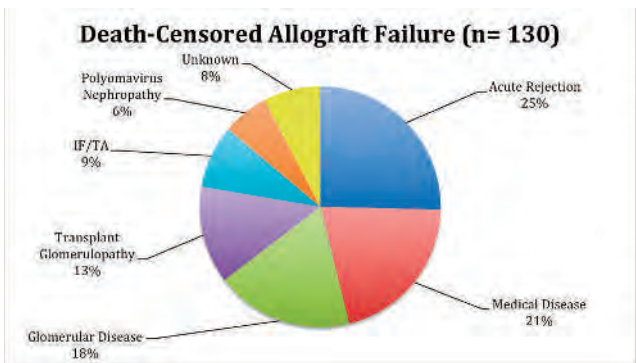
Introduction: Delayed graft function (DGF), defined as dialysis in the 1st week following kidney transplant (KT), is an early surrogate marker of organ quality and preservation. Previous studies have shown that DGF is a risk factor for graft dysfunction, acute rejection and graft loss. Rates of DGF are related to deceased donor (DD) age and category, being highest in donation after cardiocirculatory death (DCD) donors, intermediate in donation after brain death (DBD) expanded criteria donors (ECD) and lowest in standard criteria donors (SCD).
Methods: To determine the relative influence of DGF on DD subgroup outcomes, we performed a single center retrospective analysis in adult DD KT patients (pts). >85% of adult DCD and DBD/ECD kidneys were placed on machine preservation. All pts received depleting antibody induction with FK/mycophenolate ± steroids. We also stratified pts receiving dual adult kidneys (DKT) and pediatric en bloc (PEB) KTs and those receiving KTs from DDs with terminal acute kidney injury (AKI, defined as doubling of DD's admit serum creatinine [SCr] and a terminal SCr >2.0 mg/dl). Within each subgroup, we determined death-censored graft survival (DCGS) rates in pts ± DGF.
Results: Since 10/01, we performed 1499 DD KTs, including 603 DBD/SCD, 414 DBD/ECD, 261 DCD, 118 AKI, 69 DKT and 34 PEB. DGF rates were 11.8% in PEB, 21.7% in DKT, 23.1% in DBD/SCD, 23.9% in DBD/ECD, 41.5% in AKI, and 57.9% in DCD KT pts. DGF rates were significantly higher in DCD and AKI and significantly lower in PEB KT pts. With a mean follow-up of 64 months, DCGS rates in DBD/SCD (85% no DGF vs 64% DGF) and DBD/ECD (71% no DGF vs 45% DGF) KTs were significantly lower ($p<0.0001$) in pts with DGF. However, in the remaining 4 DD subgroups (DCD, AKI, DKT, PEB), DCGS rates were not different in pts ± DGF.
Conclusions: In pts receiving SCD and ECD kidneys from DBD donors, DGF continues to adversely influence medium-term outcomes. DGF was comparable in these 2 groups because of greater use of machine preservation in the DBD/ECD category. Moreover, the absence of DGF in DBD/ECD results in DCGS rates similar to pts with DGF following DBD/SCD KT. However, in DCD, AKI, DKT, and PEB DD KT pts, the negative effect of DGF on DCGS appears to be mitigated. In DCD and AKI DD KT, the mechanism of DGF may be related to terminal (and presumably reversible) warm ischemia whereas in DKT and PEB DD KT, implanting 2 kidneys may both reduce DGF and moderate its potential deleterious effects.

P.1303

The role of alloimmunity in death-censored kidney allograft failure

Mohamad Alkadi[1], Jim Kim[2], Choli Hartono[1,3], Meredith Aull[2], Thangamani Muthukumar[1].
[1]Department of Internal Medicine, Division of Nephrology, Hypertension and Transplantation Medicine, New York Presbyterian Hospital- Weill Cornell Medicine, New York, United States; [2]Department of Surgery, Division of Transplantation, New York Presbyterian Hospital- Weill Cornell Medicine, New York, United States; [3]Department of Internal Medicine, Division of Nephrology, The Rogosin Institution, New York, United States.

Introduction: Besides recurrent glomerular diseases and infection, alloimmunity is an important contributor of kidney allograft failure. In this study of kidney transplant recipients we sought to study the contribution of alloimmune factors to death-censored allograft failure.
Methods: At our center, 1670 kidney allografts were transplanted between November 1st, 2001 and December 31st, 2011. Among the 1670 recipients, 137 (8.2%) recipients developed death-censored kidney allograft failure during the follow up period (cases), excluding those with surgical complications and primary non-function of the allograft. We matched 130 of the 137 cases with 130 recipients who did not develop allograft failure during the follow up (controls). Cases and controls were matched 1:1 for (i) recipient age +/- 2 years, (ii) donor type (living or deceased) and (iii) calendar year of transplant.
We determined the cause of allograft failure in each case by reviewing the medical records and findings of the last available kidney allograft biopsy. The causes of allograft failure were divided into 7 categories: (1) acute rejection, (2) polyomavirus nephropathy, (3) transplant glomerulopathy, (4) glomerular diseases, (5) interstitial fibrosis/tubular atrophy, (6) medical condition, and (7) unknown.
We used conditional logistic regression to analyze the data. Variables that were significant at $p<0.1$ on univariate analysis were included in the multivariate analysis. Only those variables that had a significant level of $p<0.01$ were considered as independent risk factors in the final model.
Results: Only 130 cases had suitable matched controls and were included in our study. The median time from transplant to death-censored allograft failure was 29.4 months in the cases. The controls were followed for a median of 62.3 months. Alloimmunity accounted for more than a third of all causes of death-censored kidney allograft failure. Acute rejection was the main cause of death-censored allograft failure in 25% of cases (n=33), while transplant glomerulopathy was the primary cause of allograft failure in 13% of cases (n=17). Among recipients with acute rejection, non-adherence to immunosupression medcines were documented in 18 (55%). Acute rejection included acute cellular rejection (ACR, 52%), antibody-mediated rejection (AMR, 12%), and mixed cellular and antibody-mediated rejection (24%). The figure below shows the contribution of different categories to allograft failure.



The risk factors of death-censored allograft failure are shown in the table. Having an ACR episode was associated with death-censored allograft failure controlling for other variables. The odds of having at least one ACR episode were 11 times higher in cases than controls (p < 0.001).

			Univariate Analysis		Multivariate Analysis	
Variable	Cases (n=130)	Controls (n=130)	OR (95% CI)	P-value	OR (95% CI)	P-value
≥ 1 ACR episode, n (%)	51 (39%)	7 (5%)	9.80 (3.91-24.59)	0.000	11.33 (3.01-42.60)	0.000
≥ 2 for-cause biopsies, n (%)	78 (60%)	12 (9%)	17.50 (6.39-47.93)	0.000	22.57 (5.10-98.19)	0.000
≥ 3 Hospitalizations, n (%)	86 (66%)	32 (25%)	7.08 (3.68-14.07)	0.000	5.39 (1.83- 15.89)	0.002
New onset diabetes after transplant, n (%)	8 (6%)	20 (15%)	0.37 (0.16-0.88)	0.024	0.09 (0.02-0.53)	0.008
AMR episode, n (%)	30 (23%)	3 (2%)	14.50 (3.40-60.77)	0.000		
ACR within 1st year, n (%)	26 (20%)	6 (5%)	5.00 (1.91-13.06)	0.001		
BK viremia, n (%)	19 (15%)	5 (4%)	4.50 (1.52-13.29)	0.007		
CMV viremia, n (%)	17 (13%)	5 (4%)	5.00 (1.65-17.27)	0.011		
Steroid Maintenance at Discharge	47 (36%)	34 (26%)	1.72 (0.99-3.08)	0.067		
Best cr within first year < 1.5, n (%)	64 (49%)	97 (75%)	0.34 (0.19-0.59)	0.000		
HLA MM > 3, n (%)	95 (73%)	78 (60%)	1.77 (1.05-2.98)	0.032		
African American race, n (%)	38 (29%)	23 (18%)	1.88 (1.05-3.39)	0.035		
Male Donor, n (%)	85 (66%)	78 (60%)	0.64 (0.38-1.08)	0.093		
Donor Age, median year (IQR; 0.25 - 0.75)	59 (37-55)	41 (27-52)	1.03 (1.01-1.05)	0.001		
African American Recipient, n (%)	46 (35%)	36 (28%)	1.53 (0.80-2.72)	0.152		
Male recipient, n (%)	81 (62%)	72 (55%)	1.30 (0.81-2.09)	0.28		
History of pretransplant DM	29 (22%)	33 (25%)	0.83 (0.45-1.52)	0.538		
BMI ≥ 30	32 (25%)	23 (18%)	1.60 (0.84-3.05)	0.153		
Prior Transplant	19 (15%)	18 (14%)	1.07 (0.53-2.16)	0.857		
Current/Peak PRA > 50%	19 (15%)	14 (11%)	1.46 (0.68- 3.13)	0.339		
Thymo induction	111 (85%)	119 (92%)	0.50 (0.21-1.17)	0.109		
DGF, n (%)	41 (32%)	40 (31%)	1.05 (0.58-1.88)	0.882		

Conclusion: Despite advances in immunosuppression, alloimmune factors remain a significant contributor to death-censored allograft failure among kidney transplant recipients.

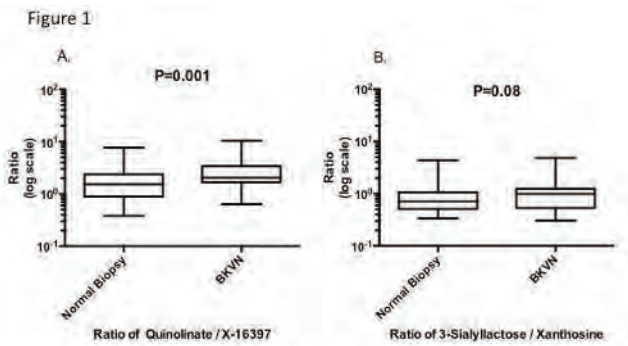
P.1304

Urine metabolomics: quinolinate, a product of tryptophan metabolism, is associated with BK virus nephropathy and intragraft inflammation in kidney transplant recipients

Darshana Dadhania[1], John Lee[1], Mohamad Alkadi[1], Thangamani Muthukumar[1], Catherine Snopkowski[1], Carol Li[1], Surya Seshan[1], Karsten Suhre[2], Manikkam Suthanthiran[1].
[1]Transplantation Medicine, Weill Cornell Medicine-NYPH, New York, United States; [2]Physiology and Biophysics, Weill Cornell Medical College - Qatar, Doha, Qatar.

Introduction: Metabolomics aims to measure all relevant small molecules (metabolites) and non-targeted metabolite profiling allows for the relative quantification of hundreds of metabolites in small volumes of biologic specimens such as urine. As both intermediate and endpoint markers of diverse biological processes in the human body, observations of altered metabolite concentrations provide access to functionally relevant read-outs of perturbed disease-associated pathways in human metabolism. We recently reported that the ratios of 3-sialyllactose to xanthosine (3-SL/X) and quinolinate to X-16397 (Q/X-16397) measured in cell free urine supernatants are diagnostic of Banff acute cellular rejection in human kidney allografts[1]. Herein, we examined whether these ratios distinguish patients with BKV virus nephropathy (biopsy positive for BKV) from those with surveillance biopsies without BKV and without histological abnormalities (normal biopsy results).
Methods: We used a comprehensive combination of non-targeted LC-MS/MS and GC-MS based metabolomics platforms (Metabolon Inc.) to analyze 107 biopsy-matched urine samples from 62 kidney allograft recipients. Among the 107 urine specimens, 33 were collected at the time of initial BKVN diagnosis (BKVN group), 14 at the time of follow up biopsy in the BKVN group and 60 urine specimens from 29 patients with normal biopsy results.
Results: Figure 1, the box and whisker plots, show that the ratio of Q/X-16397 is significantly higher in the 33 cell free urine supernatants collected at the time of initial BKVN diagnosis compared to the Q/X-16937 ratio in the 60 cell free urine supernatants collected from the 29 patients with normal biopsy results (median ratio: 2.04 vs. 1.53, P=0.001). The ratio of 3-SL/X was also higher in the BKVN group compared to normal biopsy group but this difference did not reach statistical significance (1.0 vs. 0.71, P=0.08). In the 14 patients who underwent a follow up biopsy following BKVN diagnosis, the Q/X-16937 median ratios were: 5.17 in the 3 patients with biopsies negative for BKV but positive for intragraft inflammation/tubulitis; 1.53 in the 5 patients with biopsies negative for BKV and without intragraft inflammation/tubulitis; and 3.11 in the 6 patients with biopsies persistently positive for BKV and intragraft inflammation/tubulitis.

Figure 1



Conclusion: Quinolinate is a product of tryptophan metabolism and by serving as a precursor for the biosynthesis of NAD+ may help meet the metabolic demands of activated immune cells. Our findings that the ratio of Q/X-16397 is associated with BKVN and with ongoing intragraft inflammation suggest that serial monitoring of Q/X-16397 ratio may be useful to personalize immunosuppressive therapy in patients diagnosed with BKVN.

References:

[1] Suhre K. et al. Urine Metabolite Profiles Predictive of Human Kidney Allograft Status. JASN. 2016; 27:626-636.

P.1305

Breast implants surgery in patient who had kidney transplantation

Marina Ratkovic[1], Nikolina Basic Jukic[2], Branka Gledovic [1], Danilo Radunovic [1], Vladimir Prelevic [1], Milena Dapcevic [3], Srdja Ilic[4], Danica Raicevic [4].
[1]Nephrology and Hemodialysis Department, Clinical Center of Montenegro, Podgorica, Montenegro; [2]Nephrology, Arterial Hypertension, Dialysis and Transplantation Department, Clinical Hospital Center Zagreb, Zagreb, Croatia; [3]Hematology Department, Clinical Center of Montenegro, Podgorica, Montenegro; [4]Radiology Department, Clinical Center of Montenegro, Podgorica, Montenegro.

Background: Immunosuppressed renal transplant patients have a higher incidence of carcinomas than the general population. The treatment of breast cancer in the transplant population is complicated by factors such as determining the correct dose of immunosuppressant, survival of transplant grafts, and doses of adjuvant chemotherapy.
Materials and Methods: case report study.
Results and Discussion: Female patient, 49 years old, with CKD (chronic kidney disease) diagnosed 14 years ago, with chronic glomerulonephritis. She developed ESRD (end stage renal disease) and was treated with hemodialysis for three months. Six years ago she was treated with kidney transplantation from deceased donor. After kidney transplantation she was on immunosuppressive protocol with cyclosporin (CsA), mycophenolate mofetil and prednisolone. Three years ago she was diagnosed with breast carcinoma. She was treated with right mastectomy, chemo and radiotherapy. Graft function remained good. Half of a year ago she was diagnosed with left breast carcinoma. She was treated with left mastectomy and with reconstructive surgery by bilateral breast implants surgery on her demand. In early postoperative period she developed an episode of acute graft rejection. She got fever with signs of implants inflammation and infection, sepsis, increased serum creatinine level, oliguria, anemia with echosonography signs of acute graft rejection. Patient was removed on nephrology department for further treatment. The patient was treated with pulse corticosteroid therapy with correction of immunosuppressive regimen, with wide spectrum antibiotics and other polysymptomatic therapy and with intensive therapy and nursing treatment. After two weeks of intensive therapy she recovered graft function.
Conclusion: All patients with kidney transplantation should be carefully prepared for all surgical procedures with adequate preparation with immunosuppressant dose regimen and monitored after surgical procedures.

References:

[1] Koonce, Stephanie et al. Breast Reconstruction After Solid Organ Transplant. Annals of Plastic Surgery 2015; 75 (3): 343-347.
[2] Roger Khouri, Daniel Del Vecchio. Breast Reconstruction and Augmentation Using Pre-Expansion and Autologous Fat Transplantation. Clin Plastic Surg 2009; (36): 269–280
[3] Behzad Einollahi. Genitourinary Neoplasia after Kidney Transplantation. Int J Nephrol Urol, 2010; 2(4): 491-496.
[4] Germaine Wong,Jeremy R. Chapman, Jonathan C. Craig. Cancer Screening in Renal Transplant Recipients: What Is the Evidence? Clin J Am Soc Nephrol 2008; 3: S87–S100.
[5] Kato et al. Early diagnosis and treatment of breast cancer in Japanese kidney transplant recipients: a single center experience. SpringerPlus (2015) 4:196 DOI 10.1186/s40064-015-0946-2.

P.1306

Case of rapamycine induced pancreatitis and deep venous thrombosis

Marina Ratkovic[1], Nikolina Basic Jukic [2], Branka Gledovic[1], Danilo Radunovic [1], Vladimir Prelevic [1], Milena Dapcevic [3], Srdja Ilic[5], Danica Raicevic [5].
[1]Nephrology and Hemodialysis Department, Clinical Center of Montenegro, Podgorica, Montenegro; [2]Nephrology, Arterial Hypertension, Dialysis and Transplantation Department, Clinical Hospital Center Zagreb, Zagreb, Montenegro; [3]Hematology Department, Clinical Center of Montenegro, Podgorica, Montenegro; [4]Hematology Department, Clinical Center of Montenegro, Podgorica, Montenegro; [5]Radiology Department, Clinical Center of Montenegro, Podgorica, Montenegro.

Background: Recently, evidence indicate that rapamycin may contribute to an increased risk of thrombosis. Researchers found that endothelial membrane remodeling induced by rapamycin is crucial for the adhesion of platelets to endothelial cells and thereby for thrombosis. Many investigations showed that rapamycin induces autophagy of pancreatic cells.
Materials and Methods: case report study.
Results and Discusssion: Male patient, 26 years old, was treated with preemptive kidney transplantation from living related donor. He was treated with thymoglobulin in induction therapy because of donor specific antibodies detected prior to transplantation. He received 100mg of thymoglobulin. Due to surgical complications, he had reperfusion graft injury and delayed graft function. Initial immunosuppressive protocol with thymoglobulin and tacrolimus was converted to rapamycin and dismissal of thymoglobulin. Patient was treated with LMWH (low-molecular-weight heparin) regular in preparation and after intervention. One month after rapamycin treatment he developed deep venous thrombosis of right leg. He was treated with intravenous heparin and symptomatic therapy with successful recanalization of venous vessels. Twenty days after rapamycin usage he developed abdominal pain typical for acute pancreatitis followed by increased serum concentrations of amylase and lipase and urine amylase concentrations. Patient was treated with polysymptomatic therapy with recovery of pancreas function and normalization of serum and urine concentrations of amylase and lipase. Finally, rapamycin was removed from the immunosuppressive therapy. Patient is with stabile graft function in the next year of follow up period without thrombosis episodes or episodes of pancreatitis.
Conclusion: All patients treated with rapamycin after kidney transplantation should be carefully monitored for venous thrombosis and pancreatitis events.
References:
[1] Adam D. Barlow, Michael L. Nicholson, and Terry P. Herbert. Evidence for Rapamycin Toxicity in Pancreatic b-Cells and a Review of the Underlying Molecular Mechanisms. DIABETES, VOL. 62, AUGUST 2013; 2674-2682.
[2] Zahr, Elsie et al. Rapamycin Impairs In Vivo Proliferation of Islet Beta-Cells. Transplantation 200;84 (12): 1576-1583.
[3] Christiane J. Bruns. Rapamycin-Induced Endothelial Cell Death and Tumor Vessel Thrombosis Potentiate Cytotoxic Therapy against Pancreatic Cancer. Clinical Cancer Research 2004; 10: 2019-2019.
[4] Tanemura M, Ohmura Y, Deguchi T, et al. Rapamycin causes upregulation of autophagy and impairs islets function both in vitro and in vivo. Am J Transplant 2012; 12: 102–114.
[5] Susann Stephan et al. Effect of Rapamycin Alone and in Combination with Antiangiogenesis Therapy in an Orthotopic Model of Human Pancreatic Cancer. Clinical Cancer Research 2004; 10: 6993-7000.

P.1307

Plasmapheresis and rituximab therapy in a second living-related kidney transplant patient with recurrent focal segmental glomerulosclerosis.

Marina Ratkovic[1], Nikolina Basic Jukic[2], Branka Gledovic [1], Danilo Radunovic [1], Vladimir Prelevic [1], Milena Dapcevic [3], Srdja Ilic [4], Danica Raicevic [4].
[1]Nephrology and Hemodialysis Department, Clinical Center of Montenegro, Podgorica, Montenegro; [2]Nephrology, Arterial Hypertension, Dialysis and Transplantation Department, Clinical Hospital Center Zagreb, Zagreb, Croatia; [3]Hematology Department, Clinical Center of Montenegro, Podgorica, Montenegro; [4]Radiology Department, Clinical Center of Montenegro, Podgorica, Montenegro.

Background: In focal segmental glomerular sclerosis (FSGS), the success of renal transplantation may be impaired by the frequent risk of recurrence of the disease on the allograft. In the first kidney allograft, 20 to 30% of patients develop recurrence of FSGS. Second grafts, in those who have had recurrence in their first graft, are generally accompanied by a further recurrence.
Materials and Methods: case report study.
Results and Discussion: Male patient, 32 years old, was diagnosed nephrotic syndrome and CKD in 27th year. He is from family with ADPKD. In the same year he developed ESRD and started with hemodialysis treatment. In 29th year he was treated with kidney transplantation from deceased donor. He developed multiple complications afterwards: delayed graft function, proteinuria, vein graft stenosis and ureteral obstruction treated with ureteral stent and ureteroneocystostomy. He was treated with plasmapheresis without success. He underwent 4 graft biopsies with recurrent FSGS findings with elements of acute rejection and acute tubular necrosis. He was also treated with rituximab and intravenous immunoglobulins. Due to many infection episodes and complications in the next period he underwent graftectomy one year after. He was treated with kidney transplantation from living related donor in 2014 without complications in postoperative period. Four months after transplantation he presented with proteinuria of 30 grams per day. After biopsy of transplanted kidney recurrent FSGS was pathologically confirmed. Patient was treated with plasmapheresis, corticosteroids, intravenous immunoglobulins and rituximab. Proteinuria was reduced to 0,4 grams per day and graft function is preserved.
Conclusion: Recurrence of FSGS after transplantation is relatively frequent in patients who lost a previous transplant from recurrence. In the case of living donation, the possibility of recurrence and its consequences should be clearly exposed to and discussed with the donor and the recipient.
References:
[1] Hristea et al. Successful treatment of recurrent focal segmental glomerulosclerosis after kidney transplantation by plasmapheresis and rituximab. Transplant International 2007: 20: 102–105.
[2] Park HS, et al. Plasmapheresis and rituximab in FSGS. Korean J Intern Med 2014;29:482-488.
[3] Meyer et al. Immunoadsorbtion and rituximab therapy in a second living-related kidney transplant patient with recurrent focal segmental glomerulosclerosis. Transplant International 2007; 20: 1066–1071.
[4] Carlos E. Araya and Vikas R. Dharnidharka. The Factors That May Predict Response to Rituximab Therapy in Recurrent Focal Segmental Glomerulosclerosis: A Systematic Review. Journal of Transplantation Volume 2011, Article ID 374213, 7 pages doi:10.1155/2011/374213.
[5] R. Y. Gohh, S. K. Shaffi: Long-Term Results of Early Plasmapheresis to Prevent Recurrence of FSGS in High-Risk Renal Transplant Recipients. Transplantationsmedizin 2010; 22: S 288.
[6] Xu et al: Immunosuppressive therapy in primary FSGS. J NEPHROL 2012; 25(05): 626-635.

P.1308

Complications of kidney transplantation performed through black organ markets affiliations

Marina Ratkovic[1], Nikolina Basic Jukic[2], Branka Gledovic [1], Danilo Radunovic [1], Vladimir Prelevic [1].
[1]Nephrology and Hemodialysis Department, Clinical Center of Montenegro, Podgorica, Montenegro; [2]Nephrology, Arterial Hypertension, Dialysis and Transplantation Department, Clinical Hospital Center Zagreb, Zagreb, Croatia.

Introduction: Until September 2012 many CKD patients went to third-world countries for living unrelated (paid) kidney transplantation, because of the lack of the transplantation program. These patients were deciding on the illegal purchase of the necessary transplantation in black market of organs such as India, Pakistan, or cadaveric transplantation in Russia. Complications these patients came back with were the best indicator how they were dangerous and risky.
Materials and Methods: data refer to patients from Montenegro who developed complications after living unrelated and paid cadaveric kidney transplantation abroad, in the period of last twelve years.
Results and Discussion: Among 12 patients who underwent living unrelated kidney transplantation in Pakistan, different complications were observed and treated upon arrival. There were 2 cases of severe bilateral pneumonia caused by Pneumocystis carinii, with a pleural rupture and respiratory insufficiency. One of these patients had tertian malaria, one acute Varicella Zoster infection, one pneumonia caused with AH1N1 Influenza. One patient had acute rejection and ileus in the same time; one with suprapubic cystostomy due to unresolved urethral stenosis before transplantation. One male patient developed spontaneous rupture of bladder and one rupture of both Achilles tendon. One patient developed acute adrenal insufficiency. Among two patients who underwent living unrelated kidney transplantation in India, one got severe polyoma BK infection and developed rapid loss of graft function. There have been 21 paid kidney transplantations from cadaveric donors performed in Moscow. Hyperacute rejection was noticed in two patients. Both of them underwent a re-transplantation one month after hyperacute rejection. One of them got thrombosis and arterial embolization of the graft. One developed an acute rejection three months after with unresolved urinary obstruction. In one patient abdominal aortic aneurysms was not resolved prior to transplantation, and the rupture occurred afterwards. One patient died after kidney transplantation in coma caused by Creutzfeldt–Jakob disease.
Conclusion: Our data show that over 50% of patients in group of living unrelated transplantation have returned with complications, as well as over 50% in the group of patients with deceased donor transplantation. A possible cause of high incidence of complications was the inadequate preoperative evaluation. Many of the patients were sent without treatment of post-operative complications. This gave us motivation to work on the establishment of the transplant system.
References:
[1] Sever M, Ecder M, Aydin AE, Türkmen A, I. Çaslan K, Uysal V et al. Living unrelated (paid) kidney transplantation in Third-World countries: High risk of complications besides the ethical problem. Nephrol. Dial. Transplant.1994; 9(4): 350-4.
[2] Jacob A Akoh. Key issues in transplant tourism. World J Transplant. 2012; 24; 2(1):9-18.
[3] Francis L. Delmonico. The development of the Declaration of Istanbul on Organ Trafficking and Transplant Tourism. Nephrol Dial Transplant. 2008; 23: 3381–3382.
[4] D.A. Budiani-Saberi, F.L. Delmonico. Organ Trafficking and Transplant Tourism: A Commentary on the Global Realities. American Journal of Transplantation. 2008; 8: 925–929.
[5] Gill JS, Goldberg A, Ramesh Prasad GV, Fortin M, Hansen TB, Levin A, Gill J, Tonelli M, Tibbles LA et al. Policy Statement of Canadian Society of Transplantation and Canadian Society of Nephrology on Organ Trafficking and Transplant Tourism. Transplantation. 2010; 90 (8): 817-820.
[6] The Declaration of Istanbul on organ trafficking and transplant tourism. Transplantation 2008; 86: 1013.
[7] Gill J, Madhira BR, Gjertson D, et al. Transplant tourism in the United States: A single-center experience. Clin J Am Soc Nephrol 2008; 3: 1820-1828.

P.1309

Does C1q SAB assay have any value in prediction of kidney transplantation outcome in highly sensitized patients?

Arpita Ghosh-Mitra, Sudip Roy, Nasifa Hasan, Soma Choudhury, Saheli Podder, Dilip Pahari.
HLA and Molecular Lab, Medica Superspecialty Hospital, Kolkata, India.

Introduction: With the wide spread availably of kidney transplantation, a large number of patients are now coming for second transplant. Similarly many patients are coming for operation after long years of dialysis. Such prospective recipients often become cross match (CDC with AHG augmentation) positive with their recipients. We perform SAB donor specific antibody testing to find who have truly anti HLA antibody. We have also preformed C1q binding SAB assay to find out which of the SAB antibodies complement fixing. We want to present our data with CDC positive transplantation with SAB negative or C1q SAB negative. Only in selected cases, transplantation performed with desensitization protocol with positive SAB cross match.
Materials and Methods: Complement dependent cytotoxicity (CDC) crossmatch had been performed with anti-human globulin (AHG) augmentation and DTT treatment. Single Antigen assay with C1q had been performed using Labscreen assay kit from One Lamda, USA.
Results and Discussion: A total 15 patients underwent kidney transplant with a positive CDC crossmatch (>10% positive). We have also performed C1q SAB assay all between the years 2014-2015. 4 patients have shown donor specific positive result against Class I HLA antigen and 1 patient was with no DSA against Class I. Similarly 2 patients have shown donor specific positive result against Class II HLA antigen and 8 had with no DSA against Class II. Those with a positive C1q SAB result were desensitized before transplant and negative patients underwent transplant without desensitization. Among the transplanted 4 patients with positive Class I C1q SAB, 1 patient exhibited with antibody mediated rejection after a month of transplantation. The patient with AMR did not respond to conventional therapies like plasmapheresis and IVIG treatment and underwent graft nephrectomy. All other patients either C1q Class I & II positive or C1q Class I and II negative did not have with any major episodes of antibody mediated rejection and are with stable graft function at one year.
The above data suggest that among 15 highly sensitized patients after confirming with C1q SAB, 6 patients were detected with complement fixing anti-HLA antibodies against either Class I or Class II. 9 patients among 15 were detected with negative complement fixing anti-HLA antibodies. Patients with positive complement fixing anti-HLA Class I antibodies underwent desensitization and allograft loss occurred ¼ patients (25%) of the C1q positive group. Patients with negative complement fixing anti-HLA antibodies did not require any desensitization and appeared with stable graft function without any rejection symptom.
Conclusion: Successful kidney transplant can be possible even in highly sensitized patients after prescreening with C1q SAB assay and desensitization protocol. Our study has shown that C1q positive Class I antibodies are highly associated with AMR and patients with C1q positive anti-HLA antibody should be considered as high risk for transplant rejection. Thus C1q SAB assay has become more predictive for a successful transplant.
Dr. Dilip Pahari; Medica Superspecialty Hospital Authority

P.1310

Bortezomib and antibody mediated rejection in renal allograft recipients

Tariq Farrah[1], Paul J.Phelan[1], Richard Battle[2], David Turner[2], Lorna Henderson[1].
[1]Department of Renal Medicine, Royal Infirmary of Edinburgh, Edinburgh, United Kingdom; [2]Scottish National Blood Transfusion Service, Histocompatibility and Immunogenetics, Royal Infirmary of Edinburgh, Edinburgh, United Kingdom.

Background: Antibody mediated rejection (ABMR) occurs in approximately 5% of compatible renal allografts but may occur more frequently in HLA/ABO incompatible allografts. ABMR is less responsive to conventional therapies and is associated with higher rates of allograft loss compared to T cell mediated rejection. The proteasome inhibitor, bortezomib, is a promising novel therapy for the treatment of ABMR. We report our experience of bortezomib the treatment in acute and chronic active ABMR.
Methods: We performed a retrospective analysis of renal allograft recipients who received bortezomib as part of multi-agent therapy for ABMR from 1 Jan 2013 to 1 Feb 2016. ABMR was classified by supportive pathology (microvascular inflammation, intimal arteritis) and the presence of circulating donor specific antibodies (DSA) with or without evidence of C4d staining. We collected data on allograft function, DSA levels (expressed as cumulative median fluorescence intensity, cMFI), allograft histology, graft and patient survival and adverse events.
Results: 10 patients received bortezomib for ABMR. 5 patients were diagnosed with acute active ABMR (median 8 days (6-13) post transplant) and all had pre-formed DSA. 5 patients were diagnosed with chronic active ABMR (median 1706 days (399 - 2753) post transplant) and all had *de novo* DSA. Patients also received plasma exchange (PEX) and intravenous immunoglobulin (IVIG, 100mg/kg). Median follow up from time of transplant was 728 days (233 – 3253) and from first dose of bortezomib was 473 days (226 – 881). In patients with acute active ABMR, mean percentage reduction in cMFI was 82% (+/-10) following treatment. Histological resolution of microvascular inflammation with an improvement in allograft function occured in 4 out of 5 patients on repeat biopsy. 4 patients had a functioning graft at latest follow-up with median eGFR of 50 ml/min/1.73m2 (range 19-55) with 1 graft failure due to transplant glomerulopathy. 1 patient died from opportunistic infection with a functioning graft, and 1 patient died from end stage renal disease due to graft failure. In patients with late ABMR, treatment resulted in 50% (+/-19) mean reduction in cMFI. However, 4 of 5 patients demonstrated on-going chronic active ABMR with transplant glomerulopathy on repeat biopsy. At latest follow up, 3 patients had a functioning graft with a median eGFR 65ml/min/1.73m2 (range 37-90) and 2 grafts failed with all patients surviving. No further severe adverse events were noted.
Conclusions: Our study, in a small cohort of patients, suggests that bortezomib, in combination with PEX and IVIG, is effective at reversing microvascular injury, reducing DSA cMFI, and improving allograft function in selected patients with acute ABMR. Its efficacy and role in chronic active ABMR remains to be defined. Bortezomib appears to be a safe and well-tolerated therapy in this setting. Larger, randomised controlled studies with longer follow up are needed to examine these findings.

P.1311

A flare up of de novo donor-specific antibodies following the ruptured ectopic pregnancy after kidney transplant from spousal donor

Cheol Woong Jung[1], Heungman Jun[1], Sung Yoon Lim[2], Myung-Gyu Kim[2], Kwan Tae Park[3].
[1]Department of Surgery, Korea University Anam Hospital, Seoul, Korea; [2]Department of Internal Medicine, Korea University Anam Hospital, Seoul, Korea; [3]Department of Surgery, Mongolia National University of Medical Science, Ulan Bator, Mongolia.

Introduction: De novo donor-specific antibodies (dn DSA) against the human leukocyte antigen (HLA) have been reported to be associated with poor renal allograft outcomes. In husband-to-wife transplants, the pregnancy from the spousal donor could be the significant triggering event for dn DSA.
Case Report: 28-years old woman with end-stage renal disease (ESRD) caused by IgA nephropathy underwent kidney transplant (KT) from a living unrelated spousal donor. The HLA antigens typing showed 3/6 loci mismatch and HLA-DQ locus was all mismatched. Her panel reactive antibody (PRA) to HLA class I and II were 0 percent. One year after KT, mycophenolate mofetil (MMF) was switched to azathioprine (AZA) for planned the pregnancy. She was pregnant several months later, but unfortunately she underwent emergency surgery for a ruptured ectopic pregnancy. After surgery, her serum creatinine was slightly up and PRA to HLA class II went up to 70 percent, while PRA to HLA class I remained 0 percent. Dn DSAs against HLA-DR, DQ were identified and the ranges of mean florescence intensity (MFI) were 3481 to 13510. She treated with four times of plasmapheresis, low-dose intravenous immunoglobulin (IVIG), and rituximab. Six months after desensitization, PRA to HLA class II declined to 20% and MFI also significantly reduced. In addition, serum creatinine level also has returned to normal.
Conclusion: KT increases the chances for pregnancy for women with ESRD. However, in husband-to-wife transplants, pregnancy could cause dn DSA which leads to poor graft outcome. Therefore, in these particular cases, the detailed immunologic work-ups and apposite treatment during pre and postpartum period, such as IVIG therapy should be considered.

P.1312

Relationship between de novo donor-specific anti-HLA-DQ antibody and chronic antibody-mediated rejection in kidney transplant recipients

Furusawa Miyuki, Ishida Hideki, Okumi Masayoshi, Shimizu Tomokazu, Unagami Kouhei, Tanabe Kazunari.
Tokyo Women's Medical University, Tokyo, Japan.

Background: Chronic antibody-mediated rejection (CAMR) is one of the most important factors that affect outcome of kidney allograft. It has been reported that de novo donor-specific anti-HLA antibodies (DSA) is related to CAMR.However, it remains still unclear about the relationship between the characteristics of de novo DSA and CAMR.
Objective: The aims of the present study were to: 1) clarify the de novo incidence rate, and 2) investigate the relationship between the characteristics of de novo DSA and CAMR in kidney transplant recipients
Patients and Methods: One hundred thirty-seven patients who had undergone kidney transplantation from 2000 to 2015 were screened for HLA antibody. HLA antibodies were detected using Luminex single antigen beads (One Lambda Inc.) with LABScan 100.
Results: DSA after kidney transplantation were detected in 50 patients (36%) and de novo DSA were demonstrated in 43 patients of these 50 patients (86%).In 43 patients with de novo DSA, 37 patients (86%) were possessed HLA-DQ DSA. Of these 37 patients with de novo HLA-DQ DSA, 27 patients (73%) were diagnosed with biopsy-proven acute AMR or CAMR 6 months or later post-transplant and 4 patients (11%) lost their kidney allografts.
Conclusions: De novo HLA-DQ DSA were detected more frequently compared with HLA-class I or -DR DSA in kidney transplant recipients with CAMR. Screening for HLA-DQ DSA after kidney transplantation seems to favorable to obtain better long-term outcomes of kidney allografts.

P.1313

Analysis of significance of vascular rejection in kidney transplant biopsy samples

Marek Novotny[1], Mariana Wohlfahrtova[1], Ondrej Viklicky[1], Eva Honsova[2].
[1]Dept. of Nephrology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [2]Dept. of Clinical and Transplant Pathology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic.

Introduction: Acute vascular rejection (AVR) is a severe clinical condition with detrimental impact on kidney allograft survival. Although it has been thought to be T-cell mediated process, recent clinical findings showed its association with donor specific alloantibodies (DSA) and resistance to conventional treatment. Therefore, correct assessment of AVR phenotype would be beneficial for improving long-term prognosis of kidney allograft.
Methods: We reviewed 940 patients who underwent kidney transplantation between 2010 and 2014 in order to identify those who suffered from AVR. AVR was defined as a presence of intimal arteritis (v) in both protocol and for cause biopsies. Retrospectively, the phenotype of AVR was assessed according to histopathologic finding and presence or absence of DSA. T-cell mediated vascular rejection (TCMRV) included severe tubulointerstitial inflammation (TI) and antibody mediated vascular rejection (AMRV) included features of microvascular inflammation, C4d positivity, DSA positivity in addition to v-lesion. As suspected antibody mediated rejection (sAMRV) were marked cases fulfilling 2 out of 3 features of antibody mediated rejection (AMR). Isolated v-lesion (IV) was assessed as unique group of AVR regardless of DSA presence.
Results: We identified 90 (9,6%) patients with at least one episode of AVR. 33 (37%), 21 (23%), 15 (17%) and 21(23%) patients were classified as IV, TCMRV, AMRV and sAMRV, respectively. Mean time of AVR diagnosis was 51 days after kidney transplantation. The 1-year death-censored graft survival was 100 % in IV group, 100 % in TCMRV group, 100% in sAMRV group, 94% in AMRV group. Steroid treatment was applied as a first line therapy to 25 (76%) patients in IV group, 16 (76%) in TCMRV, 2 (13%) in AMRV group and 7 (33%) in sAMRV group. Steroid-resistance, defined as need for second line treatment with more potent agents, occurred in 2 (8%) IV, 5 (31%) TCMRV, none AMRV and 3 (43%) sAMRV patients. Complex treatment of AMR consisting of intravenous immunoglobulines, plasmapheresis and rarely Rituximab and Bortezomib was the second most frequent therapy chosen for first line in 20 (22%) of patients. Antithymocyte globulin was indicated as the first line treatment in remaining 19 (21%) cases. Chronic rejection was observed in 5 (15%) patients in IV group, 2 (9,5%) in TCMRV, 5 (33 %) in AMRV, and 6 (28,5%) patients in sAMRV group during whole follow-up. Luminex was evaluated in 45 (50%) of AVR and found to be positive in 18 (40%) cases. Furthermore 8 (38%) patients in sAMRV group were evaluated for DSA but found to be negative.
Conclusion: We observed low incidence of graft failure due to AVR probably due to short time follow-up and intensive treatment. Incidence of chronic rejection was higher in AMRV and sAMRV groups. We failed to detect DSA in 8 (38%) patients in sAMRV group, carrying other features of AMR, which might be consistent with literature evidence for role of non-HLA antibodies in the process of AVR.
Supported by Ministry of Health of the Czech Republic, grant nr. 15-26519A.

P.1314

Clinical relevance of de novo donor-specific HLA antibodies (DSA) in renal transplantation

Florian Sommer[1], Teresa Kauke[2], Stefan Rüth[1], Matthias Anthuber[1].
[1]Department of Visceral and Transplant Surgery, Transplant Center Augsburg, Augsburg, Germany; [2]Department of Immunogenetics,, University of Munich, Munich, Germany.

Introduction: De novo donor-specific antibodies (DSA) are known to be correlated with poor graft outcome and the development of acute and chronic rejection.
Patients and Methods: In our prospective observation study we analyzed 218 renal transplant recipients in the period between 2006 and 2015. All patients were tested with Luminex solid-phase assay at different points in time (1, 3, 6, 12 months) after renal transplantation for the occurrence of de novo donor-specific antibodies. The data was correlated with clinical parameters and histopathological results from protocol and indication biopsies.
Results: One year after renal transplantation 37 patient (17%) developed de novo donor-specific antibodies. 67% showed positivity for DSA class 1, 22% for DSA class 2 and 11 % for both, class 1 and class 2.
Donor characteristics, such as age or gender, and characteristics of the recipient, such as age or waiting time had no influence on the development of DSA.
Factors such as the incidence of infections, (urinary tract infection, CMV or BK virus infection), cold ischemia time, differing modes of allocation and living related donation were found to have no influence on the development of DSA.
There was a strong correlation between the incidence of DSA in female recipients and poor organ quality, indicated by the elevation of creatinine prior to organ harvest.
Patients with a high immunological risk, either due to high PRA levels or former transplantations, patients with high overall mismatch, and patients whose immunosuppressive drugs had been reduced, had a significantly elevated risk of developing DSA.
Patients who developed DSA developed a significant reduction of graft function and a six times higher risk of graft failure with need to hemodialysis. Only 70% of the patients who developed DSA had a functioning graft one year after renal transplantation.
The incidence of rejection was significantly elevated one month after transplantation in the group of patients who developed DSA. One year after transplantation 49% of the patients who had developed DSA showed a histological proven rejection, in comparison to only 14% of the patients who had not developed DSA.
Discussion: Monitoring for the development of de novo DSA after renal transplantation identifies patients with a high risk for rejection episodes, decline of renal function and poor graft survival. The knowledge about the individual risk may help to stratify decisions on immunosuppressive regimes and reduction of immunosuppression and may help to reduce the risk of chronic antibody-mediated rejection (AMR).

P.1315

Clinical and pathological evaluations of combination therapy for active antibody mediated rejection in renal transplantation; a single-center experience

Masayuki Tasaki[1], Akira Kazama[1], Kazuhide Saito[1], Yuki Nakagawa[1], Naofumi Imai[2], Yumi Ito[2], Masahiro Ikeda[1], Hiroo Kuroki[1], Kota Takahashi[3], Yoshihiko Tomita[1].
[1]Division of Urology, Department of Regenerative & Transplant Medicine, Niigata Graduate School of Medical and Dental Sciences, Niigata, Japan; [2]Division of Clinical Nephrology and Rheumatology, Niigata Graduate School of Medical and Dental Sciences, Niigata, Japan; [3]Niigata Prefecture Organ Transplant Promotion Foundation, Niigata, Japan.

Introduction: Antibody-mediated rejection (ABMR) in renal transplant patients has poor allograft outcomes. However, treatment strategy has not been established yet.
Materials and Methods: We retrospectively identified seventeen patients who were diagnosed acute/active ABMR and or chronic,active ABMR according to Banff 2013 classification. The patients with clinical evidence of deteriorating grafted renal function such as the rising of serum creatinine level or abnormality of urinary examination received graft biopsies. If ABMR was suspicious by histology, anti-HLA antibodies was assessed by Luminex assay. Clinical and pathological changes were retrospectively analyzed.
Results: There were ten male and seven female patients. The age at kidney transplantation ranged from 4 years to 62 years (median, 32 years). 15 cases were living donor and two were deceased donor kidney transplantation. The median period to be diagnosed as ABMR was 44 months (range1-155 months). All patents had treatments within six months after AMBR was diagnosed. The median observation period after treatments for ABMR ranged from 4 months to 76 months (median, 22 months). The treatment for ABMR was consisted mainly of plasma exchange, low dose IVIG, rituximab, and steroid. Two patients received bortezomib. Evident improvement of renal function was shown in only one patient, and most of the patients had stable renal function after treatment. Two patients lost their graft function. Donor specific antibodies (DSAs) against HLA class I became negative in five of seven patients (71.4%). In contrast, HLA class II DSAs remained positive except for the patient treated with bortezomib. Histologic improvement were seen in scores for acute Banff components, but showed deterioration in chronic Banff components.
Discussions: The present study showed combination therapy for ABMR may contribute to stabilize of renal graft function. However, pathological results revealed that chronic damages were not improved, suggesting that chronic ABMR must be diagnosed before advanced histological damage. Additional administration of bortezomib could be considered in selected patients.
Conclusions: Combination of currently available drugs for ABMR has some effects for the stabilization of renal graft function and the healing of acute pathological damages from ABMR. However, early detection approach for chronic AMR should be established.

P.1316

Eculizumab rescue from acute antibody-mediated rejection and long-term stable graft function

Rolf Weimer[1], Philipp Boide[1], Nuray Bulut[1], Lucy Rainer[1], Hristos Karakizlis[1], Cyrill Wehling[2], Michael Kirschfink[2], Winfried Padberg[3], Fabrice Renner[1].
[1]Department of Internal Medicine, University of Giessen, Giessen, Germany; [2]Institute of Immunology, University of Heidelberg, Heidelberg, Germany; [3]Department of Surgery, University of Giessen, Giessen, Germany.

Introduction: The monoclonal anti-C5 antibody eculizumab has the potential to effectively prevent and treat acute antibody-mediated rejection (AMR). The Cornell data (Am J Transplant 2015), however, indicate that eculizumab does not prevent chronic AMR in recipients with persistently high donor-specific antibodies (DSA).
Case Reports: We here present two cases of successful eculizumab rescue from treatment resistant (steroids, immunoadsorption (IA), IVIG, rituximab, bortezomib) acute AMR in sensitized renal transplant recipients on Tacr/MPA/steroids (ATG induction) who both showed a slow but steady decline of the IgG DSA MFI values after two cycles of bortezomib and continuous eculizumab treatment resulting in long-term stable graft function (patient 1, 39 months posttransplant: S-Cr 1.4 mg/dl, eGFR 57ml/min; patient 2, 11 months posttransplant: S-Cr 1.2 mg/dl, eGFR 50ml/min). Patient 1 (deceased-donor second transplant, PRAmax 2%, pretransplant anti-HLA DQ7 IgG DSA in the single antigen assay (SAA)) experienced an acute also ATG-resistant AMR on day 44 (C4d negative; DSA in the C1q SAA positive). After a second cycle of bortezomib, the DSA nearly disappeared but completely recurred one year later so that eculizumab treatment was not terminated. Patient 2 (6 pregnancies, first renal transplant from her husband, PRAmax 0%; pretransplant multiple IgG DSA (SAA); desensitization: IA/IVIG, rituximab) showed a C4d-positive acute AMR on day 14 (SAA: IgG anti-HLA DQ7 and anti-HLA DQA1*05:05 DSA) with successful eculizumab rescue. In both cases, a temporary graft deterioration occurred due to insufficient complement inhibition which could be resolved by eculizumab dose adjustment. In a third patient (living-unrelated 4th renal transplant, ABO-incompatible, one DR/one DPB1 mismatch; PRAmax 45%; no SAA IgG or IgM nor non-HLA DSA detectable; desensitization: IA, IVIG, rituximab), an initially successfully treated acute C4d-negative AMR (day 15) was followed by a C4d-positive acute AMR at month 6, with already major chronic lesions. Eculizumab treatment (preceding steroid pulses, ATG, IA, IVIG) without bortezomib resulted in graft failure 17 months posttransplant due to chronic AMR.
Conclusion: Eculizumab may successfully be used for rescue therapy even in C4d-negative acute AMR. Failure may just be a consequence of underdosing resulting in incomplete complement inhibition. Bortezomib treatment appears to be necessary to achieve long-term stable graft function in sensitized renal transplant recipients after successful eculizumab rescue from acute AMR. Case 1 shows that continuous eculizumab treatment may be necessary in cases of DSA recurrence.
References:
[1] Cornell LD, Schinstock CA, Gandhi MJ, Kremers WK and Stegall MD. Positive crossmatch kidney transplant recipients treated with eculizumab: outcomes beyond 1 year. Am J Transplant 15: 1293-1302, 2015

P.1317

Do anti-HLA antibodies as detected by the Luminex assay have an impact on kidney allograft survival beyond 10 years post transplant?

Angeline Goh, A. Vathsala.
National University Centre for Organ Transplantation, National University Health System, Singapore, Singapore.

Although anti-human leucocyte antigen (HLA) antibodies are well-known to have deleterious effects on mid-term kidney allograft survival ie. up to 5-7 years due to the increased chronic graft rejection rates, their effect on long-term kidney allograft survival beyond 10 years remains unclear.

Using serum from multi-ethnic Southeast Asian kidney transplant recipients (KTX) currently on follow-up at the National University Centre for Organ Transplantation, a prospective cross-sectional study was performed. Each KTX had their serum checked once at least 1 year post-transplant for the presence of anti-HLA Class I and/or II antibodies using Single Antigen beads on a Luminex platform. Kidney allograft function was correlated with the presence (or absence) of these antibodies in KTX who were at least 10 years post-transplant with a functioning graft.

Amongst the 90 KTX with a functioning graft at least 10 years post-transplant, 53 (58.9%) were male, 62 (68.9%) were Chinese, 75 (83.3%) had end-stage renal disease (ESRD) due to chronic glomerulonephritis and 54 (60.0%) had received a live donor kidney transplant. These patients were transplanted between 1983 to September 2005. Only 17 KTX (18.9%) had graft dysfunction, defined as an estimated glomerular filtration rate less than 30 ml/min/1.73m2 and 22 KTX (42.3%) had proteinuria greater than 0.3g per day.

Using a mean fluorescent intensity (MFI) value of more than 1000 as a cutoff, 52 (57.8%) had anti-HLA Abs compared to 38 (42.2%) who did not. Of those with antibodies, 13 (25.0%) had Donor-Specific Antibodies (DSAs) with or without Cross Reactive Epitope Groups (CREGs), 21 (40.4%) had CREGs and 4 (7.7%) had anti-HLA antibodies that were non-DSA and non-CREG. Another 14 had only anti-Cw, anti-DP and/or anti-DQ antibodies which were unknown to be DSAs or CREGs as donor Cw, DP and DQ typing was not routinely done. The class distribution of anti-HLA antibodies in these patients was 19 (36.5%), 20 (38.5%) and 13 (25.0%) in Class I, Class II and a combination of both Class I and II antibodies, respectively.

The occurrence of anti-HLA antibodies was not significantly associated with gender, race, etiology of ESRD, age at time of transplant or the type of transplant performed. Neither was the presence of anti-HLA antibodies associated with graft dysfunction or the presence of proteinuria. MFI of antibodies was likewise not significantly different for Class I vs. Class II antibodies, DSA vs. CREG and for those with or without graft dysfunction, proteinuria or prior rejection.

We show here for the first time that there is no evidence of clear deleterious effect of anti-HLA antibodies, as detected by the single bead Luminex assay, on long-term kidney allograft survival in a multi-ethnic Southeast Asian kidney transplant population. Further studies to elucidate the individual characteristics of these antibodies, including antibody subclass typing, complement binding ability and epitope specificity are clearly warranted.

National Medical Research Council

P.1318

Long term follow up glycemic status of live kidney donors

Selene Teoh, Angeline Goh, Lee Shi Hui, Anantharaman Vathsala.
National University Centre for Organ Transplantation (NUCOT), National University Hospital, Singapore, Singapore.

Introduction: Development of diabetes mellitus (DM) following kidney donation may be associated with an increased risk of end stage renal disease due to diminished renal reserve following uninephrectomy. Hence screening for DM prior to donation and followup of donors for occurrence of impaired glycemic states is important. This retrospective study analyzed the glycemic control of live kidney donors (LKDs), prior to and after donation and evaluated their burden of cardiovascular risk factors.

Objectives: To study the course of LKDs who develop pre-diabetes and diabetes mellitus (DM) on follow-up and associated risk factors.

Methods: Study population included LKDs from 2008-2014 who were followed up for at least 6 months post-donation. Patient demographics, baseline glycemic status and clinical information were obtained from our living kidney donor registry and medical records. American Diabetes Association (ADA) guidelines were used to define diabetes and Impaired Glucose Tolerance (IGT).

Results: Of the 67 patients included in the study, majority was male (52.2%), Chinese (70.1%) and mean age of donation was 46.8±10.9 years. Mean time of follow-up post-donation was 36.4±21.5 months. At baseline, all LKDs had normal fasting plasma glucose. An oral glucose tolerance test (OGTT), mandated by presence of family history of diabetes revealed impaired glucose tolerance (IGT) in 3 LKDs but these values had normalized on repeat OGTT done at a median of 4 months later (range 3.8-11.5 months), permitting donation to proceed. Overall, at followup post donation, 2 (3%) had developed DM, 1 patient each had developed IGT and gestational DM that resolved after delivery. Of the 3 with IGT at initial screening that had resolved prior to donation, one (33.3%) developed DM 27.6 months post-donation. None of these LKDs with DM or IGT developed significant proteinuria. At the end of follow-up, 10 LKDs (14.9%) had a BMI >30 compared to 6 (8.9%) pre-donation. 80.6% of patients had hyperlipidemia compared to 25% at baseline. Both patients that developed DM had a BMI of >30.

Conclusion: The development of impaired glycemic states is infrequent in living donors due to stringent screening practices pre-donation. However, early diagnosis remains imperative especially in LKDs to mitigate potential renal complications of diabetes. Screening and long term monitoring of glycemic status especially in the presence of increasing burden of other metabolic risk factors over time remains an important aspect of care of LKDs.

P.1319

Anti-HLA antibody reduced graft survival in living-related transplants: Over 20-years experience

Kazuo MIzutani.
urology, Tokyo Women's Medical University, Medical Center East, Tokyo, Japan.

Introduction: Recently, many studies showed effects of HAL antibody to kidney transplants. However, longer behavior and impact of HLA antibody were not clear.
Objectives: In this study, the role of HLA antibodies in allograft rejection was examined utilizing a unique resource of sera collected annually and stored over a 23-year period from patients with rejected or retained grafts.
Methods: We selected 60 transplant patients who received kidney from 1984 to 1999, and whose serum samples were available annually. During 23 years, 32 patients were rejected their grafts and 28 had functioning grafts. Their samples were tested for HLA Class I and Class II antibodies by flow cytometry, ELISA, or cytotoxicity. For analyzing specific HLA antibodies, we used Labscreen Single antigen (One Lambda).
Results: HLA antibodies were found in 81% of patients who rejected grafts, compared to 50% with functioning transplants (p=0.0001). In addition, even if living-related transplants had good kidney function for a longer time, some living-related patients gradually developed HLA antibodies with stable functioning grafts.
Conclusions: n conclusion, patients who rejected transplants had HLA antibodies more frequently than those with functioning grafts. These antibodies found in the peripheral circulation, and their association with failure is consistent in long period.

P.1320

Assessment of eGFR and one-hour biopsy using in-situ-cooling double-balloon catheters in deceased kidney transplants

Kazuo MIzutani.
Urology, Tokyo Women's Medical University, Medical Center East, Tokyo, Japan.

Introduction: The shortage of deceased donor kidneys for transplantation has become a worldwide issue in the past decades. However, both availability and feasibility of marginal deceased donor kidneys are still problematic. To increase donor pool, we created a specially designed in-situ cooling system.
Objectives: The purpose of this study was to estimate availability of deceased donor kidneys, analyze donor one-hour biopsy, and better evaluate methods to estimate donor/recipient kidney function other than using donor Cr, comparing with living related transplants.
Methods: We studied 129 deceased renal transplant recipients (DD) who received kidneys from non-heart-beating donors beginning in 1984. Donors were in Maastricht Donor Categories III and IV and to minimize warm ischemic kidney damage we performed in situ cooling with specially designed double-balloon catheters. Twenty-nine living-related transplants (LD) were a control group.
Results: In the DD group average donor Cr and eGFR levels at admission were 0.3-2.1mg/dl (ave. 1.0) and 24-138 ml/min/1.73 (ave. 67) and levels before death were 0.3-15.9 (2.7) and 4-164 (34). Average recipient Cr and eGFR levels at discharge were 0.3-5.3 (1.8) and .10-133 (39). To define kidney function after transplant, the DD were classified according to recipient eGFR at discharge: <25 for the poor function group (PF: n=32) and >25 for the good function group (GF: n=95). GF had higher eGFR levels at donor hospital admission than the PF (p=0.005). There was no statistically significant difference in Cr levels of donor (at admission and before death) between those groups. Pathologically, the DD with less glomerular sclerosis of 1-hour biopsies had better graft survival than damaged subjects (p=0.015). Other histological scores were not associated with kidney survival.
Conclusions: eceased kidney transplants had excellent renal function with our double balloon catheter system. Compared with donor Cr levels, eGFR and 1-hour biopsy could be useful for donor evaluation and transplant renal function.

P.1321

Phytohemagglutinin-induced L2 mRNA as a useful marker of kidney transplants

Kazuo MIzutani.
Urology, Tokyo Women's Medical University, Medical Center East, Tokyo, Japan.

Introduction: Recently, as we have treated kidney transplant patients with multiple drug therapy, comprehensive and correct evaluation of immunosuppressed patients can not be implemented by blood level monitoring alone. In addition, the efficacy and toxicity of immunosuppressive drugs vary among patients. Currently, no diagnostic test is available to personalize the use of each drug. Even if levels of calcineurin inhibitors were suitable, some transplants had rejection episodes. Therefore, new approaches are needed for the net immunosuppressive state of the kidney transplants.
Objectives: We introduced a new concept of ex vivo gene expression analysis, where drug action was stimulated under physiological condition. This new model was applied to kidney transplants in our institutes. In this study, we report new developement such personalized immuo-monitorings and show one case with cellular rejection
Methods: Patients with kidney transplantation were recruited after the protocol was approved by the institutional review board. Blood was drawn periodically before and after transplantation and tacrolimus or cyclosporine treatment (CI). Whole blood was stimulated at 37°C for 2 hours with either solvent or phytohemagglutinin (PHA) in triplicate, then IL2 and other cytokine mRNA was quantified by the method we developed (Clin Chem 52:634, 2006).
Results: PHA-induced mRNA expression was compared before and after CI treatment in pre-transplant patients. PHA-induced interleukin-2 (IL2) mRNA expression was significantly ($p<0.05$) reduced after CI treatments. During their follow-up, one living-related transplant had cellular rejection and failed transplanted kidney. In this case, PHA-indeced IL-2 mRNA increased before rejection episode although the expression of other cytokines mRNA had not changed. After IL2-mRNA expression increased, the levels of serum creatinine increased gradually and her transplanted kidney was rejected finally. Other patients without rejection did not have any change of IL-2 mRNA.
Conclusions: The quantification of PHA-induced IL2 mRNA will be a useful tool for the development of personalized medicine diagnostics for monitoring immunosuppressive status and find rejections before increased serum Cr.

P.1322

C4d binding correlated with strong HLA antibodies in kidney transplantation

Kazuo MIzutani.
Urology, Tokyo Women's Medical University, Medical Center East, Tokyo, Japan.

Introduction: Apillary C4d was an established marker of antibody-mediated rejection in kidney transplants. Recently, Wahrmann, et al. showed that C4d can be detected on flow cytometry beads after C4 was bound to anti-HLA antibodies, and C4d fixation to antibodies against HLA-coated beads is a novel way to test for complement fixing antibodies in the serum. They postulated that this assay may detect antibodies which are complement-activating or non-complement-activating, therefore revealing the antibodies which play an important role in graft rejection.
Objectives: Our aim in this study is to check the possibility that this assay might be useful in C4d fixation to antibodies.
Methods: We applied this with FlowPRA and LABScreen to sera from patients who had either failed grafts (n=101) or functioning grafts(n=108), to check the possibility that this assay might be useful in distinguishing two types of antibodies (complement-activating or non-complement-activating antibodies).
Results: In 101 Failure patient samples, 50 sera were positive in IgG anti-HLA antibodies (HLA class I: 32 positive, class II: 37 positive). In 108 Function patient samples, 11 patients were positive in IgG anti-HLA antibodies (HLA class I: 8 positive, class II: 7 positive). Compared with the Failure patients, the frequency of HLA antibody positive patients were statistically significant. ($p<0.0001$) We found that C4d fixing antibodies were present in the sera of patients who subsequently had graft failure (39 in 101 failure cases), but found only a few C4d fixing antibodies in the sera of patients with functioning transplants (3 in 108 function cases) ($p<0.0001$). In later antibody dilution studies, we noted that C4d fixation occurs only at high titers of antibodies, and fluorescence was lost as sera were diluted further.
Conclusions: We concluded that the peak fluorescence levels of antibodies are more important that those of C4d. The quantitative aspect of antibody testing using the peak fluorescence value appears to be a valuable factor when examining anti-HLA antibodies post-transplantation.

P.1323

Effective monitoring of CMV infection by quantitative analysis of CMV mRNA. Case report in renal transplant

Kazuo MIzutani.
Urology, Tokyo Women's Medical University, Medical Center East, Tokyo, Japan.

Introduction: The virus infection is very important for renal transplants, and, there is no effective monitoring to show personalized immunosuppressive level with virus infection in transplants. We report new developement such personalized immuo-monitorings and show one case with Cytomegalovirus (CMV).
Methods: Five patients with kidney transplantation were recruited after the protocol was approved by the institutional review board. Blood was drawn periodically before and after transplantation and tacrolimus (FK) treatment. Blood was stored at 4°C overnight, then 50 mL each of whole blood was stimulated at 37°C for 2 hours with either solvent or phytohemagglutinin (PHA) in triplicate, then various mRNA was quantified by the method we developed (Clin Chem 52:634, 2006).
Results: PHA-induced mRNA expression was compared before and after FK treatment in patients before transplantation. As shown in (A), PHA-induced interleukin-2 (IL2) mRNA expression (normalized by the levels of b-actin (ACTB)) was significantly (p=0.05) reduced after FK treatment, except 1 case where baseline IL2 expression was very low probably due to the immune impairment by kidney dysfunction. FK was no effect on other inflammatory cytokine mRNA expression (A). Among 5 participants, 1 patient developed transient cytomegalovirus (CMV) infection. FK treatment inhibited PHA-induced IL2 mRNA expression before and during CMV infection. Interestingly, PHA-induced INFg mRNA expression was increased approximately 10,000 folds during and after CMV infection, and such increase was inversely correlated with the reduction of CMV positive cells (B).
Conclusion: The quantification of PHA-induced mRNA will be a useful tool for the development of personalized medicine diagnostics for immunosuppressive drugs.

P.1324

Donor specific HLA-antibodies are a risk factor for graft dysfunction in both presumptive BKVN and biopsy positive BKVN

Essa Abuhelaiqa, Jun Lee, Steven Salvatore, John R.Lee, Thangamani Muthukumar, Vijay K.Sharma, Surya Seshan, Manikkam Suthanthiran, Darshana Dadhania.
Transplantation Medicine, Weill Cornell Medicine-NYPH, New York, United States.

Background: BK virus associated nephropathy (BKVN) is a significant cause of graft dysfunction and graft failure. To prevent these complications, kidney transplant recipients are routinely monitored for BKV viremia and reduction in immunosuppressive therapies is initiated based on Presumptive BKVN diagnosis[]. Presumptive BKVN is defined as the presence of ≥10,000 BKV copies/mL of blood[2]. Reduction of immunosuppressive therapies has been linked to the development of de novo donor specific HLA-antibodies (DSA) and acute rejection. As a result, it is not clear if early intervention at the time of Presumptive BKVN, prior to significant histological injury, would impact long-term outcomes. Herein, we tested the hypothesis that recipients with Presumptive BKVN would have lower incidence of de novo DSA and improved graft function compared to recipients with Biopsy Positive BKVN.
Methods: We performed a retrospective review of kidney allograft recipients diagnosed with Presumptive BKVN (n=39) and Biopsy Positive BKVN (n=35) from 3/2008 to 7/2013 and evaluated the risk factors and incidence of graft dysfunction, defined as an increase in serum creatinine of ≥0.5mg/dL at 12 months following BKVN diagnosis. We also evaluated the frequency of de novo DSA and its relationship to graft function.
Results: The baseline variables between the Biopsy Positive and Presumptive BKVN cohorts were similar except the mean serum creatinine level at the time of BKVN diagnosis was higher in the Biopsy Positive BKVN compared to the Presumptive BKVN (Table 1, P=0.0001). Thirty-four percent of patients with Biopsy Positive BKVN and 21% of patients with Presumptive BKVN developed de novo DSA (P=0.11) during the first 6 months following BKVN diagnosis. Graft dysfunction at one-year following Biopsy Positive BKVN was significantly higher compared to Presumptive BKVN group (34% vs. 10%; P=0.01). After adjusting for biopsy creatinine values, the development of de novo DSA remained as a significant predictor of graft dysfunction at one-year (OR=4.8, 95% CI: 1.4 - 16.1; P=0.01).

Table 1.

Baseline Variables	Biopsy Positive BKVN	Presumptive BKVN	P value
Age	59± 11	57± 13	0.59
Female Gender (%)	29%	23%	0.59
AA Race (%)	29%	13%	0.09
Deceased Donor Kidney (%)	60%	54%	0.59
ATG induction (%)	91%	92%	0.89
Prednisone Maintenance (%)	20%	50%	0.06
Months Post-Transplant (mean ± SD)	15±15	11 ±14	0.13
Creatinine at time of Diagnosis	2.0 ± 0.6	1.5 ± 0.4	0.0001
Outcome Variables			
De Novo DSA	37%	21%	0.11
Graft Dysfunction	34%	10%	0.01

Conclusion: Kidney allograft recipients with Presumptive BKVN diagnosis had lower serum creatinine levels and lower incidence of graft dysfunction compared to patients with Biopsy Positive BKVN. Kidney recipients with Presumptive as well as Biopsy Positive BKVN are at risk for developing de novo DSA and de novo DSA is an independent risk factor for subsequent decline in graft function. Strategies aimed to prevent DSA following BKVN diagnosis may improve graft outcomes in patients with Presumptive or Biopsy Positive BKVN.

References:

[1] Hirsch HH et al. Reducing Immunosuppression Preserves Allograft Function in Presumptive and Definitive Polyomavirus-Associated Nephropathy. Am J Transplant 2010; 10:2615-2623.
[2] Hirsch HH et al. Prospective study of polyomavirus type BK replication and nephropathy in renal-transplant recipients. N Engl J Med 2002; 347:488-496.

P.1325

Polyomavirus associated nephropathy with concurrent recurrence of IgA nephropathy in a post kidney transplant patient: A case report

Ramona Espera-Lobaton.
Section of Nephrology, St Luke's Medical Center, Quezon City, Philippines.

The patient is a 31 year-old, single, Filipino, female, who is a diagnosed case of ESRD secondary to IgAN and Hypertension on January 2010, initially underwent thrice weekly hemodialysis until she underwent kidney transplantation (LRD) on December 2010 and was maintained on MMF, Tacrolimus and Prednisone. Post transplantion creatinine was 0.96 mg/dL. She was treated for IgA recurrence a year prior to the renal biopsy with Prednisone 30mg/day then tapered to 20mg/day. Renal function was
stable until 8 months prior to biopsy until she had several admissions due to Pneumonia and Pulmonary
TB. The creatinine remained elevated up to 2.24 mg/dL, hence she underwent kidney biopsy which showed acute and chronic tubulointerstitial nephritis associated with PyVAN, IgAN (oxford classification
M0, S1, E0, T1), moderate interstitial fibrosis and tubular atrophy and, negative C4D staining. Treatment for PyVAn was prioritized because graft loss in PyVAN is higher compared to IgAN. Tacrolimus was decreased to 1mg/day and eventually shifted to Everolimus 1.5mg/day, MMF 500 mg/day and was eventually discontinued and; Prednisone 5mg/day. She was given
Immunoglobulin 200 mg/kg and was started on Leflunomide 40 mg OD. The latter was discontinued due to hematologic side effects. The creatinine still continued to rise up to a level of 7 mg/dL, hence, she was advised hemodialysis while in the process of work up for repeat Kidney Transplantation. The diagnosis of PyVAN should be considered in post transplant patients with unexplained rising creatinine. The risk for graft loss is high if appropriate management is not administered promptly.

P.1326

Sirolimus leads to negative conversion of BK virus in renal transplantation patients

Young Nam Roh[1,2], Hye Yeon Jang[2], Sang Youb Han[2,3].
[1]Surgery, Inje Univ. Ilsan-Paik Hosp., Goyang, Korea;
[2]Transplantation Center, Inje Univ. Ilsan-Paik Hosp., Goyang, Korea;
[3]Internal Medicine, Inje Univ. Ilsan-Paik Hosp., Goyang, Korea.

BK virus nephropathy is one of the important causes of allograft failure in kidney transplantation. Although dose reduction of immunosuppressant is a treatment of choice, it is not always effective. As sirolimus has antiproliferative properties, a few reports sirolimus has BK viiral activity. We prescribed sirolimus in four patients who were resistant to dose reduction of immunosuppression, leflunomide and ciprofloxacin.
All patients received kidney from deceased donor. All patients except 1st patient was treated with basiliximab-based induction. The 3rd patient had diabetes and congestive heart failure. The 4th patient had diabetes. When BK viremia (>4 log copies/ml) occurred, antimetabolites such as enteric-coated mycophenolate sodium were stopped in all patients. If BK viremia persisted a, tacrolimus was reduced or changed to cyclosporine. Lefluonamide or ciprofloxacin, and sirolimus were treated in case of persisted BK viremia. BK viremia occurred 5-17 months after kidney transplantation. Duration to negative conversion of BK viremia was 2-15 months (Table). As allograft biopsy showed BK nephropathy and borderline change for acute T cell mediated rejection in 4th patient, enteric-coated mycophenolate sodium changed into sirolimus at the time of BK nephrpathy.
Although the cause of clerance of BK virus is not clear, sirolimus can be considered for treatment option in patients who are resistant to dose reduction of immunosuppressants.

	BK virema after KT(mo)	Highest BK (log copies/ml)	Duration (mo) to negative conversion	Cr (mg/dL) at diagnosis and clear up of BK viremia	Tacrolimus (ng/mL) at diagnosis	Sirolimus(ng/mL) at negative conversion
1	6	10.2	15	1.78>2.66	4.4	7.5
2	13	4.5	2	1.53>1.68	5.2	8.5
3	17	5.2	7	1.19>2.48	6.2	3.6
4	5	4.6	3	1.82>1.86	4.1	3.5

P.1327

Clinical outcomes of BK virus associated nephropathy in kidney transplant recipients

Woo Yeong Park, Kyubok Jin, Seungyeup Han.
Department of Internal Medicine, Keimyung University School of Medicine, Keimyung University Kidney Institute, Daegu, Korea.

Background: BK virus-associated nephropathy (BKVN) is an important risk factor causing to be an allograft dysfunction and loss in kidney transplant recipients. Currently, the reduction of immunosuppressant is a general treatment, but a long-term prognosis of BKVN has not been certain.
Objectives: We evaluated clinical outcomes and prognostic factors of graft failure in kidney transplant recipients with BKVN.
Methods: We retrospectively analyzed the medical records of 14 kidney transplant recipients diagnosed to BKVN by allograft biopsies in Dongsan medical center from 2001 to 2014. The change of allograft function and graft survivals after BKVN diagnosis were evaluated.
Results: Median time from transplantation to BKVN was 6.9 months (range, 4.6 to 8.8) and the follow-up duration after BKVN was 56.6 months (range, 11.3 to 118.2). The mean serum creatinine level and estimated glomerular filtration rate (eGFR) at diagnosis were 2.9 ± 1.3 mg/dL and 45.7 ± 17.3 mL/min/1.73m2. Acute rejection was accompanied in 10 (71.4%) patients at graft biopsies. Graft failure occurred in 8 (57.1%) patients during the follow-up period. The rate of acute rejection at the time of BKVN diagnosis was significantly higher in graft failure group than in non-graft failure group (P=0.015). eGFR at diagnosis was lower in graft failure group than non-graft failure group, but did not differ significantly between the two groups. The degree of change of eGFR before and after BKVN diagnosis showed greater tendency in graft failure group. There were no significant differences according to pathologic stage of BKVN and treatment modalities between the two groups.
Conclusions: Clinical outcomes of BKVN were very poor, and especially, BKVN patients combined with acute rejection at the time of BKVN diagnosis progressed to graft failure.

P.1328

BK polyomavirus prophylaxis with ciprofloxacin in kidney transplant recipients: A prospective study

Xiaopeng Yuan, Chuanbao Chen, Jian Zhou, Changxi Wang.
Third Division of Organ Transplant Center, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, People's Republic of China.

Objectives: BK polyomavirus (BKV) infection is a significant cause of renal graft loss. Fluoroquinolones can inhibit BKV replication in vitro and small retrospective studies have shown it to be an effective prophylactic agent against BK viremia. We prospectively observed the effect of ciprofloxacin prophylaxis on the BKV infection in renal transplant recipients.
Materials and Methods: From January 2013 to December 2014, 54 kidney transplant recipients (39 cases from donation after brain death, 15 cases from donation after circulatory death) received ciprofloxacin (250mg twice daily) for 90 days (Group 1). The rate of BKV infection was compared to the results of another prospective study conducted in our center in which 90 kidney transplant recipients were included (Group 2).
Results: At 1 year, Group 1 had a significantly lower rate of BK viruria than Group 2 (24.1% vs. 45.6%, P = 0.013). Group 1 had numerical lower rate of viremia (9.3% vs. 22.2%, P = 0.068) and BKV-associated nephropathy (1.9% vs. 5.6%, P = 0.41) than Group 2, but the difference did not attain statistical significance.
Conclusions: Ninety-day ciprofloxacin prophylaxis in kidney transplant recipients is easily tolerated and is seemly associated with a lower rate of BKV infection at 1 year. Randomized controlled clinical trials is warranted to determined the effect of fluoroquinolones on the development of BK viremia in kidney transplant recipients.

P.1329

Implantation biopsy of cardiac death donors and its implication in predicting renal graft outcomes

Xiaopeng Yuan, Chuan-bao Chen, Jian Zhou, Xiao-ping Wang, Ming Han, Chang-xi Wang, Xiao-shun He.
3rd Division of Organ Transplant Center, Eastern Campus of First Affiliated Hospital, Sun Yat-Sen University, Guangzhou, People's Republic of China.

Objectives: To determine the utility of donor implantation renal biopsy to predict the function and survival of renal grafts from donation after cardiac death (DCD) donors.
Methods: Preimplant kidney biopsy from DCD donors from July 2011 to December 2014 was retrospectively reviewed. The rate of glomerulosclerosis (GS) and Remuzzi score of the kidneys were calculated and the effects of GS and Remuzzi score on the renal graft function and survival time were evaluated.
Results: There were 71 cases of DCD donors including 14 cases of China Category II and 57 cases of China Category III. Mean donor age was 38.0 ± 10.2 years. The causes of death were brain trauma (n = 25), cerebrovascular accident (n = 38), brain tumor (n = 2), anoxia (n = 6). Needle biopsies were performed in 41 cases with glomeruli 23.7 ± 9.6 (2 strips), wedge biopsies were performed in 30 cases with glomeruli 37.2 ± 16.7. The 12 months estimated glomerular filtration rate (eGFR) of renal grafts with GS 0 - 20% was significantly higher than grafts with GS > 20% (72.9 ± 22.6ml/min vs. 48.9 ± 18.9ml/min，P < 0.001)), and eGFR of renal grafts with Remuzzi score 0 - 3 was significantly higher than grafts with Remuzzi score 4 - 5 (76.3 ± 21.4ml/min vs. 44.0 ± 10.9ml/min，P < 0.001)). With a follow-up of 14 - 40 months, Kaplan-Meier analysis showed that there were no statistical differences in graft survival between kidneys with GS 0 - 20% and GS > 20%, and between kidneys with Remuzzi score 0 - 3 and 4 - 5.
Conclusions: Kidneys from DCD donors with GS > 20% or Remuzzi score 4 - 5 have poor graft function at one year after transplantation but with excellent short-term graft survival.

P.1330

Retrospective review on the prevalence of hyperuricaemia before and after renal transplantation and the risk factors predicting hyperuricaemia after renal transplantation in a single centre

Siu Kim Chan, Zi Chan, Yiu Wing Ho, Chun Hay Tam, Wing Chung Anthony Tang, Sze Ho Sunny Wong, Chi Kwan Darwin Lam.
Medicine & Geriatrics, United Christian Hospital, Kowloon, Hong Kong.

Introduction: Hyperuricaemia is common in patients with uraemia. Urate level is related to kidney function and logically improvement after renal transplantation could be observed[1]. This study serves the purpose of reviewing the magnitude of this problem after renal transplantation and the treatment received early post-transplant period in Chinese population.

Materials and Methods: 164 new transplant patients were randomly selected from the period 2005-2014. Age, sex, urate level before transplantation, used of urate lower agents, urate level and eGFR 6 months post-transplant period together with the corresponding urate lowering agent use and the type of calcineurin inhibitor (CNI) used were recorded. Comparisons were made among female and male due to sexual difference in reference level of serum urate. 6 months post-transplant period were used because the dosage of CNI were stabilized by that time.

Results: 123 patients were included in the study. Exclusions were mainly due to insufficient data on urate level. 7 patients died before 6 months post-transplant were excluded. Only allopurinol is used.

Table 1 showed the result of the study. There was no statistically significant reduction in uric acid level before and after transplantation in terms of mean serum urate level and the number of patients having hyperuricaemia. After eliminating the effect of allopurinol use the value of urate still showed no statistical difference regardless of transplantation. Univariate and multivariate analyses both showed that Age and eGFR were independent risk factor of hyperuricaemia post-transplant in male, while for female, lower eGFR and the use of cyclosporine predict hyperuricaemia.

Discussion: Hyperuricaemia is common among patient with chronic renal failure. Renal transplantation could probably reduce serum urate level but not up to statistical significance. It appears that female patients has much higher baseline serum urate level (upper limit is 0.417mmol/L for male and 0.339mmol/L for female) and the reduction is even modest in terms of relative value. In this study we showed use of Cyclosporine predicts hyperuricaemia in female patients but not in male, the reason cannot be well answered in this retrospective cohort. Since quite a number of patients received azathioprine as the immunosuppressants, the use of allopurinol is limited during post-transplant period, however even after eliminating the effect of allopurinol use, the serum urate level still showed no statistical significant improvement after renal transplant.

Conclusion: Hyperuricaemia is common among renal transplant patients with more female patients suffering from this condition. Use of allopurinol is limited after transplantation probably because patients will have no gouty attack during the early post-transplant period when they were receiving high dose steroid therapy. Increase awareness in the condition may help to prevent overt hyperuricaemia which may potentially affect the graft function in future.

	Male			Female		
	Pre Tx	Post Tx	P value	Pre Tx	Post Tx	P value
Number	59		NA	64		NA
Mean age	51.2		NA	47.9		NA
Mean Urate level (mmol/L), all patients	0.46	0.431	0.19	0.435	0.415	0.34
Number of patients on Allopurinol	16 (27.1%)	2 (3.4%)	NA	9 (14.1%)	1 (1.6%)	NA
Mean dose of Alluprinol per day (mg)	118.75	100	NA	127.78	200	NA
Number of patients with hyperuricaemia	33 (55.9%)	32 (54.2%)	0.92	52 (81.3%)	43 (67.2%)	0.48
CNI use (Cyclosporine)	NA	39 (66.1%)	NA	NA	43 (67.2%)	NA
CNI use (Tacrolimus)	NA	20 (33.9%)	NA	NA	21 (32.8%)	NA
Number of patients never used allopurinol	42 (71.2%)			54 (84.4%)		
Mean Urate level (mmol/L), patients not on Allopurinol	0.467	0.432	0.7	0.434	0.411	0.319

Table 1: Comparison of the urate level among difference sex before and after transplantation
Tx=transplant; CNI=calcineurin inhibitor

References:

[1] Kang DH. A role for uric acid in the progression of renal disease. J Am Soc Nephrol. 2002 Dec; 13(12): 2888-97.

P.1331

Is there a link between proteinuria and increased FGF-23 levels in renal transplant patients?

Emre Tutal[1], Siren Sezer[1], Zeynep Bal[1], Bahar Gurlek Demirci[1], Gokhan Moray[2], Turan Colak[1], Mehmet Haberal[2].
[1]Department of Nephrology, Baskent University Faculty of Medicine, Ankara, Turkey; [2]Department of General Surgery, Baskent University Faculty of Medicine, Ankara, Turkey.

Introduction: Fibroblast growth factor 23 (FGF23) has emerged as a risk factor for cardiovascular disease and mortality in renal transplant (RT) recipients[1]. Post transplantation proteinuria is associated with reduced graft survival as well as an increased risk of cardiovascular events and death[2]. We aimed to measure the relationship of serum FGF-23 with renal function, bone biomarkers and proteinuria in RT patients.
Materials and Methods:A group of 160 RT recipients were included. Patients with increased creatinine levels were excluded. FGF-23 level was studied in every patient. Proteinurias (mg/day) of all patients were measured simultaneously with FGF-23. Last one year's parathyroid hormone, corrected calcium, phosphorus levels, office blood pressure measurements and demographic characteristics were also recorded from patient charts. Patients were divided into two groups according to median FGF-23 level: Group 1 (n:80) having high and Group 2 having low (n:80) FGF-23 levels.
Results and Discussion: Groups were similar in means of demographic characteristics. Group 1 had higher phosphorus (p:0.045), parathyroid hormone (p: 0.03) and lower calcium (p: 0.04) levels. Proteinuria was significantly higher in Group 1 (1433 mg/d vs 269 mg/d, p:0.0001) while blood pressures were similar. A correlation analysis revealed that proteinuria was positively correlated with creatinine, FGF-23 and parathyroid hormone levels (p: 0.004-0.0001).
Conclusion: We suggest that increased FGF-23 levels appear to be independently associated with proteinuria and could be a potential biomarker in renal transplant recipients.
References:
[1] Wolf M et al. Elevated fibroblast growth factor 23 is a risk factor for kidney transplant loss and mortality. J Am Soc Nephrol. 2011;22:956-66.
[2] Tsampalieros A et al. Evaluation and Management of Proteinuria After Kidney Transplantation. Transplantation. 2015;99:2049-60.

P.1332

Alkaline phosphatase a new marker for graft outcome and high pulse wave velocity in renal transplant recipients

Siren Sezer[1], Emre Tutal[1], Zeynep Bal[1], Bahar Gurlek Demirci[1], Gokhan Moray[2], Turan Colak[1], Mehmet Haberal[2].
[1]Department of Nephrology, Baskent University Faculty of Medicine, Ankara, Turkey; [2]Department of General Surgery, Baskent University Faculty of Medicine, Ankara, Turkey.

Introduction: Alkaline phosphatase (ALP) is a marker of bone turnover which is widely used for estimating type of bone turnover in patients with end-stage renal disease (ESRD)[1]. Recent data has implicated ALP is an initiator of calcification and pointed it to be associated with atherosclerosis[2]. In this study we aimed to analyze the relationship between renal resistive index and pulse wave velocity and ALP values in a group of renal transplantation patients with good renal function.
Materials and Methods: A group of 160 RT recipients (55 years old, 42 female, post transplantation duration median 52 months) were included. Each patient was with a current RT doppler ultrasonography for RRI measurement and pulse wave velocity were recorded. Subjects were grouped according to the post transplant first years' ALP levels as increased ALP (> 120 U/mL, n:42) and normal ALP (n: 118) groups.
Results and Discussion: Groups were similar in means of demographic characteristics, medications and creatinine, calcium, phosphorus, CaxP and PTH and creatinine clearance levels according to the first years results. Patients with increased ALP levels also had increased RRI (0.71 ± 0.06 vs 0.67 ± 0.08, p: 0.022) and pulse wave velocity measurements (8.6 ± 2.4 vs 6.8 ± 1.3, p: 0.003). A follow-up data of 3 years revealed that patients with increased ALP had higher creatinine (p: 0.03) and lower GFR levels (p: 0.02). Post transplant high ALP level correlates with atherosclerosis and high renal resistive index.
Conclusion: Alkaline phosphatase can be a new prognostic follow-up marker in terms of atherosclerosis and patients whose levels >120 IU/L show poor long term renal graft function.
References:
[1] Lau WL et al. Alkaline phosphatase: better than PTH as a marker of cardiovascular and bone disease? Hemodial Int. 2014;18:720-4.
[2] Park JC et al. Association of serum alkaline phosphatase and bone mineral density in maintanenece hemaodialysis patients. Hemodial Int. 2010 ;14:182-92.

P.1333

Ambulatory blood pressure measurement as a prognostic tool in renal transplant recipients

Emre Tutal[1], Siren Sezer[1], Zeynep Bal[1], Bahar Gurlek Demirci[1], Gokhan Moray[2], Turan Colak[1], Mehmet Haberal[2].
[1]Department of Nephrology, Baskent University Faculty of Medicine, Ankara, Turkey; [2]Department of General Surgery, Baskent University Faculty of Medicine, Ankara, Turkey.

Introduction: Hypertension is both a cause and a consequence of renal dysfunction. Blood pressure control is also important in patients with renal transplantation (RT). Increased blood pressure might accelerate loss of renal function in these patients. In this study we aimed to analyze relationship between blood pressure and graft function in a group of RT recipients.
Materials and Methods: A group of 150 RT recipients with stable renal function (no acute rejection episode or serious medical condition that require hospitalization during follow-up) were included. Each patient was evaluated for blood pressure control with ambulatory blood pressure monitorization (ABPM) at the initiation of study and after laboratory and clinical data were evaluated at 12 month follow-up period. Subjects were grouped at the end of 12 months follow-up according to loss of glomerular filtration rate (IGFR) as ≤ 10 ml/min (group 1, n:77) and lGFR > 10 mL/min (group 2, n:73).
Results and Discussion: Groups were similar in means of demographic characteristics, medications and initial creatinine and GFR levels. Basal ABPM of study groups were statistically equivalent. Both groups had higher creatinine and lower GFR levels at the end of 12 months compared to their initial values (p: 0.0001). However in group 2 patients who had more than 10 mL/min GFR loss there was a significant increase in both mean systolic (119 vs 143 mmHg, p: 0.001) and diastolic (76 vs 88 mmHg, p: 0.01) blood pressures while there was no significant change in group 1 patients. We also observed that group 2 patients had higher blood pressures compared to group 1 patients (p: 0.01). Ambulatory blood pressure results are closely related with significant decline in renal function in RT recipients.
Conclusion: Ambulatory blood pressure monitorization should be evaluated routinely and the patients' blood pressure should be targeted to the normal limits during the follow-up.

P.1334

Arteriolar hyaline formation caused by hypertension and high TG levels as well as CNI toxicity appeared to be a long-term risk factor of allograft function

Yoshihiro Itabashi, Masaki Muramatsu, Ken Sakai, Takeshi Kawamura, Yuko Hamasaki, Yusuke Takahashi, Yoji Hyodo, Hiroshi Nihei, Takashi Yonekura, Hideyo Oguchi, Seiichiro Shishido, Atsushi Aikawa.
Department of Nephrology, School of Medicine, Faculty of Medicine, Toho University, Tokyo, Japan.

Introduction: Arteriolar hyalinosis (ah) in a renal allograft is often observed. Aims of this study were to reveal the causes of ah and the effect of ah for long-term allograft function.
Materials and Methods: Of 156 living donor kidney transplant recipients who underwent in our hospital from 2002 to 2007, 66 patients who received protocol biopsy at 7 years post-transplant were enrolled in this study. All patients were administered calcineurin inhibitor (CNI) since renal transplantation. They were divided into two groups according to the presence of ah in the 7-year-biopsied specimens: ah+ and ah- group. We compared mean blood pressure (mBP), HbA1c, total cholesterol (T-cho), triglyceride, a trough level of CNI and serum creatinine (sCr) at 7 years post-transplant between both groups. Chronic tubulointerstitial changes (ci and ct) based on the Banff classification 2005 were also evaluated.
Results: Thirty-four patients (51.5%) showed ah and 32 cases (48.5%) did not. The mean age at the time of transplantation was 40.2±13.2 years old (ah+) and 43.3±10.7 years old (ah-) (p=0.294). Three patients whose original disease was diabetes mellitus, were observed only in the ah+ group. Post-transplant diabetes mellitus was showed in 7 cases in the ah+ group and in 6 cases in the ah- group. HbA1c and T-cho in both groups did not differ significantly, while mBP and triglyceride in the ah+ group were significantly higher than the ah- group (84±13 vs 75±12mmHg, p=0.001, 166±103 vs 120±53mg/dl, p=0.021). The level of sCr in the ah+ group was numerically higher than that in the ah- group (1.46±0.59vs1.24±0.38mg/dl, p=0.066)). Cyclosporine (CYA) was administered in 22 cases (65%) in the ah+ group and in 16 cases (50 %) in the ah- group. The trough level of CYA in the ah+ group was significantly higher than that in the ah- group (64.6±42.9 vs 42.7±14.1ng/ml, p=0.034). On the other hand, the trough level of tacrolimus was no significant difference in the both groups (4.6 ± 1.1 vs 4.0 ± 1.2ng/ml, p=0.171). The scores of ci and ct were 0.82±0.63 and 0.65±0.33 in the ah+ group and 0.66±0.48 and 0.56±0.55 in the ah- group (p=0.134 and p=0.08).
Discussion: Blood pressure, triglyceride and a high trough level of CYA were associated with the presence of ah. Long-term allograft function tended to be worse in the ah+ group. Chronic tubulointerstitial changes were not correlated with the presence of ah.
Conclusion: Optimal clinical management, including the dose of CNI, after transplantation may prevent the progressive formation of ah. The management may reflect to keep long-term renal graft function.

P.1335

Correlation between resistance index and urinary albumin excretion at early stage after renal transplantation

Kabei Kazuya[1], Uchida Junji[1], Nishide Shunji[1], Machida Yuichi[3], Iwai Tomoaki[1], Kuwabara Nobuyuki[1], Naganuma Toshihide[1], Kumada Norihiko[2], Nakatani Tatsuya[1].
[1]Urology, Osaka City University, Osaka, Japan; [2]Urology, Suita Municipal Hospital, Osaka, Japan; [3]Urology, Yao Municipal Hospital, Osaka, Japan.

Background: Color Doppler ultrasonography is an effective noninvasive examination for acute rejection (AR) and renal arterial stenosis in renal transplantation (RT). Ultrasonography can also provide an evaluation of flow volume and resistance in intrarenal vessels. Renal arterial resistance index (RI) and pulsatility index (PI) provide a good indication of blood vessel changes and are frequently used parameters in the clinical investigation of renal disease[1]. A previous analysis showed that microalbuminuria was a powerful risk factor for graft loss in RT recipients[2]. However, there are no available reports regarding the relationship between renal arterial RI and PI and urinary albumin excretion (UAE) in RT recipients. In this study, we investigated the correlation between RI/PI and UAE after RT.
Method: Between March 2014 and September 2015, 33 patients underwent RT at Osaka City University Hospital and were examined by color Doppler renal ultrasonography at 1 month after RT, which provided information about the diameter of the kidney, peak systolic velocity (PSV), and RI/PI (renal artery and interlobar artery). At 3 months after RT, we collected clinical parameters such as UAE, estimated glomerular filtration rate (eGFR), and episodes of AR. We retrospectively analyzed the relationship between the results of the ultrasonography (including RI/PI) and these clinical parameters.
Results: The median diameter of the kidney was 10.7 cm (range: 9.4-12.7). The median PSV was 127 cm/s (47-228). The median RI of the renal artery was 0.72 (0.52-0.88) and that of the interlobar artery was 0.67 (0.49-0.88). The median PI of the renal artery was 1.36 (0.78-1.88) and that of the interlobar artery was 1.24 (0.73-1.63). At 3 months after RT, the median UAE was 20 mg/g·Cr (3-175), and the median eGFR was 42.0mL/min/1.73 ㎡ (26.4-65.3). Twelve recipients experienced AR within 3 months after RT. The episodes of AR had no relation with RI/PI. We divided the 33 recipients into two groups according to the UAE value (microalbuminuria group: UAE≥30 mg/g Cr (n=19) and normoalbuminuria group: UAE<30 mg/g Cr (n=14)). There were no significant differences in the clinical parameters such as gender, age, donor age, eGFR, BMI, mean blood pressure, and episodes of AR between the two groups. RI/PI in the patients with microalbuminuria was significantly higher than that in the patients with normoalbuminuria (P=0.003/0.013). Furthermore, linear regression analysis indicated that increased RI/PI of the renal artery significantly correlated with UAE at 3 months after RT (r=0.550/0.500).
Conclusion: Our study demonstrated that renal arterial RI/PI at 1 month after RT was associated with UAE at 3 months after RT. Previous reports showed that UAE at an early posttransplant stage was a predictor of poor graft prognosis. RI/PI at 1 month after RT could therefore be an indication of vascular endothelial cell damage and a good predictor for early glomerular damage and graft survival.
References:
[1] Han F. Correlation of ultrasonographic measurement of intrarenal arterial resistance index with microalbuminuria in nonhypertensive, nondiabetic obese patients. *Int Urol Nephrol*. 2013; 45:1039–1045
[2] Halimi J. Low-Grade Proteinuria and Microalbuminuria in Renal Transplantation.*Transplantation*. 2013; 96: 121-130

P.1336

Serum cystatin C as a good predictor of chronic allograft dysfunction in renal transplant recipients

Sirilak Leawseng, Bancha Satirapoj, Naowanit Nata, Thanom Supaporn, Prajej Ruangkanchanasetr.
Division of Nephrology, Department of Medicine, Phramongkutklao Hospital and College of Medicine, Bangkok, Thailand.

Background: Serum creatinine is routinely used for the assessment of renal function in renal transplant recipients (RTRs), but it does not reflect true renal function because of corticosteroid-induced muscle wasting. In contrast, serum cystatin C is unrelated to muscle mass and cystatin C has been proposed as a novel marker of renal function in early chronic kidney disease. The role of cystatin C in RTRs has not been extensively verified especially in the prediction of early allograft dysfunction.
Objective: To evaluate the usefulness of serum cystatinC as a prognostic marker of chronic allograft dysfunction in RTRs with baseline normal serum creatinine.
Methods: We retrospectively analysed the RTRs with baseline serum creatinine<1.5 mg/dL and followed them until December 2015. The renal function was evaluated by serum creatinine, estimated glomerular filtration rate (eGFR) by CKD-EPI-creatinine equation and serum cystatin C. The endpoint of the study was the development of chronic allograft dysfunction which was defined as eGFR less than 60 mL/min/1.73 m2. Receiver operating characteristic (ROC) curve, Cox proportional hazard model and survival analysis were used to investigate the prognostic factors for chronic allograft dysfunction.
Results: Seventy-two RTRs were enrolled (50 men and 22 women, age 46.1±11.6 years and body weight 64.3±13.9 kg). Thirty-seven of the 72 patients (51%) developed chronic allograft dysfunction after the median follow-up 3.2 years. Baseline serum cystatin C was significantly higher in the developed chronic allograft dysfunction recipients than in non chronic allograft dysfunction recipients (1.27±0.41 vs. 1.1±0.19 mg/L, P=0.028). Serum cystatin C was the predictive factor for allograft dysfunction with a diagnostic accuracy of 0.669 (95% CI, 0.543 to 0.795; p=0.015). At a cutoff value of 1.04 mg/L, serum cystatin C was 81% sensitive and 39% specific in detecting early chronic allograft dysfunction. Comparison of the renal survival rates over the observation period revealed that a serum cystatin C level >1.04 mg/L was a prognostic marker for developing chronic allograft dysfunction (Hazard Ratio: 2.33; 95% CI 1.02 to 5.62).
Conclusions: The study demonstrates that the sensitivity in predicting chronic allograft dysfunction was high for a serum cystatin C>1.04 mg/L. Serum cystatin C could be a useful laboratory biochemical marker in predicting the allograft dysfunction in adult transplant patients.
This work was supported by a grant from the Phramongkutklao Hospital and College of Medicine, and the National Science and Technology Development Agency (NSTDA, P-13-00505), Bangkok, Thailand.

P.1337

Serum albumin level has association with both graft failure and mortality in kidney transplant recipients

Samuel Lee[1], Hee Jung Jeon[2].
[1]Surgery, Kangdong Sacred Heart Hosp. Hallym Univ. Medical Center, Seoul, Korea; [2]Nephrology, Kangdong Sacred Heart Hosp. Hallym Univ. Medical Center, Seoul, Korea.

Background: The studies concerned the association between post-transplant serum albumin concentration and post-transplant outcomes in kidney transplant recipients (KTRs) are scarce.
Methods: To evaluate the impact of serum albumin level on graft and patient survival, we performed a retrospective multi-center cohort study. A total of 2815 KTRs who underwent renal transplantation from Jan 1997 to Jan 2012 were classified into two groups according to the level of serum albumin at 1 year after transplantation (higher albumin group, ≥4.0 g/dL, n=1978 vs. lower albumin group, <4.0 g/dL, n=837). The Cox proportional hazard model was adjusted with age and gender of recipient, donor type, age of donor, diabetes mellitus, and estimated glomerular filtration rate (eGFR) at 1 year after transplantation.
Results: The mean age of the recipients was 41.7±11.3 (range, 18-73) years, and 59.1% were male. The rate of graft failure was higher in lower albumin group compared to higher albumin group (Hazard ratio [HR] 1.840, 95% confidence interval [CI] 1.367-2.477, P<0.001), even though eGFR at 1 year after transplantation was not different between the two groups (61.7±19.8 vs. 62.1±15.8 mL/min, P=0.615). Both all-cause mortality and non-cardiovascular mortality rates were higher in lower albumin group (HR 2.227, 95% CI 1.258-3.943, P=0.006, and HR 2.784, 95% CI 1.254-6.179, P=0.012, respectively). Every 1.0 g/dL higher serum albumin concentration was associated with 69.2% lower all-cause mortality (HR 0.308, 95% CI 0.196-0.483, P<0.001).
Conclusion: Serum albumin level at 1 year after transplantation is a prognostic factor for graft failure and patients' mortality in KTRs. Therefore, evaluation and management for hypoalbuminemia should be considered to improve outcomes in KTRs.

P.1338

Early-onset graft pyelonephritis is predictive of long-term outcome of renal allografts

Samuel Lee[1], Dong Ho Shin[2], Doo Jin Kim[1], Joo Seop Kim[1].
[1]Surgery, Kangdong Sacred Heart Hosp. Hallym Univ. Medical Center, Seoul, Korea; [2]Nephrology, Kangdong Sacred Heart Hosp. Hallym Univ. Medical Center, Seoul, Korea.

Urinary tract infection (UTI) is the most common bacterial infection encountered in kidney transplant recipients. Graft pyelonephritis (GPN) is associated with acute kidney injury and renal allograft scarring. However, the influence of GPN on renal allograft outcome in kidney transplant recipients remains controversial. Two hundred sixty-five kidney transplant recipients were evaluated for the impact of earlyonset GPN on renal allograft functions between January 2001 and December 2011. Early-onset GPN was defined as the first GPN episode occurring within 6 months after kidney transplantation. Thirty recipients (11.3%) were diagnosed with early-onset GPN. During the mean follow-up period of 69.1 ± 28.9 months, 56 (21.1%) recipients showed renal allograft outcomes of a > 30% reduction in the estimated glomerular filtration rate (eGFR) over 2 years. The poor outcome was significantly more frequent in the early-onset GPN group (13 patients; 43.3%) than in those without early-onset GPN (43 patients; 18.3%) (P = 0.002). Moreover, the linear mixed model revealed a significant difference in the eGFR decline rate over time between the two groups (P < 0.001). Kaplan-Meier analysis showed that renal allograft event-free survival was significantly lower in the early-onset GPN group (P = 0.006). Multivariate Cox regression analyses revealed that early-onset GPN was independently predictive of poor renal allograft outcomes (hazard ratio, 1.96; 95% confidence interval, 1.02-3.77; P = 0.04). In conclusion, early-onset GPN is independently associated with impaired renal functions in kidney transplant recipients. Thus, early-onset GPN could be a predictor for long-term outcome of renal allografts.

P.1339

New risk factor of newly onset diabetes mellitus after kidney transplantation

Michal Nowicki, Agnieszka Golebiewska-Walczak.
Dept. Nephrology and Kidney Transplantation, Medical University of Lodz, Lodz, Poland.

An increased incidence of new-onset diabetes mellitus has been observed among the kidney transplant recipients. Incident diabetes after transplantation has been associated with an increased risk of cardiovascular disease, infections and the transplanted kidney failure.
As there are no reliable biochemical markers that allow us to assess and predict the development of the disease, new markers are sought to promptly assess the risk and introduce preventive measures and appropriate management of new onset diabetes mellitus after transplantation.
The prospective longitudinal study enrolled 71 patients in the late post-transplantation phase, without the diagnosis of diabetes prior to the renal transplantation.
In the 60 months follow-up 24 patients (34%) developed NODAT.
The following factors were analyzed with respect to the incidence of NODAT: time from renal transplantation, sex, age, BMI, total cholesterol, triglycerides, PTH, serum calcium and phosphorus, proteinuria, use of specific immunosuppressive drugs, serum CRP, human glycoprotein 39 (YKL-40) and sex hormones binding globulin (SHBG)
NODAT occurred more frequently in patients treated with tacrolimus vs cyclosporin A, in older patients, those with higher serum cholesterol, higher eGFR and shorter time post-transplantation.
The subjects who had not developed NODAT had higher diastolic blood pressure and more patients in this group had been treated with cyclosporine A.
There was no statistically significant difference in the concentration of serum SHBG, YKL-40, hsCRP between the groups.
New serum markers have not proven their usefulness for predicting the development of new-onset diabetes after renal transplantation.

P.1340

Impaired Fastig Glucose according to different diagnostic criteria in kidney transplanted patients

Marcelo Taylor, Liliana M Obregon, Hugo S Petrone.
Kidney Transplant Unit, CUCAIBA - CRAI Sur, La Plata, Argentina.

Introduction. The alteration in carbohydrates metabolism is frequently observed after kidney transplantation. New onset diabetes after transplant (NODAT) has been extensively studied, although there are no reports of the characteristics and frequency of the impaired fasting glucose (IFG).
Materials and methods: We evaluated all patients who had undergone transplant in our unit in the last fifteen years with more than one year of follow-up. We considered that a patient had IFG when he met the criteria of the ADA and/or WHO, and presented less than 20 mg of meprednisone. All values obtained during the hospitalization or up to one month after the steroid pulse treatment were excluded. We considered average values and standard deviation of the variables.
Results: We studied 481 patients from a total of 596. Only 117 patients (24.32%) presented normal glycemia values during the tracking (6.1 years on average). Of the remaining 364 patients, 150 met the ADA criteria and 111 met the WHO criteria. A total of 103 patients presented NODAT (prevalence of 21.41%). The average time between transplant and IFG was 25.52 and 28.66 months depending on the considered criteria. 11.22% (n=54) met the diabetes criteria without having gone through IFG, while another 10.18% (n=49) of the patients who had IFG progressed to diabetes. Diabetic patients were significantly older than those who presented IFG, and the latter were older than those who didn't present alterations in glycemia. We observed no differences concerning mortality, dialysis re-entry, time on dialysis or hepatitis C prevalence among groups.
Conclution: IFG is very frequent in transplanted patients. Although not every patient's condition evolved into NODAT, this is a risk group for cardiovascular disease, thus precautionary measures should be taken. The number of individuals included may have contributed to the lack of significant differences between populations.

P.1341

Serum 25-hydroxyvitamin D levels with metabolic profiles in renal transplant recipients with chronic allograft nephropathy

Bancha Satirapoj, Sirilak Leawseng, Naowanit Nata, Thanom Supaporn, Prajej Ruangkanchanasetr.
Division of Nephrology, Department of Medicine, Phramongkutklao Hospital and College of Medicine, Bangkok, Thailand.

Background: Patients with end-stage renal disease who receive renal transplants have improved survival and quality of life. Unfortunately, transplant patients with chronic allograft nephropathy (CAN) often have high prevalence of metabolic syndrome and cardiovascular diseases. Vitamin D insufficiency has well-known effects on metabolic profiles and it could be a risk factor for cardiovascular diseases. The relationship of vitamin D on other indicators of cardio-metabolic risk factors in CAN patients, such as blood pressure, lipid profile and renal function, remains unknown.
Methods: This cross-sectional study was conducted on renal transplant recipients with tubulointerstitial fibrosis. Fasting concentration of plasma glucose, calcium, phosphorus, parathyroid hormone (PTH), lipid profile, creatinine, and 25-hydroxyvitamin D were measured. Vitamin D insufficiency was defined as a serum 25-hydroxyvitamin D concentration < 30 ng/mL.
Results: A total of 32 recipients were enrolled (22 men and 10 women, age 45.8±11.2 years and body mass index 22.7±3.9 kg/m2). Overall, serum 25-hydroxyvitamin D concentrations were 28.3±14.9 ng/mL and the 25-hydroxyvitamin D level was <30 ng/mL in 56.3% of patients. Recipients with vitamin D insufficiency had significantly lower duration of renal transplantation than in recipients with normal vitamin D status (5.9±5.6 vs. 12.21±7.47 years, p=0.010). The correlation analysis showed that serum 25-hydroxyvitamin D level was negatively correlated with systolic blood pressure (r = -0.422, p=0.016), total cholesterol level (r = -0.489, p=0.005), HDL-cholesterol level (r= -0.490, p=0.005) and LDL-cholesterol level (r= -0.421, p=0.016) and was not correlated with PTH, calcium, phosphate, estimated glomerular filtration rate and albuminuria.
Conclusion: This study demonstrates that renal transplant recipients with CAN frequently have low 25-hydroxyvitamin D levels, especially early after transplantation. The 25-hydroxyvitamin D levels are associated with the lipid abnormalities and the blood pressure in this population.
This work was supported by a grant from the Phramongkutklao Hospital and College of Medicine, and the National Science and Technology Development Agency (NSTDA, P-13-00505), Bangkok, Thailand.

P.1342

Using analgesics including nonsteroidal anti-inflammatory drugs in patients with chronic kidney disease (CKD 1-4) and after kidney transplantation – one center experiences

Zbigniew Heleniak, Magdalena Cieplińska, Magdalena Pryczkowska, Tomasz Szychliński, Ewa Bartosińska, Hanna Wiatr, Hanna Kotłowska, Marzena Jakimowicz-Tylicka, Leszek Tylicki, Bolesław Rutkowski, Alicja Dębska-Ślizień.
Nephrology, Transplantology and Internal Medicine, Medical University of Gdansk, Gdansk, Poland.

Introduction: Analgesics including nonsteroidal anti-inflammatory drugs (NSAIDs) are the cornerstone of pain management in patients who have inflammatory, acute and chronic pain. There is no detailed data of analgesics used by Polish people, especially in patients with a chronic kidney disease.
A lot of pharmaceuticals can be bought without prescription.
The aim of this study was to evaluate the frequency, circumstances and causes of using analgesics, as well as the knowledge of side effects of these drugs in patients with chronic kidney disease (CKD) stage 1-4 and in patients after kidney transplantation (KTx).
Materials and Methods: Cross-sectional survey study was conducted in 888 CKD patients who were under the care of the Department of Nephrology, Medical University of Gdansk in 2014.
There were statistically, more women in the group with CKD 1-4.
Details are in figure 1.

	CKD 1-4	Kidney transplantation	p
Age (years)	57.21± 19.36	50.09± 13.58	ns
Gender F (%)	282(49)	136 (33)	<0,05
BMI (kg/m2)	26.84± 5.39	25.56± 4.11	ns
The length of CKD (years)	11.51± 12.14	17.52± 10.03	ns
Bone, joint disease %	27.53	13.37	<0,05
Cancer %	10.45	4.14	<0,05
Diabetes %	23.52	22.29	ns
Hypertension %	75.08	85.03	ns
Cardiovascular disease %	30.49	18.79	<0,05
The frequency (%) of use of analgesics in the entire group			
everyday	7,7	3,5	<0,05
Few times a week	12,2	3,5	<0,05
Few times a month	14,8	11,5	<0,05
Few times a year	34,5	42	<0,05

Table 1. Characteristics of the study group.

Results: Among the study population 19,9% of patients with CKD 1-4 and 10.2% after KTx used analgesics every day or several times a week. In the group of patients with CKD 1-4 statistically more people suffered from cancer, cardiovascular and osteo-articular diseases.
The most common reasons of using analgesics were bone and joint pain (29.3%) and headache (26.23%). In 46.71% of cases the decision was taken by the patient. The possible side effects of painkillers such as deterioration of blood pressure control and renal function as well as the risk of stomach ulcers were known by 17.4, 42.3, 36.1% of responders with CKD, and 19.1, 40,8, 36,6% of responders after KTx, respectively.
Conclusion: Patients with CKD 1-4 and after KTx take analgesics quite often. The results show low awareness of adverse painkillers effects. The education is urgently required in these populations.

P.1343

The treatment of hypertension in patients after renal transplantation – one center observational study

Izabella Kuźmiuk, Zbigniew Heleniak, Dorota Adrych, Hanna Garnier, Jakub Wiśniewski, Przemysław Rutkowski, Leszek Tylicki, Bolesław Rutkowski, Alicja Dębska-Ślizień.
Department of Nephrology, Transplantology and Internal Medicine, Medical University of Gdańsk, Gdańsk, Poland.

Background: Hypertension is of the most common comorbidities in the population of kidney transplant recipients (KTR) and it affects 60 up to 85% of those patients. The antihypertensive treatment often requires a multidrug therapy and individual approach to the patient in order to achieve proper control of blood pressure and prevent its complications. The aim of the study was to evaluate the antihypertensive treatment in the group of KTR in years 2001-2014.
Materials and Methods: We analyzed a group of 96 KTR (55M, 41F) with a mean age of 56,6 years (range 25 to 76 years) transplanted in years 1987-2001 (average time after KTx - 2,9 years), who are under the control of University Outpatient Transplantation Clinic in Gdańsk from January 2001 to July 2014 (the time of the observation - 13 years). The analysis of antihypertensive treatment was based on medical documents and it consisted of a comparison of the data reported at the first and last point of observation.
Results: During the first point of observation (2001 year), the presence of hypertension and need the antihypertensive treatment was noticed in 93,5% of patients (average number of medication 2,03), while at the second (2014 year) in 100% (average number of medication 2,69). The most commonly used drugs during the first visit were: ß blockers, selective calcium antagonists, diuretics, ACE/ARB and α blockers: 69, 59, 34, 23, 9%, respectively; while during the second visit the percentage of mentioned medication was as follow: 74, 52, 49, 65, 27%. The most common antihypertensive therapy in 2001 was a two-drug therapy (ß blocker and calcium antagonist) while in 2014: triple-drug treatment (ß blocker, calcium antagonist and α blocker). The most frequently used double-drug combination in both 2001 and 2014 was a set of ß blocker and calcium antagonist: 49 and 36% respectively.
The therapy including rennin-angiotensin-aldosterone system (RAAS) blockers is one of the most common in KTRs. The usage of those medications is triple times higher in 2014 than it was in 2001: 23 and 65 % respectively. Both at the first and at the second point of observation the most frequently used RAAS blockers were as follows: angiotensin convertase inhibitors (ACE-I): 22% in 2001 and 56% in 2014, while angiotensin receptor blockers (ARB): 1 and 8% respectively.
The control of hypertension in the group of KTRs improved through years. The target values of blood pressure for general population were accomplished in 46% in 2001, while in 2014 in 53% of patients.
Conclusions:
1. Hypertension is a common syndrome occurring in KTR.
2. In the analyzed group of KTR the most frequently used antihypertensive drugs were: β-blockers, calcium antagonists, ACE/ARB, diuretics and α-adrenergic receptor blockers.
3. As the time passed ACE/ARB and diuretics were more frequently used.

P.1344

Achromobacter denitrificans pneumonia in a renal transplant patient– dose-dependent decrease of phagocytic activity as a potential mechanism for the everolimus pulmonary toxicity.

Justyna Gołębiewska[1], Ewa Bryl[2], Agnieszka Daca[2], Andrzej Chamienia[1], Bolesław Rutkowski[1], Alicja Dębska-Ślizień[1].
[1]Department of Nephrology, Transplantology and Internal Medicine, Medical University of Gdańsk, Gdańsk, Poland; [2]Department of Pathology and Experimental Rheumatology, Medical University of Gdańsk, Gdańsk, Poland.

Background: Mammalian target of rapamycin (mTOR) inhibitors inclusive regimens are associated with increased risk of pulmonary toxicity, but the underlying mechanism has not been elucidated so far.
Methods: Case report and functional analysis of peripheral blood neutrophils and monocytes. The phagocytic and oxidative burst were analyzed twice – on everolimus (EVR) treatment and 3 weeks after its discontinuation. Additionally, both assays were performed in vitro in the presence of different doses of EVR.
Results: a 68 year-old man with ESRD of unknown etiology post deceased-donor kidney transplantation 3 months earlier, presented with fever, progressive dyspnea, and productive cough for two weeks. He had been maintained on de novo EVR-based immunosuppression. There was clinical improvement with empiric antibiotic treatment (amoxicillin/clavulanic acid), but without a radiological resolution. A CT scan revealed bilateral multifocal subpleural and peribronchial patchy lung infiltrates. Both sputum and bronchoalveolar lavage cultures were positive for Achromobacter denitrificans (Ach.d), a bacterium present in soil and water and only a rare cause of human infections. A 17-day course of piperacillin/tazobactam chosen upon susceptibility profile and a reduction of the EVR dose resulted only in partial resolution of radiological abnormalities. EVR discontinuation with no additional antibiotic treatment resulted in complete recovery and resolution of pulmonary infiltrates. The ability of phagocytosis and oxidative burst generation against Ach.d and E.coli was significantly decreased on EVR treatment as compared to the control healthy person (40 vs 65% and 66 vs 81% for monocyte phagocytosis, and 5,9 vs 88% and 21 vs 88% for oxidative burst, respectively), and significantly improved after 3 weeks of EVR absence (71 vs 66% and 59 vs 40% for phagocytosis and 92 vs 6% and 95 vs 21% for oxidative burst, respectively). Additionally, these processes were significantly affected by increasing doses of EVR in vitro in control healthy donor in the dose dependent manner.
Conclusion: Dose-dependent impairment of neutrophils/monocytes phagocytic activity and oxidative burst generation might be a potential mechanism for EVR pulmonary toxicity.

P.1345

Preimplantation and intragraft molecular transcripts in serial biopsies of standard and extended criteria donor kidneys: effect of donor type and immunosuppressive protocols

Camila M.Mazeti-Felicio[1], Heloisa C.Caldas[1], Ida M.Fernandes-Charpiot[1,2], Maria Alice F.Baptista[1,2], Mario Abbud-Filho[1,2,3].
[1]Laboratory of Immunology and Experimental Transplantation, Sao Jose do Rio Preto Medical School, Sao Jose do Rio Preto, Brazil; [2]Department of Medicine/ Nephrology, Medical School - Hospital de Base – FAMERP/FUNFARME, Sao Jose do Rio Preto, Brazil; [3]Urology and Nephrology Institute, Sao Jose do Rio Preto, Brazil.

Introduction: Use of extended criteria donor kidneys (ECD) carries the fear of transplant kidneys with inferior quality and therefore of high discard rate. Clinical and histological tools available to assess non-ideal organs and predict outcomes of ECD have conflicting results. To evaluated differences in the intragraft cytokine genes expression in ECD and SCD (standard criteria donor) kidney biopsies (Bx) pre and post-transplant (Tx) and sought possible changes induced by immunosuppressive regimens (ISS).
Materials and Methods: SCD and ECD recipients (RTx) were randomized to tacrolimus (Tac) or everolimus (Eve) and Bx were performed pre-implantation (T0 Bx; n = 80) and at 15 (T15 Bx; n = 64) and 90 days (T90 Bx; n = 51) post-Tx. Subgroups SCD-Tac, ECD-Tac, SCD-Eve and ECD-Eve were analyzed for clinical outcomes and clinical data were correlated with intragraft gene expression.
Results: Overall, ECD-Eve and ECD-Tac had inferior 1-year patient survival and ECD-Tac had lower graft survival than other groups while CMV and de novo diabetes pos-Tx (PTDM) were higher in patients with Tac. After 1 year ECD-Eve had higher serum creatinine than ECD-Tac ($p = 0.03$) and acute rejection rates were higher in Eve group regardless donor type. T0 Bx of ECD showed higher expression of MCP-1, RANTES, TGF-β1 and IL-10 when compared with SCD. TGF-β1 related to the serum creatinine at harvesting while length of donor hospitalization and ECD donor type were associated with upregulation of MCP-1 and RANTES. T15 Bx of patients from both groups taking Eve had increased FOXP3 and MCP-1 and RANTES were upregulated only in the SCD-Eve group. Eve was the only variable associated with upregulation of FOXP3, MCP-1 and RANTES. Molecular profiling at T90 was similar except by an increase in FOXP3 transcripts restrict to SCD-Eve group. Independent variables associated with FOXP3 were use of Eve and DGF was associated with both MCP-1 and IL-10.
Discussion: Pre-implantation Bx of ECD kidneys have an inflammatory molecular profile clearly distinct from SCD. Post-Tx, the ISS given modifies the initial cytokine expression pattern at different time points.
Conclusion: The identification of these molecular profiles could provide insights for pre and post-Tx clinical approaches and would help to improve the fate of ECD allograft.

P.1346

Outcomes of kidney transplantation from DBD, DCD or DBCD donors: A single center experience from China

Guodong Chen, Chang Wang, Changxi Wang, Jiang Qiu, Lizhong Chen.
Organ Transplant Center, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, People's Republic of China.

Background: There are three categories of deceased donors in China now, donation after brain death(DBD), donation of cardiac death(DCD), and donation after brain and cardiac death(DBCD). The aim of this study is to compare the outcomes of kidney transplantation from these three categories of deceased donors.
Methods: We retrospectively collected the data of donors and recipients from 272 cases of deceased kidney transplantation, performed in our transplant center from July 2011 to Dec 2014, including 35 DBD donors, 62 DCD donors, and 175 DBCD donors. The recipients were followed up for at least one year. The baseline characteristics of donors and recipients were compared. The graft loss, patient death, postoperative complications and serum creatinine levels were also compared among the three groups.
Results: The mean functional warm ischemia time was much longer in DCD group compared to DBCD and DBD groups(23.4 min, 14.5min, and 0.5 min, respectively, $p<0.001$). The incidences of cardio-pulmonary resuscitation were higher in DCD and DBCD groups compared to DBD group(27.4%, 23.4%, and 5.7%, respectively,$p=0.036$). DGF rates were much higher in DCD group compared to DBCD and DBD groups(29.0%, 15.4% and 5.7%, respectively, $p=0.008$). 1 graft(1.6%) loss in DCD group and 4 grafts(2.3%) loss in DBCD group. 1 patient(0.6%) died of severe pneumonia in DBCD group. No graft loss or patient death in DBD group. The incidences of acute rejection and infection were comparable among three groups. Serum creatinine levels on postoperative 1 week, 1 month and 1 year were all higher in DCD group compared to DBCD or DBD groups($p<0.05$). Logistic multivariate regression showed that functional warm ischemia time, donor age and hypertension history were independent risk factors for DGF.
Conclusion: DGF rate and serum creatinine levels were much higher after DCD kidney transplants compared to DBCD or DBD kidney transplants due to longer functional warm ischemia time. However the graft and patient survival were comparable.

P.1347

Kidney transplantation from pediatric donors of deceased organ donation

Chen Chuanbao[1], Yuan X.Peng[1], Han Ming[1], Wang X.Ping[1], Zhou Jian[1], Jiao X.Yuan[1], Wang C.Xi[2], He X.Shun[1].
[1]Third Division of Organ Transplant Center, East Campus of First Affiliated Hospital, Sun Yat-sen University, Guangzhou, People's Republic of China; [2]Second Division of Organ Transplant Center, First Affiliated Hospital, Sun Yat-Sen University, Guangzhou, People's Republic of China.

Objective: To explore the clinical effect of kidney transplantation from donation after citizen's death (DCD) of pediatric donors.
Methods: The clinical data of kidney transplantation from DCD pediatric donors from February 2010 to October 2013 were retrospectively reviewed.
Result: There were 27 pediatric DCD donors with age of 10.5±5.2 years, including 19 males and 8 females, 16 cases of China category II and 11 cases of China category III. 6 cases of en bloc kidney transplantation and 42 cases of single kidney transplantation were performed. No primary non-function occurred. The incidence of delayed graft function, slow graft function and immediate graft function were 8.3%, 35.4% and 56.3%, respectively. Acute rejection occurred in 4 cases (8.3%). After follow-up of 5.2±6months, 1 renal graft lost due to antibody-mediated rejection, other grafts functioned well. The mean eGFR estimated glomerular filtration rate of adult recipients in 1 year was 76.1±17.7 ml•(min)-1•(1.73m2)-1.
Conclusion: The early clinical effect of kidney transplantation from pediatric donors after cardiac death is excellent. The pediatric DCD donors can be used to expand the donor pool.
Keywords: Kidney transplantation; Pediatric donors; Donation after cardiac death; En bloc kidney transplantation.

P.1348

The analysis of discarded kidneys from donors of deceased organ donation

Chen Chuanbao[1], Han Ming[1], Wang Xping[1], Yuan Xpeng[1], Zhou Jian[1], Lin Jwei[1], Zhen Ytao[1], Jiao Xyuan[1], Wang Cxi[2], He Xshun[1].
[1]Third Division of Organ Transplant Center, East Campus of First Affiliated Hospital, Sun Yat-Sen University, Guangzhou, People's Republic of China; [2]Second Division of Organ Transplant Center, First Affiliated Hospital, Sun Yat-Sen University, Guangzhou, People's Republic of China.

Objective: To analyze the discarded kidneys from donors of deceased organ donation in our center since 2011.
Methods: The data of all donors of deceased organ donation in our center from July 2011 to September 2015 were retrospective analyzed.
Results: There were 223 cases deceased donors during 2011-07 and 2015-09 in our center, 446 kidneys were procured and 46 kidneys were discarded for various reasons. The discard rate of kidney was 9.1% with exclusion of 3 uremic donors. There were 25 donors with discarded kidney, including 20 males and 5 females; the mean age was 38.0 ± 12.4 yrs (16-67yrs). Single kidney was discarded in 4 donors and both kidneys were discarded in 21 donors. The mean level of terminal serum creatine was 398.98±385.5μmol/L ((13.41～1633μmol/L)) and the mean length of stay in ICU was 17.36±25.36 d (1～126d). According to the Chinese Classification, there were 2 cases of Class I, 18 Class II and 5 Class III. The reasons for discard including donors with acute renal injury (n = 9), it was the main discard reason and occupied 36% in total discard donors. Uremic donors (n = 3), abnormal presentation in macroscopy (n = 3), donors with malignant tumor (n = 2), atheromatous plague in renal artery (n = 2) and others (n = 6). Other reasons contained 2 kidneys were discarded for paraquat poisoning in a donor, renal thrombosis were founded in two donors and discarded 3 kidneys. One donor suffered severe infection before procured and discarded 2 kidneys. The rest two donors both discarded right kidney, one for renal vein injury and the other was renal infarct. Because of the deficiency of clinical experience at the early stage of our organ donation program, there was a high discard rate of acute renal injury donor. Twenty kidneys from 10 donors were discarded from July 2011 to December 2012, 7 of them were discarded for acute renal injury and occupied 77.78% in all discarded cases of acute renal injury.
Conclusion: The main reason for kidney discard was acute renal injury at the early stage of our organ donation program.
Keywords: deceased organ donation; kidney discard; acute kidney injury; kidney transplantation
References:
[1] Huang, J;Wang, H;Fan, S T;et al. The national program for deceased organ donation in China. Transplantation, 2013. 96(1): p. 5-9.
[2] Reese, P.P;Harhay, M N;Abt, P L; et al. New Solutions to Reduce Discard of Kidneys Donated for Transplantation. J Am Soc Nephrol, 2015.
[3] Lee, M. H; Jeong, E. G; Chang, J. Y;et al. Clinical outcome of kidney transplantation from deceased donors with acute kidney injury by Acute Kidney Injury Network criteria. J Crit Care, 2014. 29(3): p. 432-7.
[4] Hall, I. E; Schroppel, B; Doshi, M. D; et al. Associations of deceased donor kidney injury with kidney discard and function after transplantation. Am J Transplant, 2015. 15(6): p. 1623-31.
[5] Heilman, R. L; Smith, M. L; Kurian, S. M.R.L; et al. Transplanting Kidneys from Deceased Donors With Severe Acute Kidney Injury. Am J Transplant, 2015. 15(8): p. 2143-51.

P.1349

Long term graft and recipient outcome of deceased donor renal transplantation at the national kidney and transplant institute

Romina Danguilan, Mark Javeson Tam, Concesa Casasola. Department of Adult Nephrology, National Kidney and Transplant Institute, Quezon City, Philippines.

Background: The number of patients with ESRD is increasing and the gap between the demand for KT and available donors is widening. Thus deceased donation is very important to the donor pool for ESRD.
Objectives: This study aims to determine the long term graft and recipient outcome of deceased donor renal transplantation at the National Kidney and Transplant Institute from 2002-2007 and to determine the donor and recipient factors that affect graft and recipient survival.
Materials and Methods: This is a retrospective cohort of deceased donor KT from January 2002 to December 2007. Data were reviewed and collected from National Kidney and Transplant Institute medical records and the Philippine Renal Disease Registry (PRDR). Recipient and donor demographic profile were expressed as frequency counts, percentages and means with standard deviation. Kaplan Meier was used to determine graft and patient survival and logistic regression to establish correlation between certain factors and survival**.**
Results: Among 1,598 KT, 1488 were from living donors and 110 from deceased donors. 91 patients were included in this study. The mean recipient age was 40.40±11.8 years and 65.9% were males. The primary renal diseases were chronic glomerulonephritis (63.7%), diabetic nephropathy (18.7%) and hypertensive nephrosclerosis (6.6%). Around 39.6% had 3 HLA mismatches and 61.5% had at least 1 DR match. Majority received induction therapy (90.1%) and 64.8% had tacrolimus-based immunosuppressive regimen. The patient survival rate at 1, 3, 5 and 7 years was 91, 89, 86 and 86 percent while graft survival was 89, 79, 73 and 68 percent respectively. Infection was the leading cause of death. Cold Ischemia Time was significantly associated with patient survival (P= 0.033) while patients with male donors had significantly better graft survival (P= 0.001)
Conclusion: There was an acceptable outcome of KT from deceased donors up to 7 years post KT.

References:

[1] Oniscu GC, Brown H, Forsythe JL. Impact of cadaveric renal transplantation on survival in patients listed for transplantation. *J Am Soc Nephrol*. 2005 Jun;16(6):1859-65.
[2] Perović S1, Janković S. vvRenal transplantation vs hemodialysis: cost-effectiveness analysis. *Vojnosanit Pregl*. 2009 Aug;66(8):639-44.
[3] Kaló Z, Járay J, Nagy J. Economic evaluation of kidney transplantation versus hemodialysis in patients with end-stage renal disease in Hungary. *Prog Transplant*. 2001 Sep;11(3):188-93.
[4] Neylan JF, Sayegh MH, Coffman TM, Danovitch GM, Krensky AM, Strom TB, Turka LA, Harmon WE. The allocation of cadaver kidneys for transplantation in the United States: consensus and controversy. ASN Transplant Advisory Group. *J Am Soc Nephrol*. 1999 Oct;10(10):2237-43.
[5] *Philippine Renal Registry 2010 Annual Report*. Department of Health, Philippines
[6] Liquete R et al. Long Term Outcome of Cadaveric Kidney Patients. *Philippine Journal of Surgical Specialties*. 1990;45(3):99-101.
[7] Magcalas W, Liquete R et al. Long Term Outcome of Cadaver Kidney Recipients. *National Kidney and Transplant Institute Proceedings*. 1996;1:1-8
[8] Ninalga HD, Danguilan R et al. Graft and Recipient Outcome of Cadaveric Transplantation at the National Kidney and Transplant Institute from 1995-2001. *National Kidney and Transplant Institute Proceedings*. 2005;1:63-70.
[9] Overio Y, Parayno A et al. Graft and Recipient Outcome of Cadaveric Renal Transplantation at NKTI from 2007 to 2010. Unpublished.
[10] *Unites States Renal Data System (USRDS) 2013 Annual Data Report*. Renal Transplantation Vol. 2 Chapter 7: 283-94.
[11] Gumber MR, Kute VB et al. Deceased Donor Organ Transplantation - A Single-Center Experience. *Indian J Nephrol*. 2011; Jul-Sep;21(3):182-185
[12] Jha, V, Chugh, S, Chugh, KS: Infections in Dialysis and Transplant Patients in Tropical Countries. *Kidney Int 2000; 57 (Suppl 74):S585-S593.*
[13] Zeier M, Dohler B et al. The Effect of Donor Gender on Graft Survival. *J Am Soc Nephrol*. 2002;13:2570-6.
[14] Salahudeen AK, May W. Reduction in Cold Ischemia Time of Renal Allografts in the U.S over the Last Decade. *Transplant Proc*. June 2008;40(55):1285-1289.
[15] Salahudeen AK, Haider N, May W. Cold Ischemia and the Reduced Long-Term Survival of Cadaveric Renal Allografts. *Kidney International*. 2004;65:713–718.

P.1350

Clinical research of kidney transplantation from cardiac death pediatric donors

Han Feng[2], Ding Chenguang[1], Tian Puxun[2], Xue Wujun[1], Ding Xiaoming[1], Pan Xiaoming[1], Yan Hang[1], Xiang Heli[1], Hou Jun[1], Feng Xinshun[1], Tian Xiaohui[1], Li Yang[1], Zheng Jin[1], Wang Xuzhen[1].
[1]Department of Renal Transplantation, Kidney Disease Hospital, the First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, People's Republic of China; [2]Organ Transplantation Institute of Xi'an Jiaotong University, Department of Renal Transplantation, Kidney Disease Hospital, the First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, People's Republic of China.

Objective: To investigate the early clinical effect of en bloc kidney transplantation and single kidney transplantation from pediatric donors with donation after cardiac death, comparing with standard DCD donor kidney transplantation (adult single renal transplantation from adult kidney donor).
Methods: A retrospective review of clinical data of donors and recipients kidney transplantation from donation after deceased cardiac donors (DCD) performed from November 2013 to December 2015 in kidney transplantation department of the first affiliated hospital of Xi'an Jiaotong university. Ages of 15 cardiac death pediatric donors: 1.5～16 years old (8 donors under 8 years, 4 donor from 9 years to 14 years, 3 donors from 15 to 16 years), donated to the same blood recipients all. Total 30 kidneys, acquiring all of them, 28 recipients(2 en bloc kidney transplantation). Protopathy of donors: 11 craniocerebral trauma donors, 1 encephalitis and 1 toxic hepatic disease donor, 2 brain gliomas donors. All operation adopted iliac fossa grafting.
Results: 28 cases of operation are performed well. The median warm ischemic time of renal allografts was 12.5 min (0－17min), cold ischemic time was 4.3 h (1.5 ～ 7.7 h). 10 donors applied lifepot to reserve organs and 5 didn't. Recipients: 17 males, 11 females; ages from 7.5 to 63 years old.; weight from 16kg to 56kg; immunosuppressive treatment protocol: ATG+CNI+MMF+Pred; postoperative complications: 4 DGF, 1 dialysis, 2 death, 2 nephrectomy. Postoperative follow-up time was from 1 to 24 months. So far, renal function of 24 recipients with renal survival (24/28=85.7%) all are normal. Comparing with standard DCD donor kidney transplantation during the same period, there are no significantly statistical differences. Kidney volume increases significantly within one month after surgery in single kidney to adult, and long axis of the kidney will be more than 10 cm after half a year.
Conclusions: Short-term out-comes of en bloc kidney transplantation and single kidney transplantation from pediatric donors are satisfactory. After specific, personalized, timely and effective perioperative treatment, there are no obvious differences with standard DCD donor kidney transplantation. Long-term results need to be further studied. It is an effective way to expand kidney source.
Corresponding Author: Tian Punxun, Email: tianpuxunxa@163.com
Xue Wujun, Email: xwujun@126.com
References:
[1] Effect of en bloc kidney transplantation from cardiac death pediatric donors.*Chin J Transplant*, 2013, 7(4):190-194.
[2] Single Pediatric Kidney Transplantationin Adult Recipients Versusstandard-Criteria Deceased-Donor Kidney Transplantation. *Journal of Urology*. 2015, 7(2):27-32.
[3] Neonatal and Pediatric Organ Donation: Ethical Perspectives and Implications for Policy. *Front Pediatr*. 2015; 3: 100.
[4] Prediction of delayed graft function after kidney transplantation: comparison between logistic regression and machine learning methods. *BMC Med Inform Decis Mak*. 2015; 15: 83.
[5] Risk factors for delayed graft function in cardiac death donor renal transplants. *Chin Med J (Engl)*. 2012 Nov;125(21):3782-5.
[6] Late Graft Loss among Pediatric Recipients of DCD Kidneys. *Clin J Am Soc Nephrol*. 2011 Nov; 6(11): 2705–2711.
[7] Utilization of small pediatric donor kidneys: a decision analysis (J). *Transplantation*.2011, 91(10):1110－1113.
[8] Current status of pe-diatric donor en bloc kidney transplantation to young adult recipients. *Actas Urol Esp*, 2013, 37(6):383-386.
[9] En bloc kidney transplantation from pediatric donors: comparable outcomes with living donor kidney transplantation. *Transplantation*, 2011, 92(5):564-569
[10] Pediatric Donation After Circulatory Determination of Death: A Scoping Review. *Pediatr Crit Care Med*. 2015 Dec 31.
[11] The Coordination and Activity Tracking in CHildren (CATCH) study: rationale and design. *BMC Public Health*. 2015 Dec 21;15(1):1266.
[12] Potential for liver and kidney donation after circulatory death in infants and children. *Pediatrics*. 2011 Sep;128(3):e631-8.
[13] Single pediatric kidney transplantation in adult recipients: comparable outcomes with standard－criteria deceased－donor kidney transplantation (J). *Transplantation*, 2013, 95(11):1354-1359.
[14] Delayed graft function in pediatric deceased donor kidney transplantation: donor-related risk factors and impact on two-yr graft function and survival: a single-center analysis. *Pediatr Transplant*. 2014 Jun;18(4):357-62.
[15] Outcomes of standard dose EC-MPS with low exposure to CsA in DCD renal transplantation recipients with DGF. *Int J Clin Pract*, May 2015, 69 (Suppl. 183), 8-15.
[16] Which is more suitable for kidney transplantation at the early post-transplantation phase in China–low dosing or standard dosing of enteric-coated mycophenolate sodium? *Int J Clin Pract*, April 2014, 68 (Suppl. 181), 1-7.
[17] Patient experiences with self-monitoring renal function after renal transplantation: results from a single-center prospective pilot study. *Patient Prefer Adherence*. 2015 Dec 7;9:1721-31.

P.1351

Clinical Investigation of kidney transplantation from cardiopulmonary resuscitation of DCD

Tian Puxun[2], Xun Wujun[1], Ding Xiaoming[1], Xiang Heli[1], Pan Xiaoming[1], Yan Hang[1], Hou Jun[1], Feng Xinshun[1], Tian Xiaohui[1], Ding Chenguang[1], Li Yang[1], Zheng Jin[1], Han Feng[2].
[1]Department of Renal Transplantation, Kidney Disease Hospital, the First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, People's Republic of China; [2]Organ Transplantation Institute of Xi'an Jiaotong University, Department of Renal Transplantation, Kidney Disease Hospital, the First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, People's Republic of China.

Objective: To investigate perioperative treatment method and the early clinical effect of kidney transplantation from cardiopulmonary resuscitation of DCD.
Methods: A retrospective review of clinical data of 34 donors and 59 recipients kidney transplantation from cardiopulmonary resuscitation of DCD performed from December 2011 to November 2015 in kidney transplantation department of the first affiliated hospital of Xi'an Jiaotong university.Analysis of cardiopulmonary resuscitation (CPR) impact on the prognosis of DCD renal transplant recipients.
Results: Under following the DCD organ donation standard and the supervision and appraisal by our hospital ethics committee, various surgical operations were implemented in the study. Perioperative treatment and nursing measures followed the international and domestic standards, combining our center clinical experience of many years. Related statistical data of donors and recipients will be Introduced in the text.
Conclusions: 1.DCD from cardiopulmonary resuscitation donation is an effective supplement to kidney source shortage. 2.Our center adheres to the following principles: If the time of CPR was less than 10 minutes and renal function was normal, we will implement organ donation directly; If the time of CPR was 10-30 minutes, we will assess blood pressure, kidney function and urine volume comprehensively and discuss the risk of operation; If the time of CPR was more than 30 minutes, we will suggest to give up donating. 3.In addition,, repair and maintenance of the donors of the primary diseases and organs function are very important. Especially, it is necessary to pay attention to potential infectious donors, observe the change of the urine and kidney function dynamically and application of hormone, heparin and urokinase before organ procurement. Application of lifepot, anti-infection and postoperative follow-up are all keys to assure outcomes of kidney transplantation.
The First Affiliated Hospital of Xi'an Jiaotong University

P.1352

Pyeloureteric junction obstruction in renal allografts

Andrej Grajn, Anthony Griffin, David Lockwood, Simon Wood, Malcom Lawson, John Preston.
Transplant services, department of surgery, Princess Alexandra hospital Australia, Brisbane, Australia.

Congenital Pyeloureteric Junction (PUJ) obstruction is present in 1/500 live births. Baggy extra renal pelvises are more frequently encountered. PUJ obstruction in kidney transplantation an uncommon problem and literature is sparse. Following transplantation the obstruction may become urodynamically significant due to diuresis, autonomic denervation, minor ureteric torsion, kink, external scarring or reduced blood supply.
We report two cases of PUJ obstruction in renal allograft recipients. In the first case, following deceased donor transplant, PUJ obstruction was diagnosed following stent removal. In the second case a 5 mm stone was in noted in baggy extra renal pelvis during live donor assessment. Back table flexible ureteroscopy was planned with standard CH6 instrument. During the procedure the ureteroscope could not be advanced though the PUJ.
Options for reconstruction of transplant PUJ obstruction include native ureteropyelostomy, Boari flap vesicopyleostomy, non-dismembered flap reconstruction, long term stenting or nephrostomy. Both of our cases were reconstructed with native ureteropyleostomy with good intermediate term outcomes.
References:
[1] Smets S, Oyen R, Coosemans W, Kuypers DR,An unusual cause of ureteral obstruction in a renal allograft.*Nephrol Dial Transplant. 2006 Dec;21(12):3593-4.*

P.1353

Patient and Graft survival and graft function three years after Living-related donation and Brain-dead donation: results from a single center

Hai An Ha Phan, The Cuong Nguyen, Manh Tuong Nguyen, Xuan Nam Nguyen, Thi Thuy Nguyen.
Kidney Disease & Dialysis Dept, Viet Duc Hospital, Hanoi, Viet Nam.

Introduction: Organ shortage is always a serious challenge for organ transplantation, as the number of End-stage patients is growing. Patients expected to have the organ, especially the kidneys, from young donors, and in some areas the living donors were considered "old" when they reach the age 60 or over.
The aim of our study was to investigate whether the source of kidneys can have impact on graft and patient survival and graft function
Materials and Methods: We conducted a retrospective study which included 46 kidney recipients who undergone the kidney transplantation from 1 Jan 2010 to 31 Dec 2012 and were followed-up at our center. Patients were divided into two groups according to organ sources: group 1 received kidneys from Living-related donors; group2 received kidney from Brain-dead donors. We compared the survival rates and the parameters relating to donors and recipients age, dose and trough level of CNI and recipients serum creatinine as a marker of graft function.
Results: Group 1 included 33 kidney recipients (22 males and 11 females); Group 2 included 19 kidney recipients (11 males and 8 females). All recipients received a triple immunosuppressive regimen. 1 patient from group 1 and 2 patients from group 2 have been moved to other cities and provinces after procedure. Among those who were on regular follow-up at our center, the patient survival rate at 1 year, 2 year and 3 year was 100%, 100% and 100% respectively. In group 1 (31), 1 patient (3.2%) lost the grafts at month 2 and another patient (3.2%) lost the graft at year 2. In group 2 (17), 1 patient (5.9%) lost the graft at year 2. The donor and recipient age was 48.0 +10.4 and 30.2 + 9.9 years old respectively for group 1 and 23.2 + 7.4 and 37.4 + 11 years old respectively for group 2. The serum creatinine of recipients from group 1 had tendency of creeping up, while the value of recipients from group 2 was stable after three years of follow-up. At baseline, serum creatinine of two groups did not differ (125.3 + 35.9 µmol/l vs 131.6 + 39.8 µmol/l, $p>0.05$), but at year three the serum creatinine of group 1 was significantly higher than that of group 2 (146.4 + 86.9 µmol/l vs 111.4 + 34.4 µmol/l).
Discussion and Conclusion: The patient survival was excellent after kidney transplantation both from living-related and brain-dead donors. However the graft survival was to be improved, in particular for the recipients from living donors. The age of donors in group 1 was much older then the age of the recipients, in opposite the recipients from group 2 were much younger than their donors. The donor age could have impact on long-term outcomes of kidney recipients even though there was good HLA matching. But the short-term survival rate of patients and of the grafts in both groups was acceptable. Donation from young brain-dead donors should be promoted.

P.1354

BK virus infection among kidney transplant recipients: data from Department of Kidney Diseases and Dialysis, Viet Duc hospital

Hai An Ha Phan, The Cuong Nguyen, Manh Tuong Nguyen, Xuan Nam Nguyen, Thi Thuy Nguyen.
Kidney Diseases and Dialysis, Viet Duc Hospital, Hanoi, Viet Nam.

Introduction: BK virus belongs to Polyomavirus family and can present in up to 90% population, locating in urothelial cells. In organ transplant patients, under immunosuppression, BKV can be reactivated and replicated. BKV infection among renal transplant recipients has wide range of manifestations, from asymptomatic coexistance to overt nephropathy, and occasionally associated with ureteral fibrosis and stenosis. Since two last decates, BKV-associated nephropathy (BKVAN) was recognized as an important cause leading to graft dysfunction and graft loss. As to date there is no effective treatment available for BKVAN except reduction in or cessation of immunosuppression.
Materials and Methods: We conducted a prospective, cross-sectional study from 1 Jan 2014 to 30 Apr 2015 to screen renal transplant recipients who were followed-up at Department of Kidney Diseases & Dialysis, Viet Duc hospital for BKV in urine and blood using Real time PCR technique to identify the prevalence of viruria and viremia, and to identify some factors that could affect this condition. Some patients with increased serum creatinine have been biopsied. The data from history, therapeutic regimens... was documented.
Results: A total of 371 renal recipients including 118 males (32,0%) and 253 females (68,0%) at the mean age of 41,2 years old (ranged from 15 to 71 years old) were enrolled into study. The prevalence of viruria was 46,8% (173 patients), but only 5,1% (19 patients) had viremia. 10 patients (2,7%) had diagnosis of BKVAN based on allograft biopsy findings. We did not find the singificant impact of mycophenolate mofetil use, Cyclosporin A level, patient's age or HLA compatibility degree on appearance of BKV in urines. However, the duration of immunosuppressive exposure seemed to be a significant risk factor on viruria ($p=0,007$). The hepatitis viral infection did not increase but reduce the risk of viruria appearance. High viral load in urines could predict viremia. Serum creatinine of patients with viremia was significantly higher than that of patients without viremia. Histopathological diagnosis had high specificity, but cannot be widely applied due to several objective reasons.
Discussion and Conclusion: Renal allograft dysfunction urged to search for BKV in urines and/or blood, in particular biopsy was recommended in order to select an appropriate therapeutic strategy to rescue allograft.
Acknowledgement to Dept of Microbiology, Hanoi Medical University, Hanoi, Vietnam
References:
[1] 1. Purighalla R, Shapiro R, McCauley J, Randhawa P
[2] 2. Binet I, Nickeleit V, Hirsch HH et al
[3] 3. Hirsch HH

P.1355

Establishing of uncontrolled donation after circulatory determination of death (UDCDD) protocol in Poland – first two cases

Marek Karczewski[1], Lukasz Kawalec[2], Lucyna Cichanska[1], Tomasz Malkiewicz[2], Zofia Adamska[1], Wojciech Czapiewski[1], Michal Stronka[1].
[1]Transplantology, General, Vascular and Plastic Surgery, Clinical Hospital Of Poznan University of Medical Sciences, Poznan, Poland; [2]Anaesthesiology and Intensive Care, Clinical Hospital Of Poznan University of Medical Sciences, Poznan, Poland.

Introduction: Despite an increase in kidney transplantation from expanded criteria donors (ECD) and living related donors (LRD) in the last decades, the supply of kidneys donors is still insufficient[1]. Only ten countries within the European Union have reported donors after cardiac death (DCD) activity[2]; Poland, until now, has not been among these countries. In this paper we describe the outcome of the first four transplantation procedures of DCD in Poland. Eligible organs are uncontrolled Maastricht I and II types, while Maastricht III donors in Poland are excluded by the law[3].
Material and Methods: Between May and July 2015, four refractory cardiac arrests were screened in the Clinical Hospital of Poznan University of Medical Sciences. Two eligible non-heart-beating donors (NHBD), Maastricht I/II, a male (44 y.o.) and a female (27 y.o.), were accepted for organ retrieval. End-tidal carbon dioxide of both donors at admission was 34 and 18 mmHg and their body temperatures were 35°C. The interval from cardiac arrest to Advanced Life Support (ALS) was one minute for both donors. ALS lasted 45 and 50 minutes with an additional five minute period of no-touch time[4]. The interval from initiation of ALS to aortic catheter placement was 135 and 158 minutes, respectively[5].
Results and Discussion: Kidneys have been transplanted into four recipients. One of the recipients' donated kidney developed primary non-function due to hypotension and renal vein thrombosis (female recipient after lung transplantation with renal failure due to calcineurin inhibitors nephrotoxicity)[6]. The other three recipients (two males and one female, aged 54 ± 15.4 years) have had delayed graft function, which lasted 18 ± 9 days. They needed 6.6 ± 5 dialyses and transfusion of 4.6 ± 3 blood bags. Their BMI was 26.24 ± 2.41 kg/m2, WIT was 35.3 ± 7.6 minutes, CIT was 1442 ± 203 minutes. GFR at one month after transplantation was 20 ± 11.5 ml/min, at three months 55.3 ± 10 ml/min and at six months 65.6 ± 16 ml/min[7]. Graft survival rate was 100% at three months after transplantation. Detailed data of the recipients are presented in Table 1.
Each year in Poland a fixed number of approximately 600 kidney procurements from donors after brain death are performed. The number of transplants from living donors (LRD) is comparatively small, approximately 55 per year[8]. The program of organ donation after cardiac arrest is a strategy to increase the pool of donors while shortening the waiting time for transplant patients on waiting lists. Still, this group of donors is not fully exploited in the world, especially in Poland. The protocol established at the Poznan University of Medical Sciences may be helpful for other transplant teams in Poland.
Conclusions: The DCD program on uncontrolled donors is extremely challenging for the transplant coordinator, the hospital transplant team and out-of-hospital emergency services. Nationwide information campaigns are crucial for the acceptance of organ after cardiac death donation by the Polish population and thus the programs future success.

No.	Sex	Age	Body mass [kg]	Height [cm]	BMI [kg/m2]	WIT [min]	CIT [min]	GFR after 1 month [ml/min]	GFR after 3 months [ml/min]	GFR after 6 months [ml/min]	Blood units transfusion
1	Male	58	87	182	26,26	30	1258	33	45	62	4
2	Female	67	61	160	23,82	44	1408	16	65	65	2
3	Male	37	78	165	28,65	32	1660	11	56	70	8

References:
[1] Port FK, Merion RM, Roys EC, Wolfe RA. Trends in organ donation and transplantation in the United States, 1997-2006. Am J Transplant 8:911-921, 2008
[2] Domingues-Gil B, Haase-Kromwijk B, Van Leiden H, Neuberger J, Coene L, Morel P, et al. Current situation of donation after circulatory death in European countries. Transplant International 2011;24:676-86
[3] Załącznik do Obwieszczenia Ministra Zdrowia z dnia 9 sierpnia 2010 r. Kryteria i sposób stwierdzenia nieodwracalnego zatrzymania krążenia poprzedzającego pobranie komórek, tkanek lub narządów do przeszczepienia, M. P. 2010r. Nr 59, poz. 784
[4] Bernat JL, Capron AM, Bleck TP, et al. The circulatory-respiratory determination of death in organ donation. Crit Care Med 2010;38:972-9
[5] B. Fieux F, Losser MR, Bourgeois E et al. Kidney retrieval after sudden out of hospital refractory cardiac arrest: A cohort of uncontrolled non heart beating donors. Crit Care 2009;13:R141
[6] Hamed MO, Chen Y, Pasea L, Watson CJ, Torpey N, Bradley JA, Pettigrew G, Saeb-Parsy K. Early graft loss after kidney transplantation: risk factors and concequences. Am J Transplant 2015;15:1632-3
[7] Nagaraja P, Roberts GW, Stephens M, Horvath S, Kaposztas Z, Chavez R, Asderakis A. Impact of expanded criteria variables on outcomes of kidney transplantation from donors after cardiac death. Transplantation 2015;99(1):226-31. doi: 10.1097/TP.0000000000000304.
[8] www.poltransplant.org.pl/statystyka_2016.html

P.1356

Identifying risk severity using proposed wuhan chart in deceased donor kidney transplantation

Kiran Jang Kunwar, Heng Li, Zeng Fuqing, Wang Zhendi.
Urology, Wuhan Union Hospital, Tongji Medical College, Huazhong University of Science & Technology, Wuhan, People's Republic of China.

Introduction and Objective: To find etiological risk factors for deceased donor kidney transplantation.
Materials and Methods: From January 2009 to December 2015, a total of 130 individuals underwent deceased donor (DBD-48, DCD-82) kidney transplantation. Any complication post transplantation within one year was noted and deceased donor re-studied to determine the probable identifiable risk factors. When reevaluating the donor from the data centre, certain factors identifiable factors like; age of donor, cause of donor death, drugs received (catecholamine, vasopressin), Creatinine < 3mg/dl, duration of anuria, duration of low BB, WIT, and TIT etc were related to more complications, therefore major focused during our study.
Results: Out of 130 cases, 56 cases reported complications like DGF, ureterocele, hydronephrosis, urinary leakage, hematoma, embolisation, chest infection, sepsis even death. Upon reevaluating the recipient and donor, we found that the following are the common risk factors for post transplantation complications in deceased donors: Patients age < 60, death related to CVS was associated with complications, serum creatinine < 3mg/dl & drugs administered prior to retrieval(prolong morbidity) is a definite risk for DGF, anuria and SBP(>60 mm of hg) for more than 24 hours increases chances of delay in graft function. Warm ischemia time (WIT) of >20 mns along with higher total ischemic time (TIT) increases risk for delay function (DGF) non-functioning of graft (PNG). We propose a chart (Wuhan Chart, see Figure 1) to evaluate the risk severity scale, greater than 7 always have an increased risk factor than with less than 7.
Conclusion: In the future, deceased donor transplantation will serve as a major organ pool. Identifying risk factors and taking extra precaution will definitely help to minimize complications. Thus, identification of possible risk factors will help to minimize complications and make transplant successful by prolonging graft and patient survival. The Wuhan Chart (Risk Severity Scale) will help to identify risk severity.

Table 1: WUHAN CHART (RISK SEVERITY SCALE)

DEMOGRAPHIC FEATURES	REMARKS	SCORE
Age (> 60/ < 60)	1/0	
Cause of Death (CVD/ Others)	1/0	
Drugs (Catecholamine/Vasopressin) (Yes/No)	1/0	
Body Temperature @ Retrieval (> 38° c/ < 38° c);	1/0	
S Creatinine at organ Retrieval (> 3.0 mg/dl/ < 3.0 mg/dl)	2/0	
Duration of Anuria (>24 hr / < 24 hr)	2/0	
Duration of Low SBP (> 24 hr / < 24 hr)	2/0	
Warm Ischemia Time (>20 mns/ < 20 mns)	2/0	
Total Ischemia Time (> 24 hr/ < 24 hr)	2/0	

P.1357

The impact of delayed graft function after kidney transplantation on long-term graft function and survival

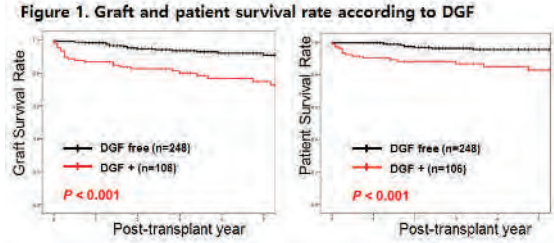
Seung Hwan Song, Jee Youn Lee, Su-Kyung Kwon, Juhan Lee, Jae Geun Lee, Kyu Ha Huh, Myoung Soo Kim, Soon Il Kim, Yu Seun Kim.
Department of Surgery and The Research Institute for Transplantation, Yonsei University College of Medicine, Seoul, Korea.

Introduction: Delayed graft function (DGF) after kidney transplantation (KT) is important factor influenced graft function and graft survival in the early period (<1year). However, the effect of DGF on long-term graft function and survival is controversial. The aim of this study is to evaluate the effect of DGF on long-term graft function and survival in KT.
Methods: A total 354 recipients who underwent deceased donor kidney transplantation (DDKT) between January 2005 and December 2014 in Severance hospital were retrospectively reviewed. Cases of age ≤ 15 years and simultaneous other organ transplantation were excluded.
The Results: Among 354 DDKT, 106 cases had episodes of DGF, and the others were in a DGF free group (n=248). The donor age, history of hypertension, serum creatinine level before donor nephrectomy, and cold ischemic time in DGF group was significantly higher than those of the DGF free group. The incidence of acute rejection (<1 years) was higher in the DGF group than DGF free group.(24.8% vs 6.5%, P<0.001)
Glomerular filtration rate (GFR) was inferior in the DGF group than DGF free group until post transplant 3 years. There was significant difference of graft and patient survival between DGF and DGF free group. DGF was an independent risk factor for graft and patient failure (HR 2.4, 95% CI: 1.23 to 4.53, P=0.01, HR 3.81, 95% CI: 1.66 to 8.75, P=0.002). The 5 year graft survival was higher in the DGF free group than in the DGF group (90.9% vs 74.8%, P<0. 001)
Conclusion: DGF had a negative influence on the long-term graft function and survival.

Table. Demographic and clinical characteristics according to DGF

	DGF free 248 (70.1%)	DGF + 106 (29.9%)	p-value
Recipient Factors			
Recipient sex (Male%)	145(58.5%)	62(58.5%)	0.997
Recipient age (years)	45.9 ± 10.3	47.7 ± 10.2	0.154
Recipient diabetes (%)	20(8.1%)	16(15.1%)	0.045
Retransplantation (%)	41(16.5)%	21(19.8)%	0.457
HLA mismatch	2.8 ± 1.6	3.1 ± 1.4	0.039
Dialysis duration (months)	85.1 ± 45.3	91.1 ± 48.2	0.265
Donor Factors			
Donor sex (Male%)	154(62.1%)	67(63.2%)	0.843
Donor age (years)	42.7 ± 13.9	47.5 ± 11.6	0.002
Donor Hypertension	49(19.8%)	31(29.5%)	0.045
Donor diabetes	17(6.9%)	8(7.8%)	0.798
Cerebrovascular accident	127(51.2%)	64(60.4%)	0.113
CPR history, Yes	103(41.5%)	41(39.0%)	0.664
Inotropics use	235(94.8%)	97(92.4%)	0.384
ICU duration (hours)	10.4 ± 12.8	7.6 ± 9.3	0.022
Serum Cr, pre-nephrectomy (mg/dl)	1.2 ± 0.7	2.0 ± 1.3	<0.0001
Cold ischemic time (mins)	306.5 ± 143.7	362.3 ± 184.5	0.006

Figure 1. Graft and patient survival rate according to DGF



P.1358

Incidence and outcome of vascular complications in kidney transplantation after expanding acceptance criteria: Single centre experience

Mahmoud Soliman[1,2], Abbas Ghazanfar[1], Atul Bagul[1], Ayman Salem[2], Ahmed Ali[1], Ashar Wadoodi[1], Sarah Heap[1], Mohamed Morsy[1].
[1]Renal and Transplantation, St George's University Hospitals NHS Foundation Trust, London, UK, London, United Kingdom; [2]Vascular Surgery Deparment, Zagazig University, Zagazig, Egypt.

Introduction: Post-transplant vascular complications are not uncommon in kidney transplantation and might lead to graft loss. The expansion of acceptance criteria to include marginal organs, donors and recipients increased vascular complications risk. We present the vascular complications and their outcome after expanding deceased donor acceptance criteria.

Subjects and Methods: A total of 203 kidney transplants performed between Jan 2013-June 2014 were reviewed. These included 65(32%) live donor (LD), 81(40%) standard criteria donor (SCD) [56(27.6%) DBD and 25(12.3%) DCD] and 57(28%) ECD [35(17.2%) DBD and 22(10.8%) DCD] kidney transplants. Seven ECD kidney pairs (3 DBD and 4 DCD) were utilised as Dual. ECD is defined as a donor ≥60 or 50-59 years old with 2 co-morbidities of hypertension, cerebrovascular accident as a cause of death, and pre-retrieval Cr >132.6 µmol/L.

Results: Thirty four (16.7%) vascular complications were reported in 32 recipients included 8(12.3%) LD, 19(23.5%) SCD and 7(12.3%) ECD Transplants ($p<0.05$; SCD v LD and ECD). respectively. Bleeding diagnosed by drains, US/CT scans, drop in Hb and BP, was the most common complication reported in 22(10.8%), of which 5(7.7%) LD, 11(13.5%) SCD [8(14.3%) DBD and 3(12%) DCD] and 6(10.5%) ECD [4(11.4%) DBD and 2(9.1%) DCD] transplants ($p<0.05$ SCD v ECD and LD). Nine (5 SCD, 3 ECD and 1 LD) were on anticoagulant/ antiplatelet. Twelve needed re-exploration. The majority occurred within 2 weeks post-transplant. Renal vein transection occurred in one SCD/DBD transplant ended with transplant nephrectomy. One dual transplant was complicated with postoperative bleeding and managed conservatively. Renal artery stenosis (RAS) was reported in 3 of 203 transplants (1.5%), in one LD (1.5) and 2 (2.5%) SCD transplants. Renal artery thrombosis was reported in one LD (1.5%) transplant successfully rescued. Renal artery aneurysm was reported in one SCD/DBD (1.2%) transplant. Renal artery kinking was reported in 2, one LD and one SCD/DBD (1.2%) transplants. Post-EVAR intra-renal cholesterol embolization in one ECD/DCD (1.8%) transplant. A case of short segment occlusion of EIA immediately distal to the renal artery occurred in one SCD/DBD (1.2%) transplant. A case of EIV stenosis distal to the transplant vein anastomosis was reported in one SCD (1.2%) and DVT occurred in SCD/DBD (1.2%) transplants.

Overall one year graft survival was 96% with 100%, 96%. 91% in LD, SCD and ECD transplants respectively. Three out of 8(4%) grafts were lost due to vascular complications (Table 1) including post-biopsy bleeding in day 12, renal vein transection in day one and post EVAR intra-renal thrombosis. Overall 1-year patient survival was 99.5%. a recipient died with DIC.

Table 1: Vascular complications, intervention and outcome

Complication	No. of cases	Intervention	Outcome
Bleeding (3post biopsy):	22 (10.8%)	- Re-exploration: 10 - Conservative: 12	Graft salvaged: 19 Nephrectomy: 1 Death: 1
TRAS	3 (1.5%)	Angioplasty: 2 Angioplasty + stenting: 1	Successful, grafts salvaged.
R.A thrombosis:	1 (0.5%)	Re-exploration & re-implantation	Successful & graft salvaged.
R.A aneurysm	1 (0.5%)	Thrombin injection.	Successful & graft salvaged.
R.A kinking	2 (1%)	-Re-exploration and change kidney position:1 - Follow up as mild kink on MRA:1	Successful, graft salvaged.
R.A embolism	1 (0.5%)	Nephrectomy.	Nephrectomy
R. V transection	1 (0.5%)	Re-exploration.	Nephrectomy
EIA occlusion	1 (0.5%)	Conservative treatment	Graft working
EIV stenosis	1 (0.5%)	Angioplasty.	Recurrence
DVT Distal EIV and Femoral artery	1 (0.5%)	Re-exploration twice for abdominal compartment syndrome + IVC filter	Improvement.

Conclusion: Post-transplant vascular complications are not uncommon with higher occurrence in the SCD subgroup. The majority were successfully treated. Bleeding constituted the majority. Post-transplant biopsy and major vascular damage can lead to loss of the graft.

P.1359

Incidence and outcome of post-transplant urological complications in kidney transplantation after expanding acceptance criteria: Single centre experience

Mahmoud Soliman[1,2], Abbas Ghazanfar[1], Atul Bagul[1], Ayman Salem[2], Ahmed Ali[1], Ashar Wadoodi[1], Sarah Heap[1], Mohamed Morsy[1].
[1]Renal and Transplantation, St George's University Hospitals NHS Foundation Trust, London, UK, London, United Kingdom; [2]Vascular Surgery Deparment, Zagazig University, Zagazig, Egypt.

Introduction: Due to organ shortage, expanded criteria donor (ECD) kidneys have been increasingly utilized to expand the donor pool. The expansion of acceptance criteria to include marginal organs, donors and recipients increases the risk of urological complications. We present the urological complications and their outcome after expanding deceased donor acceptance criteria in our centre.
Subjects and Methods: A total of 203 kidney transplants performed between Jan 2013 to June 2014 were reviewed. These included 65(32%) live donor (LD), 81(40%) standard criteria donor (SCD) [of which 56(27.6%) DBD and 25(12.3%) DCD] and 57(28%) ECD [of which 35(17.2%) DBD and 22(10.8%) DCD] kidney transplants. Seven ECD kidney pairs (3 DBD and 4 DCD) were utilised as Dual. ECD is defined as a donor ≥60 or 50-59 years old with at least two co-morbidities including hypertension, cerebrovascular accident (CVA) as a cause of death, and pre-retrieval creatinine >132.6 μmol/L. Urinary catheter is normally removed at day 2-3 post-transplant unless longer catheterisation is indicated
Results: Twelve urological complications were reported in 12(5.9%) recipients. These collectively occurred significantly in 8(14%) ECD compared to 3(3.7%) SCD and one (1.5%) LD transplants ($p<0.05$). Ureteric stricture was the most common complication occurring in 8 cases (3.9%), of which 3(4.6%), 4(4.9%) and 1(1.8%) in LD, SCD (3DBD and one DCD) and ECD (DCD) kidney transplants respectively. All except the LD transplant (12 months) occurred within 3 months post-transplant. However, there was no significant difference between groups. Intra-operative renal pelvis puncture caused by the D-J Stent in an ECD/DBD transplant that was stitched successfully. A case of anastomotic urine leak case was reported in an ECD/DBD transplant (0.5%). Obstructive uropathy (mild hydronephrosis) caused by lymphocele in reported in one (0.5%) ECD/DCD transplant. Hydronephrosis secondary to vesico-ureteric reflux occurred was reported in one ECD/DBD (0.5%) transplant. Table 1 shows the post intervention outcome. Among the 7 dual transplants mild hydronephrosis due to lymphocele was diagnosed and successfully drained.
Overall one year graft survival was 96% with 100%, 96%. 91% in LD, SCD and ECD transplants respectively. Eight grafts (4%) were lost, neither due to urological complications. Overall 1-year patient survival was 99.5%. One SCD/DBD transplant recipient died with functioning graft due to DIC secondary to gram-ve septicaemia and bleeding.

Table 1: Post-transplant urological complications, intervention and outcome.

Complication	No. of cases (%)	Intervention	Outcome
Ureteric stricture	8 (4%)	7 Surgical re-construction after nephrostomy One Nephrostomy and antegrade stenting. 1	Grafts salvaged
Obstructive uropathy by lymphocele	1 (0.5%)	Radiological drainage of lymphocele.	Successful, kidney function improved.
Ureteric stent pierced renal pelvis.	1 (0.5%)	Stent retracted & closure with 4/0 PDS intra-operatively.	Functioning graft
Hydronephrosis 2ry to VU reflux	1 (0.5%)	Conservative measures.	Mild Hydronephrosis, kidney function remained stable.
Anastomotic Urine leak	1 (0.5%)	Surgical reconstruction.	Successful, graft salvaged.

Conclusion: All post-transplant urological complications were managed successfully. Ureteric stricture was the most common complication. No grafts were lost because urological complications. The majority of them occurred significantly in the ECD subgroup.

P.1360

Pathological analysis of 544 cases of indicated renal allograft biopsies

Xutao Chen[1], Hongyang Wang [1], Wenfang Chen [2], Longshan Liu[1], Qian Fu[1], Jun Li[1], Ronghai Deng [1], Changxi Wang [1].
[1]Organ Transplant Center, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, People's Republic of China; [2]Department of Pathology, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, People's Republic of China.

Objective: To explore the histopathological features and proportion of various renal allograft biopsies for post-transplant complications.
Methods: Between January 2010 and December 2015, 544 kidney transplants received 670 percutaneous renal allograft biopsies in our hospital. Pathological diagnosis and classification were performed according to the Banff 1997, 2007 and 2013 Schema.
Results: There were 22 (4.04%) cases of acute antibody—mediated rejection and 38 (6.99%) cases of acute T cell—mediated rejection in 544 kidney transplants. Besides, 54 (9.93%) cases were diagnosed as chronic antibody—mediated rejection and 18 (3.31%) cases as chronic T cell-mediated rejection. Acute CNI-nephrotoxicity and chronic CNI-nephrotoxicity were 43 (7.90%) cases and 31 (5.70%) cases, respectively. Relapsed or new-onset nephropathy were 111 (20.40%) cases, including 83 (15.26%) cases of IgA nephropathy. BK virus-associated nephropathy (BKVAN) was founded in 45 (8.27%) cases. There was no severe adverse effect after all renal allograft biopsies.
Conclusion: Percutaneous renal allograft biopsy is safe and contributes to the diagnosis and treatment of diverse post-transplant complications.

P.1361

En bloc kidney transplantation from infant donors less than 10 kg – surgical skill, clinical management and outcome in a transplant center in China

Hongyang Wang, Jun Li, Longshan Liu, Ronghai Deng, Qian Fu, Huanxi Zhang, Suxiong Deng, Changxi Wang.
Department of Organ Transplantation, The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, People's Republic of China.

Background: Early graft loss and poor graft function limit the use of kidneys from infant donors. Between November 2012 and September 2015, we performed seven en bloc kidney transplantations from infant donors less than 10 kg. In this report, we described surgical procedures, clinical management, and transplant outcomes.
Methods: The clinical data of donors and recipients were presented. The backtable procedure was performed with distal end of the aorta and vena cava closed. The proximal end of donor aorta and vena cava was anastomosed to the internal or external iliac artery and the external iliac vein, respectively. Two ureteroneocystostomies were performed separately with placement of ureteral stents. Strict bed rest was prescribed for 5-7 days and heparin were given for 2 weeks after surgery. The central venous pressure and systolic blood pressure were kept at 6-12cm H2O and 100-130 mmHg, respectively.
Results: The age and weight of the donors were 4.9±3.6 months and 6.4±2.8 kg, respectively. The recipient-to-donor weight ratio was 6.5±2.8. All the recipients got immediate graft function. One kidney was removed due to arterial thrombosis during surgery. Urine leak occurred in 2 recipients. Bilateral ureteral obstruction occurred in 1 recipient. The size of the en bloc kidney increased to 7.3±0.7 cm 6 months after surgery. Patient and graft survival were both 100% at the last follow up.
Conclusions: As a result of successful surgery and effective clinical management, the outcomes of en bloc kidney transplantation from infant donors less than 10 kg were satisfactory in our study.
We thank Prof. Xian C. Li from Houston Methodist Research Institute, Texas Medical for his comments and revision advices on this article

P.1362

Comparison of kidney transplantation from living and deceased donation: a consecutive data analysis since implement of donation after citizen's death in China

Yunyi Xiong, Jun Li, Longshan Liu, Qian Fu, Huanxi Zhang, Weiying Deng, Suxiong Deng, Jiguang Fei, Jiang Qiu, Lizhong Chen, Changxi Wang.
Organ Transplant Center, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, People's Republic of China.

Background: Living-related donation and Chinese donation after citizen's death (CDCD) has become the two only organ donor sources of kidney transplantation in China. The aim of this study is to compare kidney transplant outcome from living-related donation and CDCD.
Materials and Methods: A consecutive data of kidney transplantation in our center from 2010 to 2015 was retrospectively analyzed.
Results: A total of 658 kidney transplant patients was enrolled, among whom 389 was from living-related donation and 269 was from CDCD. The rate of biopsy-proven rejection was 7.1% in CDCD group and 8.0% in LRKT group, with no significant difference ($p=0.666$). The rate of delayed graft function (DGF) was significantly higher in CDCD group than that in LRKT group (16% vs 2.3%, $p<0.01$). Serum creatinine at stable phase after kidney transplantation was comparable between CDCD group and LRKT group (121.3 ± 48.31 vs 118.1±25.48, $p>0.05$). The 1-year graft survival was 96.9% in CDCD group and 98.6% in LRKT group ($p=0.254$), and the 3-year graft survival was 88.4% in CDCD group and 95.7% in LRKT group ($p=0.061$). No significant difference was observed between the two groups in other complications including infection, liver function impairment, leucopenia, new-onset diabetes mellitus, and urine leakage, etc.
Conclusion: Kidney transplant outcome from living-related or deceased donation was comparable in short-term period after transplantation, although higher rate of DGF occurred in KTx from deceased donors. Nevertheless, long-term outcome needs to be further determined since a likely inferior 3-year graft survival was observed in this study.

P.1363

Conversion from mycophenolate mofetil to mizoribine and its therapeutic exposure in Chinese renal transplant recipients with leucopenia.

Longshan Liu[1], Jun Li[1], Qian Fu[1], Bin Ren[2], Gang Huang[1], Guodong Chen[1], Suxiong Deng[1], Jiang Qiu[1], Jiguang Fei[1], Lizhong Chen[1], Changxi Wang[1].
[1]Organ Transplant Center, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, People's Republic of China; [2]Department of Pharmacology, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, People's Republic of China.

Background: Mizoribine (MZ) has been reported in many countries as an effective immunosuppressant with less myelosuppressive effect than mycophenolate mofetil (MMF). The aim of this present study is to assess the efficacy and safety of mizoribine as an alternative immunosuppressant for renal transplant recipients with MMF-sensitive leucopenia, and to investigate its therapeutic exposure as well.
Patients and Methods: Nineteen MMF-sensitive leucopenia patients who underwent renal transplantation between August 2006 and November 2007 were prescribed mizoribine to replace mycophenolate mofetil. The subjects were comprised of 14 male and 5 female cases with an average age of 42 ± 10.6 years. The concomitant immunosuppressants were tacrolimus plus steroids in 11 patients, and cyclosporine plus steroids in 8 patients. All the patients presented stable graft function. Mizoribine was administered by 100 mg per day (twice daily), and pharmacokinetic monitoring was performed 14 days after MZ initiation. Peripheral vein blood samples were harvested right before MZ administration (0 h) and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10 and 12 h after the administration. MZ plasma concentrations were measured by high performance liquid chromatogram (HPLC). MZ dose was then adjusted when necessary according to its concentration and clinical features. Hemogram, renal and hepatic function were routinely measured during the follow-up.
Results: Patients were followed up for 3 to 18 months. White blood count (WBC) recovered in all the patients from 3.0 ± 0.4×109/L to 6.4 ± 0.8×109/L ($P < 0.01$). Renal graft function maintained stable and no acute rejection observed. Serum creatinine (sCr) was 119 ± 27.4 μmol/L before MZ initiation, and maintained at 114 ± 30.5μmol/L by the latest examination ($P > 0.05$). Blood uric acid increased in 10 patients and seven developed hyperuricacidemia with a level of 512 ± 58.9 mmol/L. However, normal blood uric acid level was achieved in all the seven patients when applying Allopurinol.
As pharmacokinetic features of Mizoribine in these patients, its trough level (C0) was 0.31 ± 0.16μg/mL, Cmax 1.21 ± 0.55 μg/mL, Tmax 3.5 ± 1.2 h, and AUC0-12 h was 8.4 ± 3.6 (μg/mL)•h. MZ dose was subtracted one half in one patient with high trough level and serum creatinine whose leucopenia was constant when using 100 mg per day. Meanwhile, MZ dose was increased to 150 mg per day in two patients with low C0 (0.09μg/mL) and AUC0-12 h [3.07 (μg/mL)•h].
Conclusion: Mizoribine is an alternative immunosuppressant for renal transplant patients with MMF-sensitive leucopenia except for inducing hyperuricacidemia. Dose adjustment according to its individual pharmacokinetics should be considered especially for recipients with low therapeutic exposure or with impaired graft function.

P.1364

Hemodynamics in transplant renal artery investigated by computational fluid dynamics

Hongyang Wang, Haiming Cao, Jun Li, Longshan Liu, Qian Fu, Jiguang Fei, Changxi Wang.
Department of Organ Transplantation, The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, People's Republic of China.

Background: Hemodynamics determines endothelial cell phenotype, influences vascular remodeling and affects the in-stent restenosis. This study aimed to analysis the hemodynamic changes in severe transplant renal artery stenosis (TRAS) by patient-specific computational fluid dynamics (CFD).
Methods and Results: Twelve kidney transplant recipients (KKRs) with severe TRAS, including five of whom experienced stent placement, and four normal KKRs were included in the study. All subjects were examined with color Doppler and computed tomography angiography (CTA) to enable visualization of transplant renal artery and stent, which was used to generate computational meshes. The distribution of the principal parameters was obtained by CFD using patient-specific geometries and flow analytical measurements. Compared to normal artery, the pressure different, maximal velocity and maximal wall shear stress (WSS) increased significantly in severe transplant renal artery (4215.83±2351.65 vs 46.25±29.23 Pa, 2.56±0.54 vs1.22±0.25 m/s, 221.9±76.9 vs 41.7±8.5 Pa, each $P<0.05$), while the mass flow rate (MFR) of transplant renal artery decreased significantly (0.002±0.001 vs 0.011±0.001 kg/s, $P<0.05$). When stent was placed, all the hemodynamic parameters (pressure difference, maximal velocity, maximal WSS, MFR) returned to normal. However, compared to normal artery, the stented artery had increased low WSS region.
Conclusions: Our results improve estimates of the hemodynamic changes in patients with TRAS. Stent implantation can improve the hemodynamics of severe TRAS, but the stent changes WSS disturbances in the vessel wall which may cause restenosis.

P.1365

Universal vascular index to predict organ quality during rewarming machine perfusion for kidney transplantation from donation after cardiac death

Hiromichi Obara[1,3], Yuta Morii[1], Naoto Matsuno[1,2,3], Noriyuki Morito[1], Shin Enosawa[2], Toshihiko Hirano[4], Hiroyuki Furukawa[3]. [1]Mechanical Engineering, Tokyo Metropolitan University, Tokyo, Japan; [2]Gastroenterology andTransplantation, National Asahikawa Medical University, Asahikawa, Japan; [3]National Center for Child Health and Development, National Research Institute for Child Health and Development, Tokyo, Japan; [4]Clinical Pharmacology, Tokyo University of Pharmacy and Life Science, Tokyo, Japan.

Introduction: A kidney machine perfusion is promising method to preserve organ functions and to evaluate graft quality before transplantation. Recently, there are some discussions of the perfusion temperature for a preservation of organs donated after cardiac death(DCD). Normothermic and subnormothermic machine perfusion are expected method to improve the organ obtained from the marginal donors to restore the damaged organ functions. Furthermore the rewarming perfusion from the hypothermic to the subnormothermic condition has some advantages to develop machine perfusion system. However, universal index to predict the organ quality using a renal resistance has not been established for the temperature controlled machine perfusion. This study investigates two types of the universal vasculer index of the machine perfusion calculated with the vasculer fluid dynamics for DCD kidney preservation under several temperature conditions with porcine kidney preservation experiments
Materials and Methods: Porcine kidneys were procured under several warm ischemia time conditions(0,30, 60 min) and were perfused with originally developed machine perfusion system under several temperature conditions using modified UW-gluconate solution. Each kidney was procured after cardiac arrest to control the warm ischemia time and preserved with a static cold storage(CS) for zero or two hours. After CS, the grafts were preserved under temperature controlled oxygenated perfusion from the hypothermic to the subnormothermic condition (4–8˚ C to 23˚C)(RMP). The other group was preserved under the HMP condition. The renal artery pressure and the flowrate were measured during perfusion. The two types of the new universal vasculer index of the kidney function calculated with the property of the vasculer fluid dynamics. One is the universal renal resistance and the other is the vasculer diameter index. Both parameter were compared and discussed under several conditions. Additionally, release rates of the CPK, LDH and NAG were measured in perfusate.
Results and Discussion: The pressure of the kidney vessel shows characteristic trend during the machine perfusion. Here the universal renal resistance removed the temperature effect is introduced. One of the represented measurement result of the universal renal resistance considered the temperature effect of the perfusate can calculate same values between the HMP and the RMP, but conventional renal resistance were 0.78 and 0.51 respectively. These flow characteristics indicate the corresponding to the vessel condition of the glomerulus in kidney and also the index of the vessel diameter calculated with these parameters of the pressure and the flow rates can predict the kidney vasculer condition instead of the conventional renal resistance.
Conclusion: This study suggested two types of the universal vasculer index of the machine perfusion calculated with the vasculer fluid dynamics for DCD kidney preservation under several temperature conditions.

P.1366

Outcome of kidney transplantation from expanded criteria deceases donors

Mohammad Raslan, Mostafa Al mosawi, Eyad El-halabi, Jellan Shanab, Bobby Mathew, Wael al Somaly, Mahmoud Samhan. Organ Transplant Center, Ministry of Health, Kuwait, Kuwait.

Introduction: The use of kidneys from expanded criteria deceased donors (ECD) has been generally accepted in view of the global shortage of organs available for transplantation and the increasing disparity between organ supply and demand.
Aim: To explore the outcome of kidney transplantation from ECD in comparison to that from standard criteria deceased donor (SCD).
Patients and Methods: The medical records of patients who received kidney transplants between January 2011 and December 2014 were retrospectively reviewed. 103 patients received kidney transplantation from deceased donors at Hamed Al-Essa Centre during this period. 22 (21%) grafts were obtained from ECD and 81 (79%) grafts from SCD. Recipients of ECD grafts were 12 males and 10 females aged 19 years to 65 years at the time of transplantation, and the procedure was re-transplantation in 9% of recipients. Recipients of SCD grafts were 46 males and 35 females, aged 5 years to 77 years at the time of transplantation, and the procedure was re-transplantation in 15% of recipients.
Results: Kidney transplantation from ECD was associated with 9 (41%) instances of surgical complications, and showed 50% primary graft function, 27 % delayed graft function, and 23% primary graft non-function. The mean hospital stay was 16 days. There was no recipient death but 5 grafts were lost at 1st day to 10 days post transplantation. The 1-year and 4-year survival rates were 100% and 100% respectively for recipients and 77% and 77% respectively for grafts. Kidney transplantation from SCD was associated with 29 (36%) instances of surgical complications, and showed 52% primary graft function, 42 % delayed graft function, and 6% primary graft non-function. The mean hospital stay was 22 days. Four recipients died with functioning grafts at 2 months to 6 months after transplantation, and 13 grafts were lost at 1st day to 3 years post transplantation. The 1-year and 4-year survival rates were 81% and 77% respectively for recipients and 81% and 64% respectively for grafts.
Discussion: More than 21% of deceased donor grafts were obtained from ECD. The majority (70%) of these grafts was given to older recipients and no grafts were given to children. Kidney transplantation fro ECD was associated with shorter hospital stay, similar rate of primary graft function, higher rate of primary graft non-function, slightly higher rates of recipient and graft survival rates.
In Conclusion: 1- ECD represent a viable source of kidneys for transplantation. (2) Kidney transplantation from ECD is associated with similar rates of recipient and graft survival compared to those obtained in kidney transplantation from SCD.

P.1367

Outcomes of deceased donor renal transplant recipients from a developing country

Ravi Yalamarty[1], Swarnalatha G.Guditi[1], Gangadhar Taduri[1], Uttara Das[1], Raja Karthik[1], Rajeswar Tiwari[2], Ramani M[3], Manohar K.[4].
[1]Nephrology, Nizam's institute of medical sciences, Hyderabad, India; [2]Health and Family welfare Department, Health and Family welfare, Hyderabad, India; [3]Directorate of Medical education, Directorate of Medical Education Telangana, Hyderabad, India; [4]Director, Nizam's Institute of medical sciences, Hyderabad, India.

Introduction: Renal transplantation is one of the best therapeutic modality for patient suffering from End stage renal disease.Deceased donor transplantation accounts for less than 0.08 per million population per year in India[1]. Telangana is a small state in south India comprising 35.19 million population. Government of Telangana has started programme for deceased donor organ transplantation called "Jeevandan" in 2013. This is a multicenter observational study to evaluate the outcome of deceased donor renal transplantat recipients who received organs under jeevandan programme. We report two year experience of deceased donor renal transplant recipients vis-a-vis graft survival/patient survival, graft function in terms of delayed graft function, prevalence of infections, rejection episodes.
Aim of the study: To evaluate the outcomes of deceased donor transplantation in the form of prevalence of infections, rejection rate, graft survival rate, and patient survival rate.
Collection of data and analysis: It was a multicentric observational study conducted at various hospitals in the city of Hyderabad under Jeevandan programme between january 2014 and december 2015. There is a central registry for patients who are undergoing dialysis and waiting for the deceased donor renal transplantation in Jeevandan programme. The online allocation system to the recipients was started in January 2014. From January 2014 to December 2015 there were 140 brain death donations which resulted in 240 deceased donor renal transplantations. We analyzed 130 patients who underwent deceased donor renal transplantation. Patient data were obtained by reviewing the inpatient and outpatient medical records and from patients follow up.
Results and Discussion: From January 2013 to Dec 2015 total 181 brain death donations have taken place with donation rate of 1.7 per million population per year. In Hyderabad city the donation rate came to 7.8 in the year 2015. The deceased donation rate in India is as low as 0.08 per million population per year.[1] Mean recipient age was 39.7 years. The commonest blood group was 'O'(48.36%). The commonest recipient diseases leading to ESRD were chronic kidney disease with unknown etiology (52.30%), Diabetes mellitus (25%), glomerular diseases (12.3%), tubulo interstitial diseases (6.15%). Mean dialysis duration pretransplantation was 37.4 months. Mean cold ischemic time was 3.6 hours. Induction therapy was given in 83.84% patients of which Anti thymocyte globulin (60.76%), Basiliximab (23%). Delayed graft function was observed in 35% patients. Infection prevalence was 29.5%. Rejection rate was 10.76%. Mortality rate was 10%. Over mean follow up of 2 years, patient and graft survival were 89.52% and 86.15% with mean serum creatinine of 1.1 mg/dl.
Conclusion: The deceased donation rate in our state is very high compared to India. The outcomes of deceased donor renal transplantation is comparable to live related renal transplantation.
Dr. Manisha Sahay; Dr. Satti Reddy; Dr. Soma Sekhar; Dr. Suresh Babu; Dr. Subrahmanyam; Dr. Vikrant Reddy; Dr. Hardik; Dr. Pavan Kumar; Madan Kumar; Raju; Kalyan V; Priyanka
References:
[1] G.O.MS.NO.184 Dated 16/8/2010.Data obtained from Jeevandan web site http://jeevandan.gov.in/

P.1368

Kidney donor profile index as a prognostic factor in transplants in Colombia

Alejandro Nino-Murcia[1], Fernando A.Giron Luque[1], Mauricio Sanabria[2], Laura Nino-Torres[2].
[1]Transplant Surgery Department, Colombiana de Trasplantes, Bogotá, Colombia; [2]Epidemiology, Universidad del Rosario- CES, Bogotá, Colombia.

Transplantation is the chosen therapy for End Stage Kidney Disease[3],[4]. There is a disparity between organs offered and demanded. In Colombia donation numbers are roughly static and waiting lists continue to grow[2].The Kidney Donor Profile Index (KDPI)[5] incorporates 10 deceased donor variables[4] which evaluate the kidney quality[1]. This may be a good resource or establish prognosis and optimize organ distribution[6]-[8].
A historical cohort study of deceased donors and their corresponding transplanted patients in Colombiana de Trasplantes between 01/01 2011 and 30/06/2014. Donor variables: age, gender, weight, height, race, final creatinine, donor hypertension or diabetes, cause of death and classification as a standard criteria donor (SCD) or expanded criteria donor (ECD). Receptor variables: creatinine follow up at 1, 3, 6 and 12 months, gender, age, BSA, time in dyalisis, diabetes and previous transplants.Procedure variables such as anatomical alterations, immunosuppression, duration, cold and warm ischemia times.
There were 304 deceased donor organ transplants performed during that period. 43 were discarded due to exclusion criteria. 261 transplants were analyzed. Demographics showed 65,1% male predominance in donors and 66,67% in receptors. Median donor age was 33 years (7-69 years) and 46 years (18-75 years)for receptors. There was a hispanic predominance (99,6% and 87,7% respectively). Donor median weight 71kg (±14kg) for donors and 66 kg (±12kg) for receptors. Average height was 166,8cm (120-200cm) and 164,7 cm (134-194cm) respectively. Receptor BSA average ws 1,74m^2 (1.25-2.25m^2). 80,5% corresponded to SCD and 19.5% to ECD. There was a median KDPI of 41.54%. Mortality was 6,9% and graft loss was 17,6%. Average follow up for survival was of 11,36 months (IC95% 11.042-11.686). There was 1 graft loss for every 71.78 patients/months in risk. Graft survival at 1 year post transplantation was of 88% in KDPI<70 and 82% in KDPI≥70. The survival difference was not statistically significant (LogRank p=0.143).
To our knowledge this is the first study regarding KDPI in Colombia. Longer follow ups are needed to establish a statistical significance and propose this scale as a prognostic and optimization tool for Organ allocation. There are limitations in this study of follow up due to the current Colombian health system.
References:
[1] Rao PS, Schaubel DE, Guidinger MK, Andreoni K, Wolfe R, Merion RM. A comprenhensive risk quantification score for deceased donor kidneys: the kidney donor risk index. *Transplantation* 2009;88(2):231-6
[2] Acuña L, Sanchez P, Soler L, Barrera A, Torres L, Casas L. Situación de la enfermedad renal crónica en Colombia 2014. *Cuenta de Alto Costo*
[3] Wolfe R, Ashby VB, Milford EL, Akinlolu O, Ettenger RE, Agodoa LY. Mortality in patients on dyalisis and transplant recipients: comparison of mortality in patients on dyalisis, patients on dyalisis awaiting transplantation, and recipients of a first transplant. *New Engalnd Journal of Medicine* 1999;341 (23):629-37
[4] Merion RM, Ashby VB, Wolfe R, Distant D, Hulbert-Shearon TE, Metzger R. Deceased-donor characteristics and the survival benefit of kidney transplantation. *JAMA* 2005; 294 (21):2726-33
[5] Matas AJ, Smith JM, Skeans MA, Thompson B, Gustafson SK, Stewart DE. OPTN/SRTR 2013 Annual Data Report 2013.
[6] Gandolfini I, Buzio C, Zanelli P, Palmisano A, Cremaschi E, Vaglio A. The Kidney Donor Profile Index (KDPI) of marginal

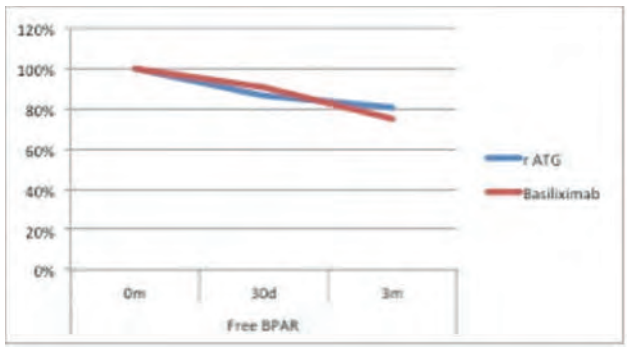
donors allocated by standardized donor biopsy assessment: distribution and association with graft outcomes. *American Journal of Transplantation* 2014;14 (11):2515-25
[7] Gupta A, Chen G, Kaplan B. KDPI and donor selection. *American Journal of Transplantation* 2014;14(11):2444-5
[8] Lee PK, Abramowicz D. Is the Kidney Donor Profile Index a step forward in the assessment of deceased donor kidney quality?. *Nephrology Dialysis Transplantion*2014;1-6

P.1369

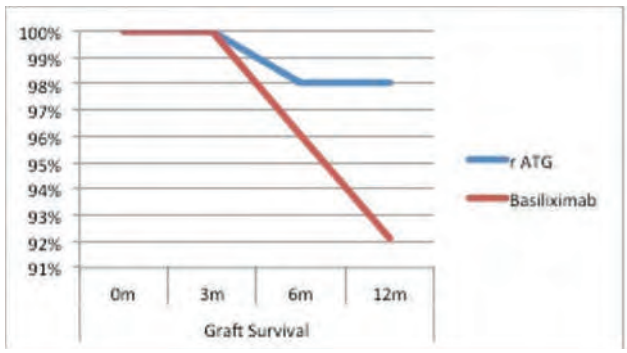
Basiliximab Vs. rabbit antithymocyte globulin (rATG) induction in "low risk" Kidney transplantation in Colombia

Sergio Salcedo, Fernando Giron, Yenny Baez, Alejandro Nino-Murcia.
Presidencia, Colombiana de Trasplantes, Bogota, Colombia.

Introduction: In high risk Kidney Transplantation (KT) patients the induction therapy (IT), should reduce biopsy proven acute rejection (BPAR) rate but IT in low risk patients is controversial, specially in Latin America because it's very expensive. We compare our "low risk" cohort in two arms: Basiliximab Vs rabbit antithymocyte globulin (rATG) induction in terms of acute rejection, graft survival, and renal function[1]-[4].
Materials and Methods: Our retrospective database were analyzed from 2013 to 2015, low risk patients (PRA<20%, first KT, >18 yo and Non African American) who completed 1 year after kidney transplant and received Basiliximab 20mg day 0 - 4 or Thymoglobuline (rATG) 2mg/kg/day in 3 continuous days, steroid therapy 500 – 250 – 125 mg/day and prednisolone 1mg/kg/day 4 days. Maintenance therapy was Tacrolimus target levels 5-7ng/ml and Mophetil Micophenolate 2g/day or Mychophenolic acid 1,44g/day. Steroids free maintenance immunosupresion.
We compared the following outcomes:
BPAR
Graft survival (GS)
Serum Creatinine
Results: The rATG patients had less BPAR Vs. Basiliximab at 3m (20% Vs. 25% RR 0,78 CI 0,4-1,55). Fig 1.



In terms of graft survival the rATG arm got better outcomes 1 year 98% Vs. 92,1% RR 0,25 CI 0,03-2. Fig 2.



rATG arm had lower creatinine levels than Basiliximab but without significance statistics.
Infections rate did not different between arms
Discussion: We followed 127 KT in 100% induction non-steroids protocol. BPAR was high in both groups but graft survival and graft function was excellent. We assumed the high BPAR risk, but in the other hand, our patients reduced the long term effects associated with steroids therapy. In our cohort rATG had better outcomes and nowadays we use this therapy in our protocol. We concluded that we don't have low risk patients because Hispanic Origen implies high risk.
Colombiana de Trasplantes Team; Ivan Salcedo
References:
[1] Clin Transplant 2013: 27: E611–E618 DOI: 10.1111/ctr.12218
[2] Transplantation 2012;94: 325Y330
[3] Transplantation Proceedings 2012; 44, 2955–2960
[4] Drugs 2012; 72 (5): 671-683

P.1370

Intensive care unit feedback about organ donation

Alejandro Nino-Murcia[1], Fernando A.Giron Luque[1], Mauricio Sanabria[2], Laura Nino-Torres[2].
[1]Transplant Surgery Department, Colombiana de Trasplantes, Bogotá, Colombia; [2]Epidemiology, Universidad del Rosario- CES, Bogotá, Colombia.

There is a disparity between organ offer and demand worldwide. A timely and appropriate identification of the potential organ donor can be improved. Emergency department (ED) and intensive care unit (ICU) doctors play a key role. Nevertheless, they rarely receive feedback. Dr Schiavelli and colleagues in Argentina started a program called "Ateneo de devolucion"[1], in order to provide feedback as an strategy to increase donation and raise awareness of its importance.
Thought this project we intended to demonstrate the benefits of an educational intervention centered in the ICUs of one of the biggest Hospitals in Bogota, Colombia. This through a theoretical review of the identification and physiopathology of brain death, the current guidelines available regarding donor maintenance, reviewing different approaches to increase organ donation and sensitizing health personnel about the importance of organ donation.
The project was first presented to the Health regulatory entity, Colombiana de Trasplantes and the Hospital Universitario Mayor in Bogotá Colombia. 8 sessions of one hour talks were scheduled onces a month, which was divided in three segments: a chart review of an effective organ donor, an educational review regarding some aspect around donation and some videos of the receptors and their families thanking the ICU staff for their efforts regarding the care of the organ donor. This meetings were openned for students, interns, residents and every member of the Hospital who wished to attend and learn about donation.
In total, 8 organ donor charts were discussed, 8 reviews regarding aspects of organ donation and 14 videos were shown with the transplanted patients and their families showing their gratitude towards the medical personnel.
This is a valid strategy to raise awareness in the ICUs and ED about the importance of donation, the impact they can achieve in a transplanted patient and their families. In Colombia deceased organ donation has been roughly the same the last years. The ICUs have not been involved before. If intensivist receive feedback from the patients, they will continue with the commitment towards donation. This is a very cost- effective strategy for every country in Latin America and third world nation.
References:
[1] Schiavelli R., Fioretti S., Martinez M., Stella M., Ponte M., Merino D. Grand Rounds to Provide Feedback on Procurement Activities. *Transplantation Proceedings*. 2012;44:2178-80.

P.1371

En bloc dual kidney transplantation: A novel technique

Dai Nghiem.
Department of Surgery, Allegheny General Hospital, Pittsburgh, PA, United States.

Introduction: Most techniques of dual transplantation are based on the sequential placement of one kidney after the other. These require more dissection, longer anesthesia time, and expose the elderly recipients to more complications. To avoid these drawbacks a two-step novel technique of *en bloc* dual kidney transplantation is described.
Material and Methods: First, using ex-vivo surgery in iced saline bath, the arterial and venous patches carrying the left renal arteries and vein were anastomosed to the cephalad end of the distal donor aorta and vena cava (Figure 1), respectively. Second, the reconstructed *en bloc* kidneys with distal aortic inflow and distal vena cava outflow were then ready for transplantation via a right regular Gibson incision. The *en bloc* kidneys were reversed to place the vena cava in medial position, concordant with the iliac vein. The distal aorta and vena cava were anastomosed end to side, respectively, to the external iliac artery and vein, 2 cm above the inguinal ligament. The vascular clamps were removed and both kidneys were revascularized simultaneously. The medial kidney was then flipped over and stacked on top of the lateral kidney. The ureters were re-implanted into the bladder through a common tunnel (Figure 2). The incision was closed without difficulties. This procedure was performed in 3 patients. In 2 other patients, the kidneys had been recovered *en bloc* with the cephalad aorta and vena cava already stapled. Thus, only step two was required, expediting the surgical procedure.
Results: All bench reconstructions were performed while the patients were being prepared by the anesthesia team. Because of the double ureter the operating time was slightly longer than the single kidney transplant procedure. At one year follow-up, renal function was normal in all patients.
Conclusions: This novel technique converts two complex vascular organs into single conduits *en bloc* grafts and requires only 2 vascular anastomoses. It allows the simultaneous transplantation of both kidneys through a regular Gibson incision. It is particularly suitable for kidneys with multiple vessels. Dual kidneys should be left *en bloc* with preservation of distal aorta and distal vena cava during organ procurement and transplanted *en bloc.*

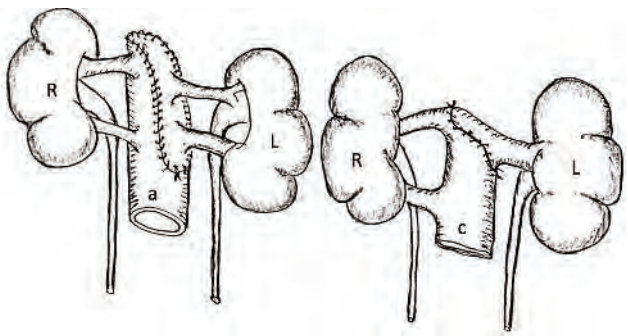


Figure1: Arterial and Venous reconstruction.
a = donor aorta, c = donor vena cava

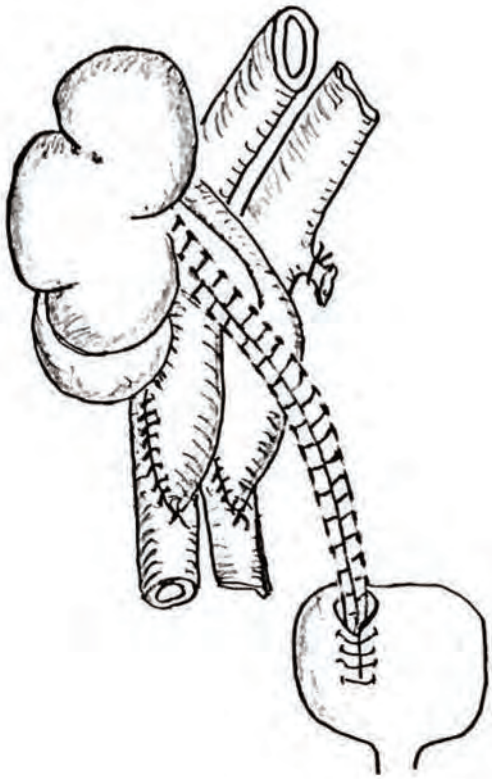


Figure2: EnBloc Transplants in situ

P.1372

A new approach to the hostile bladder in transplantation

Dai Nghiem.
Department of Transplantation, Allegheny General Hospital, Pittsburgh, PA, United States.

Leakage after uretero-neocystostomy (UNC) is the most frequent source of morbidity after transplantation, particularly in the obese patient with a deep, contracted and thick bladder. We report on a novel suture-less UNC technique used successfully in 6 such patients.
Material and Methods: Of 1616 transplants performed (1987-2007), six patients (5 male and 1 female) were identified with BMI 44 (range 39-47) and bladder capacity 110 mL (80-150), determined at the time of surgery by bladder flush with antibiotic irrigant stained with indigo-carmine at 60 cm of water pressure. After revascularization of the kidney, the bladder was localized by fine needle aspiration and grasped with 2 Allis clamps. A 2cm incision was made in the detrusor, centered on the blue needle tract until the blue-stain mucosa was reached. A small strip of detrusor was removed and the underlying mucosa was dissected to accommodate the stented ureter. Through a small opening of the distal mucosa, the spatulated tip of the ureter was withdrawn into the bladder by a mucosal horizontal mattress suture and anchored to the superior-anterior wall of the bladder. The detrusor incision was closed over the distended bladder. The Foley catheter was removed in 1 week and the patient was instructed to void every 2 hours afterwards. The stent was removed by flexible cystoscopy at 6 weeks and sulfamethoxazole discontinued.
Results: During cystoscopy the ureteral implantation, the site appeared well healed and blended into the normal bladder mucosa. During long term follow-up, no transplant pyelonephritis was experienced.
Conclusions: The stent protected the ureter from external compression by hematoma or lymphocele and internal high pressure from edema, post-operative polyuria. It allowed the anastomosis to drain and heal. The procedure is safe and easily adaptable to all patients.

P.1373

Delayed graft function in kidney transplant

Rubén Schiavelli, María J.Rosés, Roberto R.Sabbatiello, Miguel A.Raño, Daniel P.Di Tullio, Mauricio R.Pattin, Agustina E.Gautos.
Nephrology and Kidney Transplat division, Argerich Hospital, Buenos Aires, Argentina.

Introduction: Delayed graft function (DGF) is a common complication in kidney transplant, and has consequences in both short and long term outcome. Previous studies have described a high occurrence rate in our country as compared to international data, which led us to conduct this investigation. Our aim was to learn about the incidence in a transplant center, and describe risk factors for the donor (D) and recipient (R) and recipient outcome.
Materials and Method: Recipients undergoing kidney transplant (KTxR) between March 2005 and December 2012 were assessed. Age and gender of both D and R, time on dialysis, type of D, D with expanded criteria (UNOS), retrieval type (multiorgan vs monorganic), cold ischemia time (CIT), D´s kidney function, acute rejection (AR), and both R and graft survival rates.
Results: Two hundred 200 KTxR from cadaveric donors (CD) were assessed. Age was 44.70 ±12.54 years, and 55% were male. DGF was observed in 75.5% (151 KTxR); 24.5% did not exhibit DGF (49 KTxR).
Risk factors for DGF were: donor´s older age (45.2 years vs. 40 years; P=0.04), higher BMI of the D (27 vs 25 P=0.006) and longer CIT (R without DGF was 1214.26 ± 304.19 min vs. R with DGF: 1388.70 ± 373.20 min; P= 0.006).
Pre-retrieval median Cr value was higher in D of R with DGF vs. that in D of R without DGF 0.99 mg/dl (0.73-1.25) and 1.19 mg/dl (0.80-1.52) respectively, although it was not statistically significant (*P*=0.08).
No difference was observed between the median baseline Cr value in D of R without DGF vs. that in D of R with DGF; time on dialysis and retrieval type.
The Cr value at one year post-transplant was higher in pts. with DGF vs. those without DGF 1.4 mg/dl (1.1-1.75) and 1.25 mg/dl (1-1.48) respectively (P=0,022).
The DGF group exhibited a higher incidence of AR, 34% vs. 20.4% (*P*= 0.043), OR= 2.67.
The 5-year kidney survival rate was higher in R without DGF (*P*=0,04).
Conclusion: The incidence of DGF in our country is higher than in developed countries. It is interesting to note that of all the typical risk factors, CIT is still a predictive factor. Also, in this study we observed a not so studied predisposing factor such as donor´s BMI. Further studies are necessary to assess its real incidence.

P.1374

Pre-transplant deceased donor kidney biopsy is decision-making for single versus dual kidney transplantation

Kamlesh Suthar, Aruna V.Vanikar, Rashmi D.Patel, Kamal V.Kanodia, Lovelesh A.Nigam.
Department of Pathology, Lab. Medicine, Transfusion Services and Immunohematology, Institute of Kidney Diseases & Research Centre and Institute of Transplantation Sciences (ITS), Ahmedabad, India.

Introduction: Deceased donor kidney transplantation (DDKT) is a viable option for increasing donor pool. Pre-transplant deceased donor (DD) kidney wedge biopsy (KWB) evaluation has debatable role in deciding potential donor kidney suitability. We compared the outcome of single kidney transplant (SKT) versus dual kidney transplant (DKT) in our DDKT program vis a vis baseline biopsy findings.
Material and Method: This retrospective study included pre-transplant histopathological evaluation of DD-KWB performed between Jan'11 to Nov'15. Biopsies were divided in 2 groups, SKT-group included 15 biopsies from 9 DDs and DKT-group included 16 biopsies from 8 DDs. Patient-donor demography was comparable. There were 15 recipients (11 males, 4 females) in SKT-group with mean age, 48 years and mean donor age, 57 years (7 males, 8 females). There were 8 recipients (5 males, 3 females) with mean age, 50 years in DKT-group with mean donor age, 61 years (4 males, 4 females). Mean kidney size was 10.5 x 4.82 cm in SKT-group and 10.18 x 4.82 cm in DKT-group. Baseline serum creatinine (SCr-mg/dL) was 2.02 in SKT and 2.45 in DKT. Frozen sections of 3-4 µ thickness stained with hematoxylin and eosin were evaluated on formalin fixed tissues processed after immediate snap-freezing at -220 to -240c, 3µ thick paraffin sections prepared after 4 hours processing were stained with Periodic Acid Schiff and Gomori's trichrome stains also. Biopsies with ≥25 glomeruli and ≥2 medium caliber vessels were considered adequate. Morphological findings were correlated with gross examination and outcome in terms of graft/ patient loss and SCr.
Results and Discussion:

Table-1: Histopathological findings in wedge biopsy

Group	Single Kidney Transplants (N-15) (15 recipients)	Dual Kidney Transplants (N-16)(8 recipients)
Unremarkable	6	0
Acute tubular necrosis	5	2
Mesangial proliferation	1	12
Tubular atrophy and Interstitial Fibrosis	1	0
Focal global sclerosis in 20-40%	2	2

Majority of SKT biopsies revealed unremarkable morphology (Table-1). In DKT-group glomerular mesangial proliferation was the commonest finding. DKT had better outcome than SKT. Over mean follow-up of 21.8 months of SKT, 13.3% grafts, 20% patients and 6.7% combined graft and patient loss was recorded. Over mean follow-up of 10.57 months in DKT, 12.5% graft loss and 100% patient survival were observed. One graft was lost in DKT due to rejection. Mean SCr at 1 and 3 years post-transplantation were 1.4 and 2.61 in SKT and 1.41 and 2.43 in DKT. DDKT from older donor or kidney with glomerulosclerosis has inferior posttransplant outcome[1],[2]. In DD below 40 years, 54% renal biopsy findings were reported as normal whereas only 7% biopsies were reported normal from donors above 40 years[3]. In our study pre-transplant biopsy with 20- 40% glomerulosclerosis was found in 2 biopsies each of both groups. SKT group lost the grafts whereas DKT group has functioning grafts. Remuzzi et al suggested a scoring system based on pre-transplant biopsy for selection of a kidney for DKT[4]. Thus well accepted score index including histopathological evaluation of kidney biopsy are helpful to decide kidney allocation for SKT vs DKT for better outcome.
Conclusion: DD Kidney allocation based on histopathology of pretransplant biopsy helps to improve DDKT outcome when kidney size is normal in donors above 40 years. Dual-kidney DDKT has better outcome than single-kidney DDKT.
We thank histopathology department lab technicians for preparing tissue sections and slides, Nephrologists Dr. H.V.Patel and Dr. V.B. Kute for providing clinical information and Mrs. Aanal Mehta for statistical evaluation
References:
[1] 1. Rao, K.V., Kasiskc, BL, Anderson, R.C. et al. Influence of cadaveric donor age on posttransplant renal function and graft outcome. Transplantation.1990; 49: 91.
[2] 2. De Vusser K, Lerut E, Kuypers D, Vanrenterghem Y, et al. The predictive value of kidney allograft baseline biopsies for long-term graft survival. J AmSoc Nephrol. 2013: 24; 1913-23.
[3] 3. Cahen R, Dijoud F, Couchoud C, et al. Evaluation of renal grafts by pretransplant biopsy. Transplant Proc. 1995: 27: 2470.
[4] 4. Remuzzi G, Grinyo J, Ruggenenti P, et al. Early experience with dual kidney transplantation in adults using expanded donor criteria. Double Kidney Transplant Group (DKG). J Am Soc Nephrol. 1999: 10(12): 2591-98.

P.1375

Excellent long-term outcomes of renal transplantation from donation after cardiac death donors: A single center experience

Masashi Takenaka[1], Mamoru Kusaka[1], Naohiko Fukami[1], Taihei Ito[2], Hitomi Sasaki[1], Takashi Kenmochi[2], Ryoichi Shiroki[1], Kiyotaka Hoshinaga[1].
[1]Urology, Fujita Health University School of Medicine, Toyoake, Japan; [2]Transplantation and Regenerative Medicine, Fujita Health University School of Medicine, Toyoake, Japan.

Introduction and Objective: The most effective treatment of end-stage renal disease (ESRD) is renal transplantation (RTx), however a severe donor shortage has significantly limited this strategy. Therefore, donation after cardiac death (DCD) has the potential to significantly increase the number of organ donors.
In this study, we investigated the outcome of RTx from DCD donors in our institution.
Patients and Method: This study included 132 patients with ESRD who underwent RTx from DCD donors between October 1990 and January 2016 at our institution. The mean age of the recipients was 44.5±0.9 (range, 15 to 63) years, and 64% were male. The mean age of the donors was 48.5±1.5 (3 to 75) years, and 64% were male. The mean period of the dialysis before RTx was 130±7 (12 to 401) months. Mean of the warm ischemic time was 9.9±0.9 (1.0 to 57) minutes and the mean total ischemic time was 926±42 (205 to 2603) minutes. The mean follow-up time was 137±8 (1 to 306) months.
In this study, we focused on the recipient and donor characteristics, graft and patient survivals and complications. In addition, the recipients were stratified into 2 groups according to their graft status: 82 patients received grafts from standard criteria donors (SCD) and 50 patients from expanded criteria donors (ECD). We compared the outcomes of RTx from DCD with SCD and ECD.
Results: The graft survival rates of RTx from DCD donors at 5, 10, 15, and 20 years were 93.7%, 77.2%, 67.5% and 67.5%, respectively. The patient survival rates of RTx from DCD donors at 5, 10, 15, and 20 years were 93.5%, 86.8%, 81.8% and 76.4%, respectively (Figure 1). The mean nadir-serum creatinine level was 1.37±0.04 (0.5 to 3.8) mg/dl. The mean period of the acute tubular necrosis was 8.7±0.8 (0 to 42) days. The rate of acute cellular rejection and primary non-function were 56 cases (42.4%) and 3 cases (2.3%), respectively.
The graft survival rates at 5, 10, 15, and 20 years were 97.4%, 85.5%, 81.8% and 81.8%, respectively, in SCD vs 89.2%, 63.6%, 39.4% and 39.4%, respectively, in ECD ($p<0.0001$) (Figure 2). The patient survival rates were 92.5%, 86.2%, 81.8% and 78.3%, respectively, in SCD vs 95.1%, 87.6%, 81.7% and 68.1%, respectively, in ECD (p=N.S.). The mean nadir serum creatinine level was significantly lower in SCD than in ECD (1.26±0.05 vs 1.56±0.09 mg/dl, $p<0.003$).
Conclusion: This study shows that the long-term outcome of RTx from DCD donors is excellent at our institution. In addition, the graft survival was significantly higher following RTx from DCD with SCD than ECD. RTx from DCD donors has the potential to significantly increase the donor pool.

Figure 1

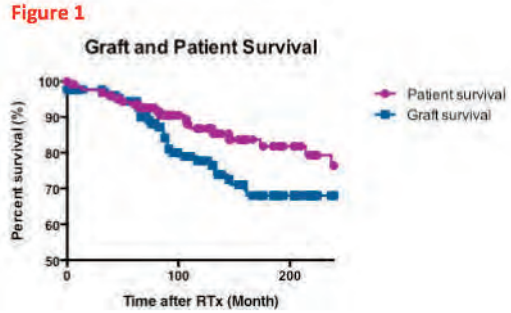
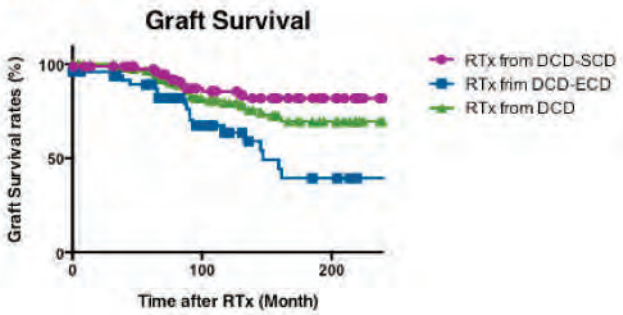


Figure 2



P.1376

Comparison of hypothermic and normothermic pulsatile machine perfusion in an ex-vivo porcine kidney model

Natalie Vallant[1,2], Frederick Tam[1], Bynvant Sandhu[1], Nienke Wolfhagen[1], Vassilios Papalois[1].
[1]Renal and Transplant Department, Imperial College London, London, United Kingdom; [2]Department of Visceral-, Transplant- and Thoracic Surgery, Medical University Innsbruck, Innsbruck, Austria.

Background: Normothermic machine perfusion offers great promise to preserve, preassess and even precondition donor organs ex vivo. However, as more experimental studies are needed to establish the optimal conditions for this to occur, hypothermic machine perfusion should not be overlooked as simple though effective way of preserving and preconditioning organs. In an ex-vivo model using porcine kidneys, we aimed to directly compare the two methods.
Methods: 6 porcine kidneys coming from 3 donor pigs retrieved in an abattoir were retrieved and statically stored on ice for 24 hours. Afterwards, they were either perfused hypothermically or normothermically for 4 hours using an RM3 pulsatile perfusion machine. Thereafter, both kidneys were reperfused with whole blood for 2 hours. ABG measurements were used to calculate oxygen consumptions. Physiological parameters, like the resistance index, flow rates and urinary outputs were compared between the two preservation methods.
Results: Hypothermically perfused kidneys showed a significant higher urinary output (mean 410,7ml ± 52ml SD vs. mean 112ml ± 13,53ml SD, p=0.0078) during reperfusion than normothermically perfused kidneys. The oxygen consumption, resistance index and flow rates did not differ significantly between the two treatment groups.
Conclusion: Hypothermic machine perfusion is a well established method for preservation and preconditioning of organs coming from extended criteria donors and further evidence is needed to proof the superiority of normothermic machine perfusion in kidney preservation.

P.1377

Extraction of green fluorescent protein labelled mesenchymal stem cells to investigate their mechanisms of action on ischemia-reperfusion Injury in a rat kidney transplant model

Natalie Vallant[1,2], Jacques Behmoaras[1], Jeong-Hun Ko[1], Theresa Page[1], Frederick Tam[1], Ana Garcia-Diaz[1], Bynvant Sandhu[1], Charles Pusey[1], Vassilios Papalois[1].
[1]Renal and Transplant Department, Imperial College London, London, United Kingdom; [2]Department of Visceral-, Transplant- and Thoracic Surgery, Medical University Innsbruck, Innsbruck, Austria.

Background: In solid organ transplantation, Ischemia- Reperfusion Injury (IRI) is an unavoidable event which contributes to enhanced graft immunogenicity and early graft dysfunction. Mesenchymal Stem Cells (MSCs) are multipotent, self renewing cells with immunomodulatory, antiinflammatory and tissue repair capacities. Therefore, the application of exogenous MSCs to ameliorate IRI is being investigated in several animal-, preclinical and clinical studies and beneficial effects are reported. However, the mechanisms of action are poorly understood, in particular, delivered MSCs have to be distinguished from preexisting MSCs within a graft. This study aimed to investigate whether MSCs from GFP transgeneic Wistar Kyoto (WKY-GFP) rats express the green fluorescence throughout passaging as a tool to trace them at application in a rat kidney transplant model, and to examine the in vitro immunomodulatory and antiinflammatory actions of these cells when compared to MSCs coming from the wildtype WKY animals.
Methods: Bone marrow derived MSCs were extracted from the femurs and tibias of male WKY- wildtype and -GFP+ rats, respectively. MSCs identity was confirmed using flow cytometry for CD44, CD90, CD45 and CD34 in both cell types. In vitro, the effects of MSC culture supernatants and cells from passage 0-10 on LPS-induced cytokine production from WKY wildtype macrophages were investigated using qPCR.
Results: MSCs from WKY-wildtype, as well as from WKY-GFP+ rats could be successfully differentiated in culture. The expression of GFP in cells from GFP+ rats was strong and green fluorescence was present up to passage 10. MSCs were shown to be plastic adherent, to express CD44 and CD90 and to lack expression of CD45 and CD34. Furthermore, the cells could be differentiated into adipocytes and osteocytes.
Conclusions: MSCs from transgeneic WKY rats positive for the expression of GFP show strong green fluorescence throughout passages 0-10 and therefore, application in a rodent model of kidney transplantation will be promising to reveal possible beneficial effects of these cells for organ preconditioning in solid organ transplantation.

P.1378

Kidney transplant from deceased donors with several stages of acute kidney injuries: A comparison with standard donors and expanded criteria donors

Punlop Wiwattanathum[1], Vasant Sumethkul[1], Atiporn Ingsathit[1,2], Nuttapon Arpornsujaritkun[3], Wiwat Tirapanich[3], Sopon Jirasiritham[3].
[1]Division of Nephrology, Department of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; [2]Section for Clinical Epidemiology and Biostatistics, Research Center, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; [3]Vascular and Transplant unit, Department of Surgery, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.

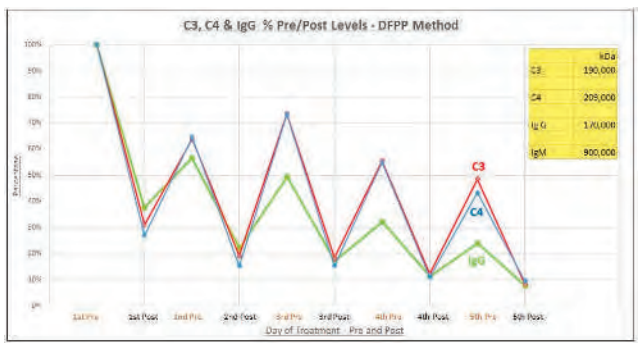
Background: Kidney transplantations from non-ideal decease donors are other potential option in the presence of organ shortage. Acute kidney injury (AKI) donor and expanded criteria donor (ECD) are important sources of deceased donors. However, there are several challenging issues about the outcomes of using kidney form AKI donors or ECD. This can lead to the discard of using deceased donors with high terminal serum creatinine (Cr). We therefore evaluate the outcomes of kidney transplant from deceased donors with several stages of AKI and compare with that of standard criteria donors and expanded criteria donors.
Methods: This retrospective study included 111 deceased donor kidney transplant recipients. Deceased donor were classified as standard criteria donor (SCD), AKI donor and expanded criteria donor (ECD). AKI was diagnosed and classified based on change of serum Cr by Acute kidney injury Network (AKIN) criteria. Primary outcome was one-year eGFR calculated from Cr by CKD-EPI. Multivariable analysis was done by adjusting factors such as type of DDKT, % PRA, cold ischemic time, the presence of delay graft function, the use of induction therapy and the presence of acute rejection. Significant factors that can affect the primary outcomes were then identified.
Results: ECD group had a significantly lower eGFR at one year (33.9 ± 17.3 ml/min) when compared with AKI group (56.6 ± 23.9) and SCD group (63.6 ± 19.9) [$p < 0.001$]. For AKI group, one-year eGFR was also indifferent among AKIN stage 1, 2 or 3. Patients with AKIN stage 3 had progressive increase of eGFR from 49.6 ± 27.2 at discharge to 61.9 ± 29.0 ml/min at one year. From Kaplan-Meier analysis, AKI donor showed better two year graft survival than ECD (100% VS 88.5%, $p = 0.006$). Interestingly, AKI group had a stable eGFR at one and two year. The two year eGFR of AKI group was not significantly different from SCD group (56.6 ± 24.5 VS 58.6 ± 23.2 ml/min, $P = 0.65$).
Conclusions: Kidney transplantation from deceased donors with variable stage of acute kidney injuries was associated with favorable two years allograft function. The outcomes were comparable with kidney transplant from SCD. This information support the option that deceased donors with AKI is an important resource of organ for kidney transplantation even in the presence of stage 3 AKI.

P.1379

Effects of plasmapheresis and antigen- specific immunoadsorption on complement levels in antibody-incompatible live renal transplant

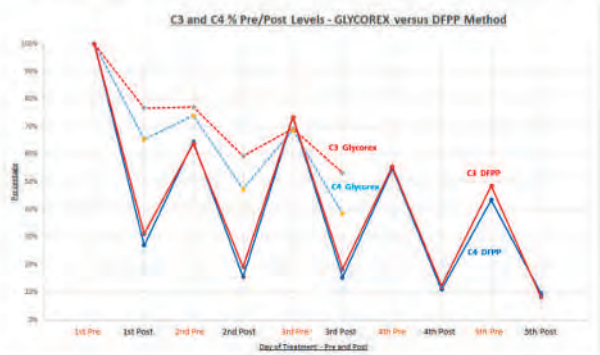
Archana Jain[1], Nithya Krishnan[1,3], David Briggs[2], Sunil Daga[1,3], Daniel Mitchell[3], Robert Higgins[1,3].
[1]Renal Unit, University Hospitals Coventry & Warwickshire NHS Trust, Coventry, United Kingdom; [2]H&I, NHSBT, Birmingham, United Kingdom; [3]Clinical Research Institute, University of Warwick, Coventry, United Kingdom.

Background: About 25% of patients on the renal transplant list have donor specific HLA or ABO Blood Group antibodies that affect early graft success. Double filtration plasmapheresis (DFPP) or Glycorex Immunoadsorption (IA) are therapies designed to reduce donor specific antibody levels prior to transplantation. Complement is an important part in the antibody mediated hyperacute rejection process. A better knowledge of the effects of DFPP and IA on complement levels would aid our understanding of pre- transplant recipient conditioning and early graft function.
Aim and Objective: Given the similar molecular weights of C3, C4 and IgG, we hypothesise DFPP treatment reduces these levels to a similar extent. How does the rate of complement depletion compare with antibody depletion? Does IA also affect complement levels if the column is designed only to remove antibody to blood groups?
Methods: This was a non-randomised prospective study. 11 eligible patients were treated with DFPP and 3 patients with IA. IgG, C3 and C4 levels were recorded pre and post treatment over 3-5 days. C3 and C4 were measured by the standard service laboratory methods. Absolute levels pre and post treatment were converted into percentage falls. A paired T-Test was used to assess statistical significance for continuous data.
Results: The mean pre to post level reduction of C3, C4 and IgG by each session of DFPP was 75%, 77% and 64% respectively. The effect of each DFPP treatment on removing IgG, C3 and C4 was approximately the same, but C3 and C4 re-synthesis between DFPP



IA also affected complement levels, although it caused much smaller pre to post level reduction in C3, C4 and IgG of 23%, 38% and 16% respectively. However, this lesser rate of removal by IA did not seem to stimulate rapid re-synthesis. Hence after two IA sessions, complement pre-levels fall to about 70% starting level and are at the same level compared to that effectively achieved on DFPP.

Figure 2 - The effect of Immunoadsorption (IA) on removing C3 and C4



Conclusion: DFPP seems to remove C3 and C4 as predicted from their molecular weights. IA has a similar cumulative effect on complement levels, at least during the initial phase. This could be due to binding to sepharose or the effects of tubing in circuit. A course of DFPP does not progressively deplete C3 and C4 as much as it reduces IgG. This could be because the rate of re-synthesis of C3 and C4 is more rapid. The removal of complement by IA does not seem to trigger re-synthesis.

P.1380

Dengue fever in patients with renal transplantation analysis – cases report

Ling-Hsien Lee[1], Hsin-Lin Lin[2], Hung-Jen Tang[3], Yu-Feng Tian[1,4]. [1]Dept. of Transplantation Medicine, Chi Mei Medical Center, Tainan City, Taiwan; [2]Department of Pharmacy, Chi Mei Medical Center, Tainan City, Taiwan; [3]Dept. of Infectious Disease, Chi Mei Medical Center, Tainan City, Taiwan; [4]Div. of Surgery, Chi Mei Medical Center, Tainan City, Taiwan.

Introduction: The kidney transplant patients got dengue virus infection, which can cause harmful effects in situation of immune-compromised, and dengue shock, even threatening their life.
Method and Results: This study is gathering data with 4 renal transplant patients (Female: 2, Male: 2) as dengue cases and diagnosed during August, 2015 and September, 2015. The average age of them are 48.5 ± 15.5 years. (All of them are dead-body transplant patients) For this study, the drug used with Csyclosporine or Tacrolimus and add Myfortic and Prednisolone in combination with a ratio of 1:1. There are four kidney transplant patients are staying in hospital caused by dengue fever. The average days of hospital staying are 4.8 ± 2.2 days, and no any occurrence of hemorrhagic dengue case. Its dengue symptoms are bellows: fever (100%), bone paining (50%), headache (50%), muscle paining (25%), and chest tightness (25%), but zero mortality. The data showed that, all cases which due to dengue fever were increased creatinine from 1.15 ± 0.04 mg / dl to 1.26 ± 0.09 mg / dl (but p> 0.05), the mortality rate is 0%. Thrombocytopenia data showed 46.4 ± 15.2%, and no significant abnormality of liver function. GOT increased from 17.1 ± 1.6 U / L to 43.7 ± 25.4 U / L; GPT increased from 15.7 ± 1.7 U / L to 32.4 ± 17.8U / L (but p> 0.05).
Summary: Dengue fever and general flu are similar in symptoms, when transplant patients infected with dengue fever, they need to stop using Myfotic, if platelet decline rate is higher, and the staying hospital days would be increased, but ultimately it did not any effect for creatinine on kidney transplant patients. (GOT and GPT, p>0.05)
Chi Mei Medical Center

P.1381

Management of highly sensitized patients in kidney transplantation: prospective single center strategy

Martine De Meyer[1], Dominique Latinne[2], Catherine Lambert[2], Luc De Pauw[1], Antoine Buemi[1], Tom Darius[1], Eric Goffin[3], Nada Kanaan[3], Michel Mourad[1].
[1]Abdominal Surgery and Transplantation, Cliniques Universitaires Saint Luc, Brussels, Belgium; [2]Haematology, Cliniques Universitaires Saint Luc, Brussels, Belgium; [3]Nephrology, Cliniques Universitaires Saint Luc, Brussels, Belgium.

Introduction: Many candidates for kidney transplantation are highly sensitized (HS) with HLA antibodies in their serum. They are at a high risk for immunological complications including alloantibody mediated rejection and early graft loss. The aim of the study is to evaluate the safety and the efficacy of a prospective therapeutic strategy combining plasma exchanges (PE) and intravenous immunoglobulin administration (IVIG).
Materials and Methods: Between November 2007 and July 2015, 60 HS patients were enrolled in a prospective strategy including PE and IVIG (Group1). PE started just before surgery and was repeated postoperatively from day 5 during one month. IVIGs (2g/Kg body weight) were given during the first 48 hours after surgery. They were compared to 120 other not sensitized patients (PRA: 0%) (Group 2). Thirteen other HS patients were not included in the prospective strategy for medical reasons and were also evaluated (Group 3). All the patients were transplanted during the same period and received a standard immunosuppression combining tacrolimus, Mycophenolate Mofetil and steroids.
Results: No difference was observed between the three groups in relation to donor and recipient ages, HLA mismatches and total ischemia time. At the end of the follow-up period (median 34,4 months, range 3-98), patient survival was similar in the three groups. Graft survival was comparable between group 1 and 2 but significantly lower in group 3 (Log-Rank test, P=0,002). A similar free rejection graft survival is observed in group1 and group 2. However, a significant lower free acute rejection survival was observed in group 3 (Log-Rank test; P=0,02).

Graft survival rate			
	Group 1	Group 2	Group 3
6 months	95%	100%	84%
12 months	95%	99%	74%
60 months	87%	86%	52%

Conclusion: The therapeutic strategy based on PE and IVIG administration as described above has been shown effective in highly sensitized patients leading to reduced graft rejection and graft loss. Further studies comparing several strategies are still needed to assess the benefit in terms of morbidity, cost and efficacy.

P.1382

Living kidney transplantation from older donor aged 60 and older

Kiyokazu Akioka, Tatsuki Ishikawa, Masafumi Osaka, Yayoi Kadotani, Kaori Okugawa, Katsunori Nakano, Yoshio Osaka, Kuniyuki Tsuchiya, Hirotaka Sako.
Surgery, Omihachiman Community Medical Center, Omihachiman, Japan.

Introduction: From the shortage of donor, marginally appropriate donors might be considered in order to extend the donor criteria. Here we report an outcome after living kidney transplants from living donors aged 60 and older, who had mild hypertension or diabetes.
Patients and Methods: From Dec. 2007 to Jan. 2016, total of 43 living related kidney transplants had been done in our hospital, 12 donor ages were over 60. Six of 12 donors had hypertension and five of 12 donors had diabetes. Renal function of the recipients and donors, and their outcomes and prognoses were investigated after the transplantation.
Results: Only one of 12 recipients of older living donor had lost his graft caused of acute rejection and recurrence of nephritis 21.4 months after transplant. 11 of 12 recipients of older donor had still moderate graft function. Serum Cr (sCr) of older donor their selves at the hospital discharge was 1.08mg/dl on average, and that at one year after the operation was 1.07mg/dl on average.
Only one of 31 recipients of younger living donor aged 59 years had lost her graft caused of prolonged gynecological bleeding. Allograft mortality of recipients of old donor was almost same as that of younger living donor.
In 12 older donors, two of them died after the donation, one from interstitial pneumonia and another from advanced colon and cholecystic cancer. Cancers were diagnosed at 17 months after the donation although 76 years old donor had had CE-CT and total colonoscopy as pre-operational screening. Ten of 12 old donors were still alive with moderate renal function, even though some of them had mild hypertension and diabetes.
Discussion and Conclusion: Older donors with pre-operational complications such as mild hypertension or diabetes could be also considered as marginal donor, if their complications were well controlled and the functions were appropriate enough within the consensus statement of the Amsterdam forum on the care of the live kidney donor. Malignancy after the donation was observed very short term, even though he was oldest living donor. More careful observation should be required for older donors respectively.

P.1383

Post-nephrectomy inflammatory syndrome: A common complication post minimal access live donor nephrectomy?

Erin McIlveen[1], Marc Clancy[1], Grant Baxter[2].
[1]Kidney Transplantation, Queen Elizabeth University Hospital, Glasgow, United Kingdom; [2]Radiology Department, Queen Elizabeth University Hospital, Glasgow, United Kingdom.

Introduction: Living kidney donation involves exposing a healthy individual to risks of major surgery. Therefore, it is imperative that it has as little morbidity as possible. As part of routine departmental audit the authors noted that a significant number of patients were re-presenting with non-specific abdominal symptoms post laparoscopic nephrectomy. The aim of this study was to investigate this. Little is known about the abdominal inflammatory response which occurs post operatively.
Materials and Methods: A retrospective data collection was performed from a prospectively maintained database, on all hand-assisted laparoscopic live donor nephrectomies that were performed between 2009 and 2014 (n=150). Data was collated on immediate post-operative complications, inflammatory markers and imaging. Similar data was collated on the re-presentations to hospital. A blinded consultant radiologist then re-reviewed all post-operative CT abdomen/pelvis (CTAP) and CT pulmonary angiogram (CTPA) scans (n=33) to look for common findings.
Results: 150 hand assisted live donor nephrectomies were performed between June 2009 and December 2014 with a conversion rate to open of 2%. 113 (75.3%) left and 37 (24.7%) right. Median hospital stay was 4 days. Mean CRP on day 2 was 177.98mg/l and day 3 158.02mg/l. 12 (8%) patients underwent immediate imaging. 54 (36%) patients re-presented within 60 days of operation. Re-presentations occurred at a median 14 days post-op and stayed a median of 2 days. 52 (96.2%) presented with abdominal/loin pain and/or pyrexia/rigors. Mean CRP on readmission was 103.6mg/l (n=44) however the mean white cell count was within normal limits. 41 (73.21%) patients underwent imaging on re-presentation. Of these, 27 (96.43%) ultrasound scans were normal and 14 (82.35%) CTAP scans showed 'inflammatory change' most commonly in the renal bed, anterior abdominal wall and omentum. 29 (53.7%) resolved without any specific treatment apart from symptom relief. There were no peri-operative deaths or ICU admissions.
Discussion: Of the 150 live donor nephrectomies that were performed in our unit, 36% re-presented at a median of 14 days post-op with variable, often non-specific abdominal or systemic symptoms. The reason for these re-presentations is unproven, however it is consistent with a self-limiting systemic inflammatory response syndrome. Based on CTAP findings (82.35% showed inflammatory change at a median 14 days post-op) this is likely due to fat necrosis in residual perinephric fat. Our unit applied a very low threshold for readmission. All patients improved without long-term consequences.
Conclusion: A significant number of the live donor nephrectomies re-presented post-operatively. It is thought to be due to a self-limiting post-nephrectomy inflammatory syndrome. Although there is no long-term sequelae, removal of any suspected ischaemic perinephric fat at nephrectomy may prevent such readmissions in the future.

P.1384

Living longer and better after kidney transplant in southern Spain

Javier de Teresa Alguacil, Carmen de Gracia Guindo, Almudena Pérez Marfil, Raquel Castellano Carrasco, Pilar Galindo Sacristán, José Manuel Osorio Moratalla, Juan Bravo Soto, Antonio Osuna Ortega.
Nephrology Unit, Virgen de las Nieves University Hospital, Granada, Spain.

Introduction: Kidney transplant remains the best therapeutic option for patients with end-stage renal disease, offering patients a longer and possibly more satisfying life. Quality of life, as defined by the World Health Organization, is an individual's perception of their situation within their cultural context and value system in relation to their goals, expectations, personal standards, and vital interests. In Spain, more than 55,000 patients have received a kidney since initiation of the technique in 1965. During 2014, there were 2,678 kidney transplants performed and 4553 people waiting for a kidney transplant.[1]
Materials and Methods: In an outpatient clinic in Granada, Spain, we interviewed kidney transplant recipients to analyze the subjective quality of life and sociocultural level 1 year after transplant versus before transplant, measuring responses against completed level of education, work-life situation, number of hospital admissions, sexual impotence, and recipient and graft status.
Results and Discussion: Among the 600 kidney transplant recipients interviewed, 96.4% of patients (n = 477) described their subjective health-related quality of life 1 year after transplant as "better than pretransplant," 2.2% said it was equal, and 1.4% said it was worse. Most patients had completed at least high school (29.4%; n = 176) or primary school (27.9%; n = 167), although 4.3% of patients were illiterate. Only 24.4% of responders were working, mostly as homemakers (14.9%), farmers (10.7%), or builders (5.9%). One year after transplant, 6.8% presented with sexual impotence. Of 600 responders, 93.5% were alive with a functioning graft, 5.7% had returned to dialysis, and 0.4% had died during the first year after kidney transplant.
Most patients (96.4%) reported having more energy, a less restricted diet, and fewer complications with a transplant compared with their pretransplant response. Indeed, former findings have also indicated lower rates of anxiety after kidney transplant.[2]
Conclusion: Overall, despite the strict control of medication and medical supervision, kidney transplant recipients had a better quality of life compared with before transplant, similar to the general population.[3] Fortunately, growing public awareness of living donor transplant has resulted in increasing numbers of potential donors, a welcome response to organ shortage. Kidney transplant recipients have been given a second chance because of the generosity of donors and their families; they should make their goal to stay positive, to stay healthy, and to live life to the fullest.
References:
[1] 1. Spanish National Transplant Organization. Memoria de actividad. http://www.ont.es/infesp/Memorias/Memoria%20trasplante%20renal%202014.pdf. Accessed March 1, 2016.
[2] 2. Pascazio L, Nardone IB, Clarici A, et al. Anxiety, depression and emotional profile in renal transplant recipients and healthy subjects: a comparative study. Transplant Proc 2010;42:3586 -90. doi: 10.1016/j.transproceed.2010.08.056 pmid:21094820.
[3] 3. Villeneuve C, Laroche ML, Essig M, et al. Evolution and determinants of health-related quality of life in kidney transplant patients over the first 3 years after transplantation. Transplantation 2016;100: 640-7. doi: 10.1097/TP.0000000000000846.

P.1385

Identifying the main causes of kidney allograft loss: a large cohort study in Spain

Javier de Teresa Alguacil, Carmen de Gracia Guindo, Raquel Castellano Carrasco, Pilar Galindo Sacristán, Jose Manuel Osorio Moratalla, Almudena Pérez Marfil, Juan Bravo Soto, Antonio Osuna Ortega.
Nephrology Unit -UGC, Virgen de las Nieves University Hospital, Granada, Spain.

Introduction: Kidney transplantation is the best therapy available for most patients with end stage renal disease (ESRD). The mortality rate is higher in these patients than in general population, mainly caused by cardiovascular disease (CV). The ratio of mortality either by infectious or tumoral diseases has highly increased due to the fact that there is an older population of donors and recipients, who continue to have chronic immunosuppressive treatment.
The specific causes of kidney allograft loss remain unclear[1]. We investigated these causes in 1446 kidney transplant recipients to determine potential interventions.
Our objective was to identify the major causes of mortality of renal transplant recipients in our transplant center. We described the global frequency of death with functioning graft (DWF), as well as the main causes of mortality in our patients.
Methods: We used single-center data from Granada University Hospital (Spain).
It is a descriptive and transversal study in 1446 kidney transplant recipients followed-up in outpatient clinic, where frequency of death and most common causes were analyzed.
Results: During the follow-up, death with function (DWF) was the single most commonly observed cause of graft loss, 244 grafts, 16, 9% of all transplants.
The frequency of death before post-transplant discharge was 1.5 % (N=22), before 1 year post -transplant 3.3% (N=48) and global mortality (16, 9%) %)(N=244).
The first cause of death was Cardiovascular (36,2%), followed by infections (22, 3%) and malignancies (17, 5%).
There were no differences in the causes of death that occurred during the first year and more than 1 year post- transplant.
63,5% of the patients had at least one hospitalization after trasplantation, which 9,7% did at ICU.
The most frequent maintenance for immunosuppressive regimen was tacrolimus + micofenolate (MMF) + prednisone (24,9%)
The remaining functioning grafts were lost due to a variety of causes, including glomerular diseases (recurrent, transplant glomerulopathy, nonrecurrent), interstitial fibrosis/ tubular atrophy (IF/TA) and acute rejection (either celular or antibody-mediated).
We saw an increase in time to DWF, we attribute it to improved medical practices.
Conclusions: Patient's death with a functioning graft (DWF) is still the most common cause of graft loss[1]. Being the first cause of death: cardiovascular, followed by infections, malignancies and others.
The transplant community must focus research on cardiovascular deaths and antibody-mediated rejection with superior immunosuppressant protocols and better prophylaxis and treatment of infections.
References:
[1] El-Zoghby Z,Stegall M,Lager D et al. Identifying Specific Causes of Kidney Allograft Loss. *Am J Transplant* .2009;9:527-535

P.1386

Donors with stone-bearing kidneys are eligible for living kidney donation: a systematic review and cohort analysis

Jeff Lafranca[1], Said F Durmaz[1], Ognen Ivanovski[1], Michiel GH Betjes[2], Jan NMI Jzermans[1], Frank JMF Dor[1].
[1]Department of Surgery, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands; [2]Department of Internal Medicine, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands.

Background: Since living kidney donation can still not meet the demand for donor organs, extended criteria donors are increasingly included, such as donors with one or more kidney stones. Little is known about the outcome of these donors, as a possible future risk exists that recurrent stones, obstructions, and infections could injure the remaining kidney. Furthermore, the outcome of renal transplant recipients that retrieve a kidney with stones is of great importance as inadvertent transplantation could also impair recipient outcome.
Methods: Comprehensive searches were carried out in several databases up to December 2014 to search for relevant articles. We evaluated current guidelines for donors with stone disease and data to review to which extend stone-bearing kidneys are eligible for living kidney donation. Furthermore, the cohort of 1555 donor nephrectomies and transplantations in the center of the authors was analysed for outcome of donors and their recipients.
Results: Of the 3115 articles found, 18 met the inclusion criteria. Based on the literature search, both in attitude and in practice there is a shift in accepting more donors with (a history of) kidney stones. The prevalence in the literature ranges from 0,6% to 7%. In our cohort, the prevalence of kidney stones was 2,3% (36 live kidney donors). 21 donations of stone-bearing kidneys took place, of which 3 donors developed a stone-related event, which all passed without intervention. Three of the recipients developed a stone-related complication, which were all successfully treated. [FD1]
Conclusion: We conclude that asymptomatic stone-bearing kidneys seem to be suitable for donation and transplantation. Both donors and recipients have excellent outcome. However, a sufficient follow-up is required to confirm these outcomes in the long-term. We would advice that current guidelines are revised to remove stone-bearing kidneys as an absolute contra-indication for donation and transplantation.

P.1387

Recurrent focal segmental glomerular sclerosis after renal transplantation; prevention and treatment with Rituximab

Kenta Futamura, Manabu Okada, Takahiro Nagai, Takayuki Yamamoto, Takahisa Hiramitsu, Makoto Tsujita, norihiko goto, Syunji Narumi, Yosihiko Watarai.
Department of Transplant Surgery, Nagoya Daini Red Cross Hospital, Nagoya, Japan.

Introduction: The recurrence rate of primary focal segmental glomerular sclerosis (FSGS) after renal transplantation is relatively high, even with immunosuppressant use. Rituximab has been introduced as an effective agent in some patients with FSGS. However, the demography of patients who are susceptible with Rituximab remain unclear.
Materials and Methods: Twenty-eight patients with primary FSGS who had undergone renal transplantation between 2003 and 2014 at our hospital were retrospectively investigated. Recurrent FSGS after transplantation were treated with steroid pulse (500mg for 3 days) and plasma exchange (Conventional group; n=2). Rituximab was additionally administrated with these modalities recently (RTX group; n=6). Definition of treatment effectiveness is complete or partial remission. Pretransplant Rituximab and plasma exchange protocol was introduced to investigate efficacy for FSGS recurrence (PreRTX group; n=7).
Results: Among these 28 patients, 8 (38.1%) had recurrent FSGS after transplantation. Recurrence rate of PreRTX group did not significantly improve (42.8% vs. 23.8%, p = 0.317). Response rate of RTX group was higher than that of conventional group (66.7% vs 40.0%). Risk factors of FSGS recurrence were not identified in this study. Rituximab deemed effective in patients who were diagnosed at younger age (13.5 years vs. 24.5 years; p=0.113), were female (25% vs. 100%; p=0.116), had higher serum albumin level at the treatment (3.19 g/dl vs. 3.0 g/dl; p=0.342) or smaller amount of proteinuria (4.75 g/day vs. 6.65 g/day; p=0.285), and underwent fewer plasma exchange (0.5 times vs. 3.5 times; p=0.051), although none of these trends reached statistical significance.
Conclusions: Rituximab is effective for recurrent FSGS after renal transplantation. However, pretransplant Rituximab does not significantly reduce recurrence of FSGS in adult renal transplant candidates. Prospective study with a big population is essential to determine predictive parameters of therapeutic effect of Rituximab.
References:
[1] Araya CE.*Journal of Transplantation*.2011:374213.
[2] Modini Vinai.*Pediatric Transplantation* 2010:14:314-325
[3] Park H.S. *Transplantation*,2012;94:828-829

P.1388

50-year donor and recipient survival after non-twin living donor kidney transplantation

Oscar Serrano, Arthur J.Matas.
Surgery, University of Minnesota, Minneapolis, MN, United States.

We report 2 non-twin kidney transplants (1 sibling donor, 1 distant relative) with 50-year graft survival (on azathioprine/ prednisone immunosuppression).
Case 1: The first recipient, transplanted at 8 years old, completed university studies and held a full-time job. As an adult, the recipient had the following medical problems: orthopedic fractures occurring while playing sports (age 33), peptic ulcer disease (age 49), medically-controlled type 2 diabetes (age 64), hyperlipidemia and hypertension (age 66), and atrial fibrillation (age 72). Multiple basal cell (first diagnosed at age 62) and squamous cell carcinomas (70) were surgically-excised. Serum creatinine was 1.1 mg/dl at 30 years, 2.0 at 40 years, and 3.0 at 50 years post-transplant. The recipient returned to hemodialysis treatment 53 years after transplant. At a recent visit, the patient reported exercising regularly and denied any complaints exclusive of musculoskeletal aches. The donor is alive and in good health at 72 years old. The last eGFR was 94 mL/min/m2 at 51 years post-transplant. The only post-donation medical problem is hyperlipidemia, which is medically-controlled.
Case 2: The second recipient, transplanted at 19 years old, completed university studies, married and had 2 healthy children. The recipient worked fulltime until age 66. Medical problems included: gastroesophageal reflux disease (age 36), detached retina (age 63), cataracts surgery (age 65), hyperlipidemia (age 72), and a myocardial infarction (age 75). Multiple basal and squamous cell carcinomas (first diagnosed at age 61) were surgically-excised. Serum creatinine was 1.0 mg/dl at 30 years, 1.1 at 40 years, and 1.3 at 50 years post-transplant. At a recent visit, the recipient reported physical and mental health to be at the highest level since transplant. The donor is currently 81 years old and enjoys good health with normal blood pressure and an eGFR of 62.5 mL/min/m2 measured 48 years post-transplant. The only medical problem has been hyperlipidemia (age 80).
Conclusion: We present 2 patients whose lives were transformed by a kidney transplant at a time when kidney transplantation was in its infancy. Their long-term graft survival and relatively normal lives are a testament to the capabilities of transplantation and illustrate the improvements that have occurred in the management of kidney transplant patients.

P.1389

The impact of viral replication in recipient urine, oral wash and blood before transplant on post-transplant infection

Priya Verghese, E. Filtz, D. Schmeling, A. Matas, H. Balfour, Oscar Serrano.
University of Minnesota, Minneapolis, United States.

Post-transplant (tx) viral infection threatens recipient and graft survival. We hypothesized that virus in body fluids of recipients pre-tx would play a role in post-tx viral infection. We conducted a 5-year prospective study in organ tx recipients and tested them for cytomegalovirus (CMV) and Epstein-Barr virus (EBV) antibody (Ab), oral, urine and blood CMV, EBV and BKV PCR pre-tx and periodically for 5 years post-tx.

Incidence of Viral Replication in Urine, Oral Wash						
	ALL ORGANS (n=220)		KIDNEY (n=111)		LIVER (n=67)	
	PRE-TX	POST-TX	PRE-TX	POST-TX	PRE-TX	POST-TX
CMV Viruria	2 (1%)	47 (21%)	1 (0.009%)	0	1 (2%)	0
CMV Oral wash	4 (2%)	0	2 (0.02%	3 (0.03%	0	1 (2%)
CMV Viremia	2 (1%)	0	0	32 (29%)	1 (1.5%)	8 (12%)
EBV Viruria	1 (0.5%)	4 (2%)	1 (0.009%)	2 (0.02%)	0	2 (3%)
EBV Oral wash	68 (31%)	31 (14%)	39 (35%)	14 (13%)	23 (34%)	12 (21%)
EBV Viremia	10 (5%)	40 (20%)	3 (0.03%)	16 (14%)	4 (6%)	20 (30%)
BKV Viruria	28 (13%)	49 (22%)	15 (14%)	32 (29%)	6 (9%)	10 (15%)
BKV Oral wash	0	3 (2%)	0	3 (0/03%)	0	0
BKV Viremia	0	31 (14%)	0	17 (15%)	0	6 (9%)

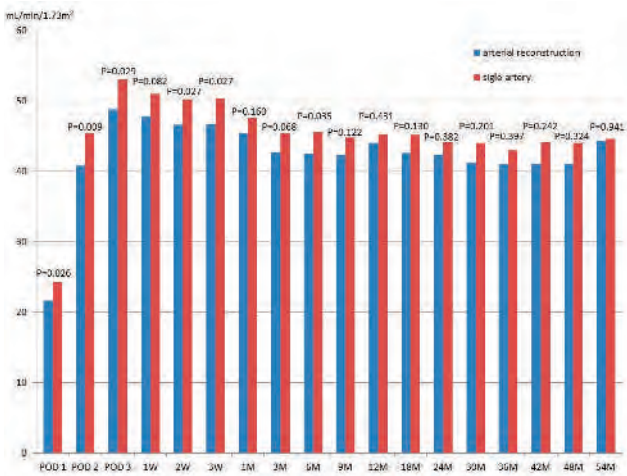
There was significantly less post-tx EBV viremia if recipients had EBV (mainly in oral wash) pre-tx in kidney (p 0.003) and all organ tx (p 0.02). Pre-tx CMV replication was rare. BKV replication pre-tx was associated with post-tx urinary BKV in kidney (p 0.02), liver (p 0.01) and all recipients (p 0.005) but not BKV viremia (p 0.98) regardless of organ type.Conclusions: Pre-tx viral replication did not affect post-tx EBV, CMV or BKV disease.Recipients shedding EBV pre-tx had less EBV viremia regardless of recipient-donor serology. Recipients shedding BKV pre-tx had more BK viruria but not viremia.

P.1390

The impact of graft thin upper pole artery ligation on living donor kidney transplantation

Takahisa Hiramitsu, Kenta Futamura, Manabu Okada, Takayuki Yamamoto, Makoto Tsujita, Norihiko Goto, Shunji Narumi, Yoshihiko Watarai.
Transplant and Endocrine Surgery, Nagoya Daini Red Cross Hospital, Nagoya, Japan.

Objective: The aim of this study is to investigate the impact of graft thin upper pole artery ligation on living donor kidney transplantation retrospectively.
Summary Background Data: The impact of graft thin upper pole artery ligation on living donor kidney transplantationhas been rarely reported.
Methods: Between January 2008 and April 2014, 532consecutive living donor kidney transplants in adult recipients were performed at our hospital. 389kidney grafts with a single artery (single artery group) and17kidney grafts withgraft thin upper pole artery ligation (arterial ligation group) were enrolled in this study. The characteristics of enrolled donors, recipients, and their grafts were initially evaluated. Thereafter, perioperative and postoperative recipients' estimated glomerular filtration rate (eGFR), and complication rates among two groups were investigated.
Results: The characteristics of enrolled donors, recipients and kidney grafts did not differ significantly. And perioperative and postoperative recipients' eGFR and complication rates were also similar in arterial ligation group and single artery group, respectively.



	Arterial ligation group	single artery group	P value	95% confidence interval
numbers	17	389		
Warm ischemic time (sec)	172.0	139.5	<0.05	20.42 to 50.15
Total ischemic time (min)	157.4	90.6	<0.05	51.22 to 82.78
Initial urination (min)	21.5	20.7	0.716	-3.28 to 1.68
Complications				
Artery thrombosis (%)	0.0	1.0	0.746	-0.027 to 0.018
Urine leakage (%)	0.0	1.0	0.708	-0.029 to 0.020
Ureteric stricture (%)	0.0	0.5	0.85	-0.017 to 0.020
Delayed graft function (%)	0.0	0	0.318	-0.036 to 0.027
Bleeding (%)	0.0	1.5	0.415	-0.016 to 0.009
Lymphocele (%)	1.8	1.2	0.88	-0.018 to 0.020
Acute cellular rejection (%)	0	2.5	0.457	-0.018 to 0.008
Antibody mediated rejection (%)	0.0	1.8	0.509	-0.035 to 0.017

Conclusions: Graft thin upper pole artery ligation can be a safe procedure in living donor kidney transplantation.

P.1391

Efficacy and feasibility of radiotherapy for localized prostate cancer in renal transplant recipients: a single-center experience

Junpei Iizuka, Yasunobu Hashimoto, Tsunenori Kondo, Toshio Takagi, Tomokazu Shimizu, Masayoshi Okumi, Hideki Ishida, Kazunari Tanabe.
Department of Urology, Tokyo Women's Medical University, Tokyo, Japan.

Introduction and Objective: Radiotherapy is considered as an alternative to prostatectomy as primary radical treatment for young men with localized prostate cancer. However, irradiation is not proposed for renal transplant recipients (RTRs) as much as for healthy adults because of the risk of post-radiotherapy complications due to fragile tissue peculiar to RTRs. Few reports discuss irradiation for RTRs with localized prostate cancer. This study aimed to evaluate the efficacy and feasibility of radiotherapy as a radical treatment of prostate cancer in RTRs.
Methods: All patients who underwent radiotherapy for localized prostate cancer as primary treatment between May 2005 and September 2015 were identified (n = 486). Of these patients, five who had a prior history of renal transplantation were included in this study. Two patients were treated with intensity-modulated radiotherapy, two were treated with low-dose rate brachytherapy, and one was treated with combination high-dose rate brachytherapy (HDR) and external-beam radiotherapy. Intermediate- to high-risk patients according to D'Amico risk stratification underwent a 6-month androgen deprivation therapy (ADT), and 6-month ADT was added as adjuvant therapy for high-risk patients.
Results: Median (range) age, prostate-specific antigen (PSA) level, time from renal transplantation to prostate cancer diagnosis, and follow-up period were 70 years (54–71 years), 17.3 ng/mL (5.3–29.4 ng/mL), 6 years (4.2–26 years), and 43 months (8–69), respectively. The biopsy Gleason score was 6 in two patients, 7 in one, and 9 in two. One patient was low-risk, one was intermediate-risk, and three were high-risk. Three patients had a functioning graft, and two had restarted hemodialysis after transplantation. All the patients completed radiotherapy. Elevated PSA level recurred in one patient treated with HDR 2 years after irradiation. However, four patients remained free of PSA progression without any additional treatment. Severe acute and chronic genitourinary and gastrointestinal toxicity were not observed during the follow-up period. The renal function of the patient with a functioning graft as measured by serum creatinine level was stable during and after irradiation.
Conclusions: Radiotherapy is feasible and acceptable as a minimally invasive treatment in the carefully selected RTRs with localized prostate cancer. Even in RTRs, radiotherapy should be considered as a good treatment option for localized prostate cancer.

P.1392

A safer 2-layer method of inguinal hernia repair for the renal transplant recipient

Nobuhiro Ishido, Yuki Yokota, Satoshi Komoto, Nobuhito Kubota, Syuya Yano, Takahiro Okamoto, Yoshihiko Kadowaki.
Department of Surgery, Kobe Red Cross Hospital, Kobe, Japan.

Inguinal hernia is a serious problem which makes quality of life lower. But inguinal hernia repair of the renal transplant recipient is a surgical procedure that tends to be avoided because of the surgical difficulty and the non-fatal aspect. Several cases have been reported of post-operative morbidities such as graft-ureteral necrosis due to contact with mesh-plug, or intraoperative injury of grafted ureter or urinary bladder due to incorrect recognition of anatomical structure caused by firm adhesion from the preceding surgery of kidney transplantation. The Lichtenstein method of hernia repair is known as a first choice for a renal recipient because of the absence of dissection of the preperitoneal space, but hernia recurrence or annoying chronic pain after surgery had been reported in some cases. Recently, the 2-layer method of hernia repair such as Prolene Hernia System (PHS) has gotten attention as a superior method with regard to curability and minimum complications. But PHS is inappropriate for renal recipients because of the necessity of wider dissection of preperitoneal space and postoperative harmful effects of adhesion against visceral tissues. In this case, we got an excellent result of an innovative hernia repair on the renal transplant recipient by the modified 2-layer method using Bard Ventralex ST. Bard Ventralex ST, a newly developed prosthesis for treating incisional hernia, requires minimum dissection of preperitoneal space, and a unique hydrogel barrier on its posterior layer, based on the Sepra Technology, swells to minimize tissue attachment to the visceral side of the mesh and resorbs within 30 days, providing visceral protection during the critical healing process, which keeps post-surgical complications low. Combined with a lightweight on-lay mesh support to strongly reinforce the rear wall of the inguinal canal, hernia recurrence is low. We report the safety, usefulness and applicability of this innovative 2-layer method of inguinal hernia repair.
References:
[1] Kobayashi S, et al.: Urinary bladder injury during inguinal herniorrhaphy in a renal transplant patient: report of a case. *Surg Today*. 2000 Jan; 30: 101-103
[2] Magnusson J, et al.: Lichtenstein, Prolene Hernia System, and UltraPro Hernia System for primary inguinal hernia repair: one-year outcome of a prospective randomized controlled trial. *Hernia*. 2012 Jun; 3: 277-285
[3] Pierides G, et al.: A prospective randomized clinical trial comparing the Prolene Hernia System and the Lichtenstein patch technique for inguinal hernia repair in long term: 2- and 5-year results. *Am J Surg*. 2011; 202: 188-193
[4] Tollens T, et al.: Prospective analysis of ventral hernia repair using the Ventralight ST hernia patch. *Surg Technol Int*. 2013 Sep; 23: 113-116.

P.1393

Results of paired kidney exchange transplantation: initial experience of a single institution

Feza Karakayali[1], Umit Ozcelik[1], Aydincan Akdur[2], Huseyin Y.Bircan[1], Sedat Yildirim[2], Gokhan Moray[2], Mehmet Haberal[2].
[1]Transplantation Center, Baskent University, Istanbul, Turkey; [2]Transplantation Center, Baskent University, Ankara, Turkey.

Introduction: Paired kidney exchange (PKE) transplantation has gained popularity worldwide as the best alternative for renal recipient candidates who are sensitized to their donors or with ABO incompatible donors. In this study we present our initial experience of PKE transplants.
Materials and Methods: We started our PKE transplantation program in July 2015. Various incompatible pairs were matched depending upon the availability of suitable donors and compatible recipients. Matching and donor allocation was done manually. Donors were also matched for age and glomerular filtration rate as far as possible. Donor and recipient pairs were allowed to meet before renal transplant. All recipients received antithymocyte globulin induction therapy at the time of transplantation and for 3 days posttransplant. Tacrolimus (target level 8-10 ng/mL), mycophenolate mofetil (1g twice a day) and prednisolone were started as immunosuppressive therapy and tacrolimus was maintained with a target level of 6-8 ng/mL for 3 months after the operation. All the patients were followed up twice weekly for the first 2 weeks, once weekly for the second 2 weeks, once every 2 weeks in months 2 and 3. Data were collected from medical records, including demographic data, follow-up serum creatinine, acute rejections, graft and patient loss, and infections.
Results: Five pairs were matched from July 2015 to January 2016 and we performed 10 PKE (5 women, 5 men) transplants. Mean recipient age was 44.9±13.6 y (range: 23-61y) and mean donor age was 52.3± 9.6 y (range: 38-64y). Three of the donors were fathers, 3 were husbands, 3 were wives and 1 was the mother. Mean HLA mismatch number was 5±1 (range: 3-6). Reasons for an exchange transplantation were ABO incompatibility in 8, and positive crossmatch and presence of donor specific antibodies in 2 patients. All were two-way donations. Median waiting time for getting a suitable donor after registration was 3 months. Two of the recipients had prior failed transplants and desensitization with plasmapheresis was needed for panel reactive antibody positivity. One patient had undergone preemptive kidney transplant. Mean serum creatinine level at 1st and 3rd post-transplant months were 1.03±0.37 and 0.97±0.25 mg/dL, respectively. There were only 2 early acute rejection episodes treated with pulse steroids and two urinary tract infections treated with oral antibiotics. In 1 patient, ePTFE vascular graft reconstruction to external iliac artery was needed because of an arterial intimal dissection during surgery.
Conclusion: There is a growing need to maintain a nationwide registry to promote PKE across different centers. This will be to the benefit of many desperate patients, it will expand the donor pool, and decrease the need for expensive desensitization protocols and ABO incompatible transplants.

P.1394

Results of BK virus screening in renal transplant recipients

Ümit Özçelik[1], Hüseyin Yüce Bircan[1], Mehtap Aydın[2], Eyyüp Külah[3], Feza Yarbuğ Karakayalı[1], Mehmet Haberal[4].
[1]General Surgery, Baskent University, Istanbul, Turkey; [2]Infectious Diseases and Clinical Microbiology, Baskent University, Istanbul, Turkey; [3]Nephrology, Baskent University, Istanbul, Turkey; [4]General Surgery, Baskent University, Ankara, Turkey.

Introduction: BK virus nephropathy (BKVN) has appeared as an important cause of renal graft loss with the use of potent immunosuppression. The aim of this study is to identify BKVN with regular BK virus (BKV) screening for early treatment.
Materials and Methods: This prospective study includes kidney transplant recipients from December 2012 to December 2014 who underwent kidney transplantation at the Baskent University Istanbul Hospital. Blood samples were collected for BKV DNA PCR from the patients at the postoperative 1, 2, 3, 6, 9 and 12. months. Incidence of BKV, rejection episodes, renal biopsy results, treatment of BKV and results of the treatments, mortality and graft loss rates were evaluated.
Results: A total number of 57 patients (26 women, 31 men) with a mean age of 41.5 (range: 18-63 years) underwent kidney transplantation. 54 (94.8%) of them transplanted from live donors and 3 (5.2%) of them from cadaveric donors. Antithymocyte globulin (ATG) induction therapy was administered at the time of transplantation as well as 3rd days thereafter and tacrolimus (target level 8-10 ng/mL), mycophenolate mofetil (1g twice a day) and prednisolone were started as immunosuppressive therapy and tacrolimus maintained with a target level of 6-8 ng/mL three months after the operation. A total number of 12 (21%) BKV positivity was detected at the postoperative one year period. They were seen as one at the 1st month, one at the 2nd month, 5 at the 3rd month and 4 at the 6th month. Immunosuppressive drug doses reduced in all patients. Ten of them (83.3%) became BKV negative in three months but in 2 patients (16.6%) biopsy proven BKVN was developed. The first patient became BKV positive at the 3rd month and BKVN detected at the 6th month, two months after type 2A acute cellular rejection (ACR) and one month later acute antibody mediated rejection (ABMR) was developed, after the treatment of rejections patient died due to sepsis at the 9th month. The second patient developed type 3 ACR and ABMR at the postoperative 2 weeks and pulse steroids, ATG, Plasmapheresis and intravenous immunoglobulin (IVIG) treatment was given. BKV positivity and BKVN with type 2A ACR was detected at the postoperative 6th month. Cidofovir started after treatment of rejection but graft loss occurred at the postoperative first year due to BKVN. BKVN negative 3 of 10 (30%) patients with BKV positivity developed rejection episodes. Totally, BKV positive 5 of 12 patients (41.6%) developed rejection episodes whereas 8 of 45 (17.7%) BKV negative patients developed rejection episodes. BKV positive one of 12 patients (8.3%) died due to sepsis whereas 3 of 45 (6.6%) BKV negative patients died due to Guillain-Barre Syndrome, fungal brain abscesses and disseminated intravascular coagulation.
Discussion: BKVN is an important cause of renal graft loss. We found BKV positivity as 21% and BKVN rate as 3.5% in our study. We recognized that BKV positive patients developed more rejection episodes.
Conclusion: Routine BKV screening after renal transplantation is very important for the diagnosis and treatment of BKVN.
References:
[1] [BK virus infections in kidney transplantation]. Lanot A, Bouvier N, Chatelet V, Dina J, Béchade C, Ficheux M, Henri P, Lobbedez T, Hurault de Ligny B. Nephrol Ther. 2016 Jan 27. pii: S1769-7255(15)00688-4. doi: 10.1016/j.nephro.2015.11.003. [Epub ahead of print] French.
[2] BK virus nephropathy in renal transplant recipients. Jamboti JS. Nephrology (Carlton). 2016 Jan 18. doi: 10.1111/nep.12728. [Epub ahead of print] Review. PMID: 26780694

[3] BK Virus Associated Nephropathy, a Cause of Early Renal Allograft Dysfunction: A Single Centre Study. Shrestha S, Kerr PG, Kanellis J, Polkinghorne KR, Brown F, Yii M, Mulley W. Kathmandu Univ Med J (KUMJ). 2015 Jan-Mar;13(50):140-5.
[4] Results of repeat renal transplantation after graft loss from BK virus nephropathy. Geetha D, Sozio SM, Ghanta M, Josephson M, Shapiro R, Dadhania D, Hariharan S. Transplantation. 2011 Oct 15;92(7):781-6. doi: 10.1097/TP.0b013e31822d08c1. PMID: 21836535

P.1395

Investigation of body mass index and cases of living renal transplantation performed in this department

Yu Kihara, Hitoshi Iwamoto, Takayoshi Yokoyama, Osamu Konno, Yuki Nakamura, Shigeyuki Kawachi.
Department of Digestive and Transplantation Surgery, Hachioji Medical Center, Tokyo, Japan.

Introduction: Japanese people have a lower obesity rate than do those in the West; however, the rate has increased since the past 10 years. In Japan today, 30% of men and 20% of women aged 20 years and over are classified as obese, with a body mass index (BMI) of at least 25%. With this background, the obesity rate is expected to continue increasing, and we anticipate operating on obese organ transplant patients more frequently. Obese patients are generally said to have a higher surgical risk; however, there are few reports on transplant patients in Japan. Thus, we retrospectively analyzed the relationship between BMI and the success of living renal transplantation performed in this department.
Subjects and Methods: We investigated the survival rate, graft survival rate, complications, and similar parameters in 143 cases of living renal transplant performed in this department from January 2003 when basiliximab was introduced until August 2014. A BMI of at least 25 was classified as obese (25–30 being obesity class 1 and at least 30 being obesity class 2); 18.5–25, as normal; and 18.5 and under, as underweight. Postoperative immunosuppressive therapy used four drugs: a calcineurin inhibitor (tacrolimus or cyclosporine), mycophenolate mofetil, methylprednisolone, and basiliximab.
Results: No significant difference was found in the survival rate or graft survival rate between the four groups. The underweight group tended toward a more favorable graft survival rate; however, the obesity class 2 group tended toward a more favorable survival rate. With respect to the rate of complications, both obese groups exhibited a higher frequency of complications such as acute rejection and subcutaneous abscesses. No significant difference was found in the length of the dialysis period leading up to the transplantation or in the perioperative hospitalization period. The obesity class 2 group tended toward a lower rate of cytomegalovirus infection. Renal function (s-Cr and 24 CCr at the time of discharge) tended to be more favorable with lower BMI; however, the difference was not significant.
Discussion: Although no large difference in survival or graft survival was seen, operating on obese cases is difficult, and there is a higher risk of rejection and complications, which requires caution. To increase long-term graft survival, we believe preoperative body weight control to be important for increased success. As the occurrence of acute rejection was much higher in the high-BMI group, the currently used dosing protocol requires review. Going forward, we consider it necessary to devise a new dosing protocol based on body weight.
Conclusion: No significant difference was found in the survival rate or graft survival rate between the four groups. However, obese cases exhibited a higher frequency of complications such as acute rejection and subcutaneous abscesses.

P.1396

Long-term renal allograft survival after sequential liver-kidney transplantation from a single living donor

Kumiko Kitajima, Katsuyuki Miki, Yuichi Ogawa, Kotaro Kai, Akihito Sannomiya, Toru Murakami, Kazuhiro Iwadoh, Ichiro Koyama, Ichiro Nakajima, Shohei Fuchinoue.
Surgery, Kidney Center, Tokyo Women's Medical University, Tokyo, Japan.

Introduction: Combined liver-kidney transplantation (CLKT) is well-established as a definitive therapy with the potential to provide complete recovery for certain liver-kidney diseases, although the results might be contingent on the cause of transplantation. The purposes of the present study were to review the long-term outcome of renal allografts in combined liver and kidney transplant (CLKT) patients from single living donors and to investigate the beneficial factors, compared with solitary renal transplantation.
Materials and Methods: Thirteen patients (3 adults, 10 children) underwent sequential liver (LTx) and kidney (KTx) transplantations from single living donors between August 1996 and July 2014 at our center. The indications for KTx were oxaluria (n=7), autosomal recessive polycystic disease (n=3), and others (n=3). The initial immunosuppressive agents used for LTx (tacrolimus and steroids in four patients, tacrolimus, steroids and mycophenolate mofetil [MMF] in nine patients) were continued with dose modifications after the successive KTx except in one patient in whom MMF was discontinued at 3 months after LTx because of leukopenia. The outcomes of the renal allografts were reviewed.
Results: KTx was performed between 1.7 and 47.0 months after the LTx. One patient died two months after the KTx because of sepsis. The overall patient survival rate was 92.3% at 10 years. In 12 of the 13 surviving patients, the renal allografts were found to be functioning in 11 patients, based on the serum creatinine levels estimated glomerular filtration ratio, after a mean follow-up period of 89.3 100.6 months (range: 10 – 205 16-221 months). Only the first patient, who underwent transplantation in 1996, experienced allograft loss 17.1 years after transplantation because of chronic antibody-mediated rejection. The death-censored renal allograft survival rate at 10 years was 100%, which was better than that of KTx alone (84.9%) in Japan. Overall, only one case of biopsy-proven acute renal rejection requiring intensive treatment occurred within the first year of the post-transplant period. The incidence of acute renal rejection was also lower after LTx/KTx than after KTx alone at our center (8.4% vs. 13.6%, respectively, ns).
Discussion: Immunological protection conferred by the preceding liver allograft may have contributed to the long-term outcomes of the renal allografts. In addition, the donation of double organs from a single living- and related-donor may have a favorable impact on the graft survival rate.
Conclusions: In the CLKT recipients, a better long-term renal allograft survival was obtained in comparison with the solitary renal transplant patients. In the future, investigations of factors affecting the long-term outcome of renal allografts, including details of the involvement of de novo DSA, will be needed.

P.1397

Impact of serum creatinine levels at 1 year on 5-year graft survival

Syed Haider Nawaz Naqvi[1], Mirza Naqi Zafar[1], Anwar Naqvi[2], Adib Rizvi[2].
[1]Pathology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [2]Urology and Transplantation, Sindh Institute of Urology and Transplantation, Karachi, Pakistan.

Introduction: Long term renal graft survival is a priority due to shortage of organ donation. We have examined the impact of serum creatinine (Scr) at 1-year post transplant on 5-year graft survival.
Methods: A retrospective cohort study included 773 adult living-related renal transplant recipients from 2007 to 2009 with a minimum follow-up period of 5 years. Demographics and post-transplantation follow-up data including immunosuppression regimens, rejection episodes, and survival rates were evaluated. Renal function in the first year was expressed as Scr at one year. Patients were divided into 4 cohorts based on Scr at the end of the first year: G1, Scr <1.0 mg/dl; G2, 1.01mg/dl <Scr <1.5mg/dl; G3, 1.5mg/dl < Scr <2.0 mg/dl and G4, Scr >2.01mg/dl. Comparisons between groups used the X2 test for qualitative parameters and analysis of variance for continuous variables. Other statistical tests were applied as appropriate. Statistical significance was accepted as $p < 0.05$.
Results: Five year graft survival for G1 was 98% as compared to 76% in G4 ($p<0.001$). Recipients of G4 encountered acute rejection episodes in 21% of cases as compared to 7.3% in G1 ($p=0.001$). Donors were older in G4 (42.07±10.4 years) as compared to G1 (30.1±8.5 years) ($p=0.001$). A third of the donors in G1 were 6 antigen matched as compared to 7% in G4.
Conclusion: Prediction of 5-year graft survival is possible by the serum creatinine level at 1-year post transplant. So this parameter can be used for filtering patients who require close monitoring in follow-up. Donor age, relationship and HLA match also play a role in 5-year survival of renal graft.

P.1398

A living donor risk model for predicting kidney allograft and patient survival in an emerging economy

Mirza Naqi Zafar[1], Germaine Wong[2], Tahir Aziz[3], Khawar Abbas[1], S Adibul Hasan Rizvi[4].
[1]Pathology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [2]Sydney School of Public Health, University of Sydney, Sydney, Australia; [3]Nephrology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [4]Urology and Transplantation, Sindh Institute of Urology and Transplantation, Karachi, Pakistan.

Background: Living donor kidney is the main source of organs in low to middle income countries. We aimed to develop a living donor risk model that predicts graft and patient survival in an emerging economy.
Methods: This study used information from the Sindh Institute of Urology and Transplantation (SIUT) database (n=2,283 recipients and n=2,283 living kidney donors) and Cox proportional hazard analyses to create a composite score that predicts graft and patient survivals. Model discrimination was assessed using the area under the receiver operating curve (c-statistic).
Results: Donor factors age, creatinine clearance, nephron dose (estimated by donor / recipient body weight ratio) and human leukocyte antigen (HLA) match were included in the living donor risk model. The adjusted hazard ratios (HRs) for graft failures among those who received a kidney with living donor scores (reference to donor score of zero) of 1, 2, 3 and 4 were 1.14 (95%CI: 0.94 –1.39), 1.24 (95%CI:1.03–1.49), 1.25 (95%CI:1.03–1.51) and 1.36 (95%CI:1.08–1.72) [p-value for trend =0.05]. Similar findings were observed for patient survival. Using the model to predict graft and patient survival, the c-statistics was 0.52 (Cl: 0.46–0.58) and 0.50 (CI: 0.42–0.56) respectively.
Conclusions: Similar to findings in high income countries, our study suggests that donor characteristics such as age, nephron dose, creatinine clearance and HLA match are important factors that determine the long term patient and graft survival in low income countries. However, the overall predictive value of our current model for graft and patient survival is low, suggesting other crucial, but undefined factors may play a role in determining the overall risk of graft failure and mortality In living kidney donor transplant recipients.

P.1399

High prevalence of post transplant donor specific HLA-DQ antibody in live related renal transplantation. Is it time to extend typing to HLA-DQ in renal transplantation?

Mirza Naqi Zafar[1], Khawar Abbas[1], Rabia Fawad[1], S Adibul Hasan Rizvi[2].
[1]Pathology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [2]Urology and Transplantation, Sindh Institute of Urology and Transplantation, Karachi, Pakistan.

Introduction: The detection of HLA antibodies against the allograft is important as they are considered the major cause of transplant glomerulopathy. In the recent years new technology to detect HLA antibodies has given newer insight to antibody mediated acute and chronic rejection. This study presents screening for HLA antibodies post transplantation in stable transplant recipient in follow-up in outpatient settings. The aim of this study was to determine the HLA classes of post transplant donor specific antibody (DSA) in live related renal transplant recipient.
Patient and Methods: HLA antibodies were analyzed in 212 renal transplant recipient 1-24 month post transplant. Samples were collected in routine follow-up in out patient. All recipients were HLA antibody negative by flow cross match and Luminex pre-transplant. All rejections were confirmed by biopsy. Antibody screen and single antigen screen was undertaken by Luminex. Outcomes were compared between recipient with DSA and No DSA for rejection, HLA match, Serum Creatinine and Graft Survival.
Results: Of the 212 recipient screened for HLA antibodies 20 (9.4%) were positive for Class I and 34 (16%) for Class II. DSA were found in 31 (14.6%). DSA developed in 2 (7%) of the recipient within 3 months, in 4 (14%) within 6 and 25 (79%) after 6 months. DSA against Class I in 10 (4.7%) and Class II in 27 (13%). Within Class I, against HLA A in 9 (90%) and HLA B 1 (10%), while within Class II, HLA-DR in 11 (41%), HLA-DQ in 15 (55%) and HLA-DR + HLA-DQ in 1 (3.7%). Rejections were diagnosed in 16 (52%) of DSA group vs 48 (26%) in No DSA group ($p< 0.05$). The mean HLA match was 3.39 ± 0.9 in DSA vs 3.67 ± 1.2 in Non DSA (p 0.231). The mean S. Creatinine was 2.42 ± 1.4 mg/dl in DSA vs 1.6 ± 0.8 in Non DSA (p 0.001). Graft survival in DSA group at 1, 3, 5 years was 97%, 90% and 57% vs 93%, 92% and 78% in the No DSA group.
Conclusion: In OPD follow-up 14.6% of the recipient had DSA mainly against Class II HLA-DQ antigens. Although rejection episode were higher in patients with DSA but the short term survival at 1, 3 years was similar to non DSA group. High frequency of HLA-DQ antibodies warrant typing for HLA-DQ as routine specially in live related transplant to determine the long term consequences of DSA against HLA-DQ antigens.

P.1400

Association between dipstick proteinuria and allograft outcomes in living donor kidney transplant recipients

Ji Yoon Choi[1], Oh Jung Kwon[2].
[1]Department of Surgery, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; [2]Department of Surgery, Hanyang University Hospital, Seoul, Korea.

Background: Proteinuria is one of the important factors suggestive of kidney function impairment. Previous epidemiologic studies had demonstrated that greater than trace amounts of protein on a casual urine dipstick may be an important predictor of long-term clinical outcomes in general population. In kidney transplant (KT) recipients, there were few data concerning it.
Methods: Thus, we retrospectively analyzed 238 living donor KT recipients to investigate impact of dipstick proteinuria on allograft outcomes. All KT recipients were divided into 2 groups according to dipstick proteinuria: control group (n=190), negative; case group (n=48), ≥ trace. Cox's proportional hazard model with time-dependent covariates was used to encompass compounding effect of covariates that change over time, including vintage and allograft survival time.
Results: As compared with controls, the change of estimated glomerular filtration rate was prominent in recipients with dipstick proteinuria after 3-year post-KT (-1.5 ± 7.1 vs. -4.4 ± 7.4 mL·min-1·1.73 m-2·year 1,P<0.0174). In Kaplan Meier analysis, KT recipients in control group had a better dialysis-free survival as compared with cases(201± 6 vs. 164 ± 10 months; log-rank P=0.0118). In Cox proportional hazard models, trace or more dipstick proteinuria was closely associated with long-term allograft loss (HR=1.964, 95% CI=1.152-3.348), and further adjustment for age and gender did not attenuated this association (HR=1.764, 95% CI=1.050-3.065).
Conclusions: Our results may suggest that presence of small amount protein in urine may be the first sign of deteriorating allograft renal function.

P.1401

Is the variability of renal arterial resistance index measurements useful parameter of late graft function after renal transplantation?

Ji Yoon Choi[1], Seung Jo Choi[2], Oh Jung Kwon[2].
[1]Department of Surgery, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; [2]Department of Surgery, Hanyang University Hospital, Seoul, Korea.

Background: The renal arterial resistance index (RI) by duplex Doppler ultrasound after kidney transplantation reflects the exacervation of interstitial edema. The influence of RI after renal transplantation on its predictive power has not been sufficiently evaluated. We performed retrospective analysis of RI and its power to predict renal allograft failure or death with special emphasis on the time point and the variability of RI measurements.
Methods: RI measurements were obtained from 107 transplanted patients on postoperative day (POD) 1 and POD 7 from January 2000 to November 2013 at Hanyang university medical center. All patients with RI measurements were retrospectively stratified into three groups according to the RI value ; Group 1 : index of < 0.70 (n = 73(68.2%) on POD 1 and n= 82(76.6%) on POD 7), Group 2 : index between 0.70 and 0.85 (n = 30(28.0%) on POD 1 and n= 22(20.6%) on POD 7), and Group 3: index of ≥ 0.85 (n = 4(3.7%) on POD1 and n= 3(2.8%) on POD 7). The graft function of kidney was estimated by glomerular filtration rate(eGFR), serum creatinine(s-Cr) and delayed graft function(DGF).
Results: RI measurements on POD 1 were not predicted the end point, whereas the RI obtained at POD 7 showed a significant predictive value for renal transplant failure or death in a univariate approach [P =0.0001]. Patients with the Group 3 on POD 7 showed the highest incidence of DGF[P =0.0001], eGFR[median value = 17.00, P =0.004] and s-Cr[median value = 5.20, P =0.0001], among three groups. The analysis of the change in RI value showed that the increased RI index between POD 1 and POD 7 was significant for a dismal outcome ; DGF[P =0.0001]. Survival analysis of each three group on POD 7 was as in the following ; acute rejection episodes : 4.1%(n=3) in Group 1, 13.3%(n=4) in Group 2, 25%(n=1) in Group 3 and incidence of DGF : 1.3%(n = 1) in Group 1, 6.6%(n=2) in Group 2, 100%(n=4) in Group 3 and during follow up period 13 graft losses occurred in patients.
Conclusion: RI measurements on 7 days after transplantation appeared useful to predict allograft outcomes. And the variability of RI measurements in transplanted kidney suggested a valuable parameter of the graft function of kidney. Sequential renal duplex ultrasonography can be useful for identifying high risk group for subsequent development of graft failure.

P.1402

Short-term changes in renal function before and after nephrectomy of living kidney donors

Ji Yoon Choi[1], Min Kyung Nam[2], Oh Jung Kwon[2].
[1]Department of Surgery, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; [2]Department of Surgery, Hanyang University Hospital, Seoul, Korea.

Background: Normal renal function has been recognized as an important portion of the living donor's healthy after kidney donation. The purpose of this study was to evaluate the short-term changes in renal function of living donors before and after kidney donation.
Methods: Of 145 living kidney donors from January 2001 to December 2013, we analyzed the data of 58 donors completed the examinations of before nephrectomy and 1 month, 6 months, and 12 months follow-up examinations after nephrectomy.
Results: Of 58 donors, female were 53.4%. The mean age was 38.6±12.35 years. In the relationship with recipient, sibling (29.3%) and children (25.9%) were predominant. As the indicators of renal function, BUN, Cr, eGFR, and CCr were analyzed. Before kidney donation, the mean and standard deviation (SD) of BUN, Cr, eGFR, and CCr were 12.85±3.34mg/dl, 0.85±0.15mg/dl, 97.76±15.36, and 91.74±15.59ml/min respectively. At 12 months after nephrectomy, BUN 15.53±3.48mg/dl, Cr 1.12±0.21mg/dl, eGFR 71.55±15.8, CCr 68.04±17.15ml/min. Reviewing the differences in kidney function according to the general nature, Cr in accordance with the gender difference is, but eGFR ratio there was no difference. Group of donation by children and under the age of 30, eGFR ratio results showed significant differences between the highest scores. Analyzing results of an average score 66 points in eGFR ratio after 1month in donation, there was statistically significant good score a group more than 66 in eGFR ratio such as Cr(before donation, after 6months), BUN(after 1month) and eGFR(after 12months).
Conclusions: In spite of compensation reaction, living kidney donation resulted in reduced renal function after nephrectomy and prolonged renal function recovery till one year after nephrectomy. It is required for living kidney donors to be managed continuously and systematically before and after nephrectomy.

P.1403

Intra-operative fluid management for robotic kidney transplantation: an initial experience

Manisha Modi[1,2], Bina Butala[1,2], Geeta Parikh[1,2], Kalpana Vora[1,2], Beena Parikh[1,2], Pranjal Modi[1,2], Veena Shah[1,2], Vivek Kute[1,2], Himanshu Patel[1,2], Hargovind L.Trivedi[1,2].
[1]Anaesthesiology, Smt. G. R. Doshi and Smt. K. M. Mehta Institute of Kidney Diseases and Research Centre, Ahmedabad, India; [2]Transplant Anaesthesia, Dr. H.L. Trivedi Institute of Transplantation Sciences, Ahmedabad, India.

Introduction: Robotic platform has recently been introduced for kidney transplantation surgery. There is paucity of information about fluid management during robotic assisted kidney transplantation. Our aim of presentation is to describe intraoperative fluid therapy based on central venous pressure monitoring in Trendelenberg position and pneumoperitoneum in renal transplant recipients undergoing robotic assisted surgery.
Materials and Methods: Thirty-five adult patients (M/F 28/7) aged 37.4± 11.01 years weighing 56.42±9.94 kg scheduled for elective living donor kidney transplantation were enrolled. All patients underwent hemodialysis on the day of surgery. Balanced general anesthesia was given and volume controlled mechanical ventilation given. Radial artery was cannulated for blood pressure monitoring and internal jugular vein was cannulated for central venous pressure monitoring. Baseline cvp was measured in supine position, thenafter cvp tracing was observed for fluid guidance. After pneumoperitoneum and head low position cvp were noted and deducted from the baseline value. Hemodynamics, Etco2, temperature and Spo2 were monitored. All patients were given 30 degree trendelenburg position. Main outcome measures were urine output after vascular clamp release, volume overload, facial oedema and post exutubation respiratory distress. Duration of surgery was 3.14±0.79 hours.
Results: All patients required nitroglycerine infusion for blood pressure control.Spo2 remained 100% throughout the surgery. About 500ml of 0.9% normal saline/hour was infused to all patients to keep cvp between 10-15 mm of Hg.No hypercarbia/acidosis were observed. They established good urine output after vascular clamp release. None of the patients had facial oedema, volume overload and postexubation respiratory distress. All patients were extubated well after surgery.
Conclusion: Adequate volume replacement is essential to ensure graft function after kidney transplantation surgery on the other hand, steep trendelenburg position and pneumoperitoneum can make fluid management a complex issue. Restricted crystalloid solution avoids brain edema without compromising renal allograft function in patients who are undergoing robotic assisted kidney transplantation surgery.

P.1404

Preemptive kidney transplantation in Japanese patients: A propensity score-matched study

Masayoshi Okumi, Yasuyuki Sato, Kohei Unagami, Toshihito Hirai, Tomokazu Shimizu, Hideki Ishida, Kazunari Tanabe.
Urology, Tokyo Women's Medical University, Tokyo, Japan.

Background: Evidences indicate that compared with non-preemptive kidney transplantation (non-PKT), preemptive kidney transplantation (PKT) is associated with advantages in outcomes. However, the exact mechanism underlying this beneficial effect of PKT remains to be elucidated. Because any randomized controlled trial investigating whether PKT reduces risk of adverse outcomes in KT recipients is virtually nonexistent, we postulated that the effects of PKT might be overestimated with respect to the better outcomes.
Objective: The aim of this study was to attempt to estimate the precise effect size of PKT on outcomes among patients who underwent living KT using propensity score-matching (PSM) methodology.
Patients: We analyzed 1060 Japanese adult recipients who had undergone living KT and received tacrolimus-based immunosuppression from 2000 to 2013.
Results: Of 1060 patients, 95 (9.0%) patients received PKT. According to the PSM procedure, we were able to match 93 patients. Marked difference in transplant year was observed between the two groups in the unmatched cohort. More than 80% of patients in the PKT group received transplant in the recent 5 years. As a result, the PKT group had a significantly shorter follow-up period than that of the non-PKT group. In contrast, the two groups were well matched with respect to all baseline characteristics in the matched cohort. Before PSM, there was a significant reduction in graft failure (absolute difference, -6.2%; 95% CI, -9.6% to -2.8%; P=0.026) and cardiovascular event (absolute difference, -6.7%; 95% CI, -10.7% to -2.7%; P=0.026) for patients who received PKT. There was no difference regarding other outcomes between the two groups. In a matched population, no reduction in graft failure (absolute difference, -3.2%; 95% CI, -8.7% to 2.2%; P=0.444) or cardiovascular event (absolute difference, -2.2%; 95% CI, -8.0% to 3.7%; P=0.721) was seen in the PKT group compared to the non-PKT group. No beneficial effect was observed regarding other outcomes among patients who underwent PKT. In a matched cohort, the mean eGFR two weeks after transplant was similar between the PKT group (45.2 mL/min/1.73 m2) and the non-PKT group (46.5 mL/min/1.73 m2) (P=0.559).
Conclusion: Our present study showed that PKT was not beneficial compared to non-PKT in adult patients who received living donor KT after evaluation in a propensity score-matched cohort study design.
Katsunori Shimada, PhD. (STATZ Institute, Inc., Tokyo, Japan); Astellas Pharma Inc. (Tokyo, Japan)

P.1405

Who is at risk in living kidney donation?

Rajesh Sivaprakasam, Vittoria Mastantuono, Giulia Ottaviano, Kate A.E.Nicholls, Evaldo Favi, Carmelo Puliatti, Roberto Cacciola.
Renal Transplantation, Bartshealth NHS trust, London, United Kingdom.

Background: In recent years a number of reports have raised some concerns on the safety of living kidney donation and on long term renal functions following Donor Nephrectomy (DN). In this study on we have reviewed the outcome of our Living Donors (LD) with more than 3 years from donation. We analysed demographic, adherence to Follow-Up (FU) and renal functions at 6-8 weeks, 3 and 5 years post donation.
Methods and Results: We have identified 161 LD from June 2008 to March 2012. Of them 56 (34%) were transferred out or lost to FU. All 27 LD (17%) lost to FU were Non-White (NW); p=0.0001.

Total Number of Living donors	n = 105
Age	45 Mean (21-76)
Female	66 (63%)
Ethnicity	
Any White (AW)	63 (62%)
Non-White (NW)	42 (38%)
Any Black (AB) 28 (17%)	
Any Asian (AA) 37 (21%)	
Adherence to Follow Up Visits	
50-100%	83 (79%)
<50%	22 (21%)

Demographics, adherence to FU and renal functions were analysed on the remaining 105 LD. Expectedly, at first FU visit at 6-8 weeks in all 105 LD we observed a reduction of eGFR from pre-donation. There was no eGFR variation between 3 and 5 years.
The predicted eGFR decline of 0.9 ml/min/1.732 per year was observed in 11 LD (10.5%); in 3 of them the eGFR decline was greater than expected. All the other 94 LD (90%) had improved eGFR. Proteinuria varied from Trace to 2+ in 31 LD (29%) and was not linked to reduced eGFR. BMI was not recorded in enough patients to allow an accurate analysis. On multivariate analysis NW ethnicity was associated with eGFR reduction; p 0.001.
Age and gender were not associated with decline of functions post donation and there were no cases of diabetes post donation.
Discussion: In our unit we observe a remarkably ethnic diverse population that applies to both AW and NW groups. The rate of LD lost to FU is high; however all were LD from abroad who returned home after the donation. The overall adherence to FU is below our expectations and should be improved.
Ethnicity was the only variable with strong statistical difference associated with a decline of eGFR, also beyond the natural senescence of renal functions as indicated in the UK guidelines for LD kidney transplantation. This observation may be of concern when considering our rate (17%) of donors from abroad.
Interestingly, LD of AW ethnicity tends to improve their eGFR over the course of the years. In light of these observations a national evaluation of the Renal Functions of LD at 3-5 years and beyond may be necessary, as it may indicate different eGFR thresholds for living kidney donation according to ethnicity.

P.1406

Effect of tacrolimus trough-level variability on new-onset diabetes after transplantation in renal transplantation

Rajesh Sivaprakasam, Esam Aboutaleb, Flavia Gentile, Carmelo Puliatti, Roberto Cacciola.
Renal Transplantation, Bartshealth NHS Trust, London, United Kingdom.

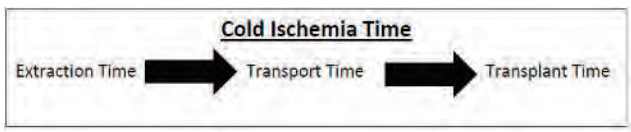
Introduction: Tacrolimus based immunosuppresive regimes are widely used as a maintenance therapy following renal transplantation. Trough-level variability (TLV) has been shown to affect the graft and patient survival after renal transplantation and closes monitoring of the trough levels are essential.
New-Onset Diabetes mellitus After Transplantation(NODAT) is a well recognised side effect of calcineurin based imunosuppressive régimes and causes are multifactorial and patients with increased risk, the patients may develop NODAT within 3 monts after transplantation. Therefore, we intend to assess the association of tacrolimus trough-level variability at 6 and 12 weeks with NODAT.
Methods: This observational study was performed in a single renal transplant centre and all adults who underwent renal transplantation over 7 years were included and the data collected were ; Donor variables – age, sex cause of death, type of donor, cold-ischaemic time, CMV mismatch and recipient variables - age, sex, cause of end-stage renal failure, duration of renal replacement therapy, induction & maintenance immunosuppressive régimes, time to diagnosis of NODAT, delayed graft function, graft outcome with a minimum follow up of 1 year. All patients who had conversion between CNI agents, multiple organ transplantation were excluded from the study.
Tacrolimus trough-level variability (TLV) was calculated using the co-efficient of variation formula and the TLV results are expressed as a percentage. We divided the study cohort into two groups bases on the TLV – Low (<50%) and High (> 50%) and the data was analysed for TLV association with NODAT and also, the differences between the groups using SPSS.
Results: A total of 639 patients underwent renal transplantation during the study period and 59 (9.23%) were diagnose to have NODAT during the study period. 8 patients were excluded because of CNI conversions and incomplete data. A multivariate analyses showed an association of trough-level variability with NODAT (HR- 1.25, CI 1.16-1.78, p=0.002) and high tacrolimus TLV was associated with earlier onset of NODAT (p=0.027). In contrary to recent reports, the high tac TLV wasnot associated with graft outcome.
Outcome: Our study shows an association between Tacrolimus trough-level variability and NODAT and an earlier onset with high TLV. Our study is limited by small study cohort, therefore, further validation using a larger sample is essential.

P.1407

What component of the cold ischemia time is important?

Rajesh Sivaprakasam, Vittoria Mastantuono, Giulia Ottaviano, Kate A.E. Nicholls, Evaldo Favi, Carmelo Puliatti, Roberto Cacciola.
Renal Transplantation, Bartshealth NHS trust, London, United Kingdom.

Background: Cold Ischemia Time (CIT) is the only modifiable factor in transplantation. Its importance is indisputable and valuable consideration has recently been given to the initial part of CIT called "Extraction Time". In this study we analyse the final part of CIT calculated from "Kidney Arrival" in the transplant unit to "Kidney Out of Ice". We have defined this period of CIT as Transplant Time (Tx Time) and propose the division of CIT in three components; Extraction time, Transport Time and Transplant Time. We have also evaluated the impact that Virtual Cross Match (VXM) has on Tx Time and on the overall CIT.
Methods and Results: We obtained data on CIT, time of Kidney Arrival (KA) and Kidney Out of Ice (KOI) from our prospectively kept database and NHSBT transport services. The data of 226 out of 240 (94%) Deceased Donor Renal Tx (DDRTx) performed in our centre between 1/1/12 and 1/4/15 were collected and analysed. Overall the mean Tx Time was 579min; it represented 68% of the mean CIT of 843min. Out of 226 there were 112 (49.5%) DDRTx performed with VXM. In this group the mean Tx Time was 483min and CIT 760min. All retrospective XM were negative. In the remaining 114 DDRTX the mean Tx Time was 673min and the mean CIT was 924min. The Tx Time as defined above is a statistically significant component of the CIT versus the other parts of the CIT; $p < 0.0001$ (X2). The VXM group had a statistically significant lower Tx Time and CIT compared to the group were prospective XM was performed; $p < 0.0001$. The same significance was observed between DCD and DBD.
Discussion: The Tx Time represents an important component of the CIT. We propose to divide the CIT in three components: Extraction Time (Cold perfusion in the donor to kidney in the box), Transport Time (Kidney in the box to Kidney Arrival at the transplant centre) and Tx Time (Kidney Arrival to Kidney Out of Ice). Analysing these components of the CIT will allow clinicians and transplant service commissioners adequate intervention aimed to reduce CIT and optimise outcomes. Allocation and acceptance of kidneys may be influenced by these parameters; as well as indicating specific intervention required for improving organ procurement, transportation and organisation of the transplant centres. The established use of VXM in our unit allowed significant reduction of the Tx Time and CIT. Further studies will be required to evaluate what impact the proposed components of CIT may have on the functions of DDRTX.



P.1408

Psychological problem in kidney transplant recipients

Man Ki Ju, Sunyoung Son, Im-Kyung Kim.
Transplantation Surgery, Gangnam Severance Hospital, Seoul, Korea.

Background: Kidney transplantation is a well-established treatment for end-stage renal disease. As recently increased interest in quality of life after transplantation, the need for research on mental health have been raised.
Method: We investigated anxiety and depression in kidney transplant recipients using the Hospital Anxiety Depression Scale(HADS). Clinical and demographic data were collected from questionnaires. The data were collected from August 2014 to November 2014 at two medical centers in Korea.
Results: Of the 105 patients, 53.9% were male and the mean age was 46.6±13.24. The average period after transplantation was 38.26±47.7month. Anxiety and depression scores were found to be in the normal range (6.32±4.27, 6.01±3.99). Scores of ≥11 on either subscale are considered a significant case of psychological morbidity. In this research, The patients with significant severity on anxiety was 15.7% and the patients with significant severity on depression was 11.3%. There was a correlation between anxiety and duration of dialysis(r=-.183, p=.051).
Conclusion: The result of this study, we found that patients with kidney transplantation have psychological problem. Therefore, further study is needed for confirming the phenomenon and determining the factor that affect the mental health. And Screening for anxiety and depression in kidney transplant recipients is essential. Appropriate treatment of mental illness may improve patients' quality of life.

P.1409

Impact of early hospital readmissions after kidney transplantation on graft function

Man Ki Ju, Im-Kyung Kim, Sunyoung Son.
Transplantation Surgery, Gangnam Severance Hospital, Seoul, Korea.

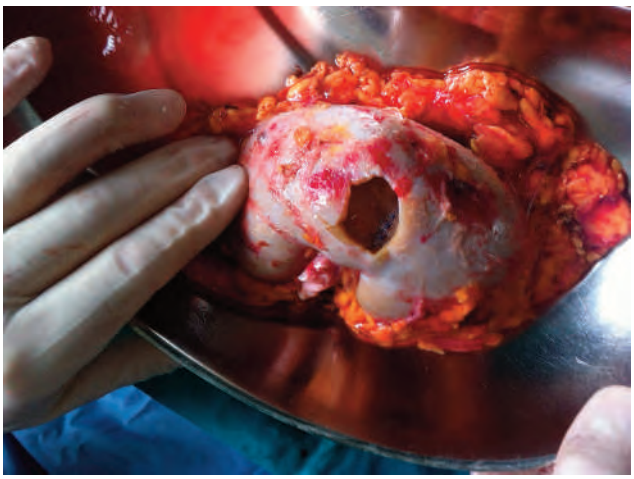
Early hospital readmissions are common after kidney transplantation. We investigated the relationship between early hospital readmissions and clinical outcomes in a single-center retrospective study that included all adult kidney transplant patients between March, 2009 and June, 2015. The early hospital readmissions within the first 30 days were numbered and the diagnosis ascertained. Patients were grouped as None and Readmissions. Clinical outcomes and patient and death-censored graft survival were compared. Among 103 patients, 32 (31.1%) patients had once or more readmissions within 30 days. Surgical complications, electrolytes imbalance, and acute rejection were common cause of readmission. There were no differences in baseline characteristics between two groups. Patients with early readmissions had lower renal function at post-operative 3, 6, and 12 months ($p = 0.002$, 0.020, and 0.013). There was no difference of graft function 12 months after transplantation between None and Readmission groups. 5-year graft and patient survival also showed no difference between two groups ($p = 0.424$ and 0.442).
In conclusion, early readmission after kidney transplantation affected lower graft function until 1 year after kidney transplantation. However, long-term effect on graft function is limited in this study.

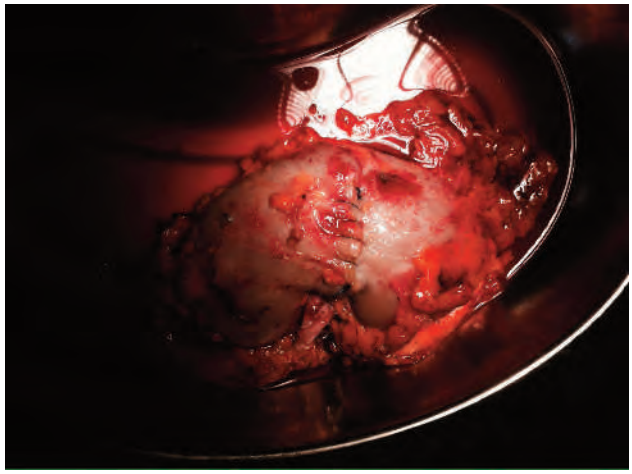
P.1410

Renal transplantation after laparoscopic radical nephrectomy due to neoplastic disease - case report

Marek Ostrowski[1], Jarosław Szumiłowicz[2], Anna Prekwa[1], Anita Suwałaa[1].
[1]Department of General Surgery and Transplantation, Pomeranian Medical University, Szczecin, Poland; [2]Department of Vascular Surgery and Angilogy, Pomeranian Medical University, Szczecin, Poland.

A 36-year-old doctor, came to the Clinical Department of Urology and Urological Oncology of Pomeranian Medical University because of the recognized left kidney tumor on computed tomography. Due to the suspicious nature of the tumor (carcinoma clarocellulare), the patient was qualified for laparoscopic left radical nephrectomy. During laparoscopic nephrectomy procedure, hilus of kidney neoplasm with a diameter of 3 centimeters was found. The kidney was removed. On a side table, the kidney was rinsed with Custodiol fluid and macroscopic evaluation of the tumor`s morphology was conducted. Then, the tumor with capsule was removed and the incision was closed with single sutures. The kidney was placed on a Lifeport perfusion machine.
Kidney tumor was sent to the Depatment of Anatomical Pathology for histopathological examination. The initial result indicated oncocytoma. The patient did not agree to autotransplantation of the removed kidney. On the other hand, she proposed a kidney transplant for her mother undergoing peritoneal dialysis for over four months. After fulfilling all the necessary formalities (informed consent of the donor and recipient, crossmatch test) after 16 hours of removal, the kidney was transplanted into the patient's mother. After the transplant, a proper function of the kidneys was restored.
In a postoperative period following complications were observed – anastomotic vesicoureteral leak, which was surgically cured, (most likely caused by too early administration of rapamicin) and anastomotic stenosis of renal artery treated with stenting. The final result of histological examination, obtained 14 days after transplantation, was a chromophobic cancer without capsule infiltration. The recipient was informed of the possible consequences of transplantation.
The case was presented to an oncology committee. At present, after 14 months, renal function is normal with creatinine level of 1[1]. In both the donor and the recipient, no evidence of neoplastic disease is observed. Currently, the recipient receives immunosuppressive agents: Mycophenolate mofetil, Everolimus, Methylprednisolonum.





References:
[1] Kidney cancer: Laparoscopic nephrectomy as outpatient surgery? Sidaway P. Nat Rev Urol. 2016 Jan 27. doi: 10.1038/nrurol.2016.20.

P.1411

Obstacles facing living related donor kidney transplant program at an urban non-transplanting center, UK.

Husham Rasheed, Kashif Iqbal, Karen Hodgson, Jyoti Baharani .
Renal Medicine, Heart of England NHS Foundation Trust, Birmingham, United Kingdom.

Background: Living Related Donor (LRD) kidney transplantation is the treatment of choice for most renal patients. Better graft survival in comparison to deceased donor transplantation and avoiding dialysis when performed preemptively; are the biggest advantages. The West Midlands, UK has a low live donor and preemptive kidney transplant rate compared to national figures. We conducted this study in our center to evaluate the LRD program particularly looking into how we can enhance living donor transplantation.
Methods: All recipient referrals for LRD kidney transplant at our center, between 2013 and 2014 were reviewed. Hurdles were ascertained for those LRD which did not actually proceed to kidney transplant. These were categorized into 9 modifiable and non-modifiable obstacles (outlined below).
Results: In the two years between 2013-2014 there were a total of 110 recipient referrals for LRD kidney transplant, out of which 27 (24.5%) proceeded to have LRD transplant, 2 had cadaveric transplant and 81 recipients (73.6%) did not proceed.
We found almost an equal combination of modifiable and non-modifiable obstacles that led to this outcome. The modifiable obstacles were donor or recipient being unwilling to proceed (29.6%), delayed work up process (9.8%) and issues around excessive BMI (8.6%). Donor or recipients being unsuitable (17.2%) or unfit (14.8%) to proceed were the main non-modifiable obstacles.

Modifiable Obstacles	N=39	Non-Modifiable Obstacles	N=42
Not willing to proceed in LRD Program	24	Recipient and donor non suitability to proceed	14
Donor still in workup	8	Unfit from cardiovascular point	12
Overweight issue	7	Overseas work up issue (cost, relationship)	8
		Recipient death	3
		Referred to another center	4
		Stable eGFR of recipient	1

Conclusion: At our center, around three quarters of those coming forward to be considered for live kidney donation did not actually proceed to surgery. A combination of modifiable and non-modifiable obstacles are responsible for the non-fruition of donation.
Recipients or donors who were unwilling to proceed despite medical suitability had a variety of family and emotional issues, like pressure and the feeling of guilt. These are complex psychological barriers that would require a dedicated Clinical Psychology service.

P.1412

Successful direct-acting antiviral therapy for renal transplant recipients with hepatitis C virus infection

Tamotsu Tojimbara[1], Jun Yashima[1], Hiroyuki Shirai[1], Satoshi Teraoka[1], Ichiro Nakajima[2], Shohei Fuchinoue[2].
[1]Transplant Surgery, International University of Health and Welfare, Atami, Japan; [2]Surgery in Kidney Center, Tokyo Women's Medical University, Tokyo, Japan.

Hepatitis C virus (HCV) infection contributes to unfavorable outcomes in renal transplant recipients. We evaluated the treatment outcomes of HCV-infected renal transplant recipients who received direct-acting antiviral (DAA) therapy.
Patients: We treated 2 HCV-RNA-(genotype 1b)-positive renal transplant recipients (Patients #1 and #2) who received daclatasvir (60mg/day) and asunaprevir (200mg/day). None of the patients had mutations (L31 and Y93) in the *NS5A* gene. Another recipient (Patient #3) with positive HCV (genotype 2b) was treated with sofosbuvir (400mg/day) and rivavirin (400mg/day). Patient #1 was a 60-year-old man with end-stage renal disease (ESRD) due to diabetic nephropathy. In 2009, he underwent living-donor renal transplantation from his wife. Following unsuccessful pegylated-interferon therapy, the patient received DAA treatment in 2014. Before DAA therapy, his HCV-RNA level was 6.0 log IU/mL. The patient underwent 8 weeks of DAA therapy, which was then discontinued because of deteriorating of allograft function. Patient #2 was a 55-year-old man with ESRD due to diabetic nephropathy. He had received a living-donor renal graft from his brother in 2007. DAA treatment was initiated 93 months after renal transplantation, in 2015. At the initiation of therapy, his HCV-RNA level was 6.9 log IU/mL. Patient #3 was a 46-year-old man with ESRD due to chronic glomerulonephritis. He underwent deceased donor transplantation in 2015. DAA treatment was initiated 4 months after renal transplantation, when the patient's HCV-RNA level was 7.4 log IU/mL.
Results: HCV-RNA was undetectable in all 3 patients within 8 weeks after the initiation of DAA therapy. All the patients, including Patient #1 who received DAA for 8 weeks, achieved sustained virological response 12 weeks after the therapy. Although the renal allograft function of Patient #1 deteriorated 8 weeks after DAA initiation (serum creatinine level increased from 2.9 to 4.1 mg/dl), his allograft function recovered when DAA therapy was stopped. No serious adverse effects, including liver and kidney allograft dysfunction, developed in the other patients during and after DAA therapy.
Conclusion: The DAA therapy was successful for treating our 3 HCV-infected renal transplant patients. Although physicians should be aware of the possibility of complex drug interactions, DAA therapy for HCV-positive renal transplant recipients may provide promising short-term outcomes. Further studies are needed to assess the long-term affects of DAA therapy on patient and allograft survival in this patient population.

P.1413

Right-sided laparoscopic live-donor nephrectomy: results and technical aspects

Pablo Troncoso, Javier Dominguez, Mireya Ortiz, Elizabeth Galdames, Sandra Vilches.
Urologia y Trasplante Renal, Pontificia Universidad Catolica de Chile, Santiago, Chile.

Introduction: Many surgeons still prefer the left kidney (LK) for laparoscopic live donor nephrectomy (LLDN) due to the fact that the renal vein is longer and in initial experiences right kidneys (RK) harvested by laparoscopy presented renal vein thrombosis, attributable to a short renal vein. In the open surgery era, the use of RK was typically around 25 to 30%. In some instances RK should be harvested, particularly when LK presents complex vascular anatomy or when RK has a non critical pathology and thus maintaining the principle of keeping the best kidney with the donor. The aim of this study was to evaluate our experience with Right-LLDN
Methods: All LLDN performed in our center were included in this analysis. Indications for use of the RK, surgical technique in donors and recipients, evolution and surgical complications were reviewed.
Results: In eighty-eight LLDN, twenty-two were of the RK (25%). Average age was 42 years in both, left and right donors. Indications for the use of RK were: two or more left renal arteries with only one right artery (13), left renal artery with a very early bifurcation (6), presence in the RK of a simple cyst or a small stone (2) and a smaller RK that the contralateral (1). Donor surgery was performed in the usual manner of a pure laparoscopic nephrectomy. Operative time was similar in right or left LLDN. Renal vein was controlled by vascular stappler or double hem-o-lok. One left donor was converted to open surgery because of intraoperative bleeding (1,1%). In most of the recipients the renal vein was long enough and the transplant was performed routinely. In six out of twenty-two recipients of a RK the renal vein was less than 15 mm in length and it was extended with donor gonadal vein harvested during the LLDN as we have described previously[1]. Other three patients required ligation of the internal iliac vein to improve exposure. Mean cold ischemia time, including graft preparation, was 108 mins (42-253) in LK and 80 mins (22-240) in RK (n.s.). This minimal difference was due to the vein extension. Mean time of vascular anastomosis was 39 (25-55) and 45 (27-60) mins for RK and LK; respectively (p-value: 0.029). In a recipient of a RK an area of ischemia in the upper pole was shown in the postoperative doppler ultrasound which was managed with anticoagulation without further progression. There were no other vascular complications or graft loss in this series. Recovery of renal function was excellent in all recipients. The average creatinine values in the recipients of a LK at discharge, 3 and 6 months were 1.06 ± 0.1 mg/dL, 1.23 ± 0.14 and 1.19 ± 0.1 and in the recipients of a RK were 0.99 ± 0.075, 1.286 ± 0.15 1.2 ± 0.13 (n.s.)
Conclusions: Selection of the appropriate kidney for donation should be based on the same criteria that traditionally governed open donor nephrectomy. The shorter renal vein may require additional techniques to facilitate vascular anastomosis. In this series, this strategy allowed to minimize vascular complications.
References:
[1] Troncoso P. Renal vein extension using gonadal vein: a useful strategy for right kidney living donor harvested using laparoscopy. Transplant Proc. 2009;41:82-84.

P.1414

Laparoscopic right donor nephrectomy: Analysis of different technique

Anant Kumar, Manoj Kumar.
Urology, Renal Transplantation and Robotics, Max Super Speciality Hospital, Delhi, India.

Introduction: Right laparoscopic donor nephrectomy (RLDN) is still not done in many centres due to short renal vein and high complication rates. Herein, we will describe various techniques to make it safe operations with good results.
Material and Methods: From 1999 till July 2015, we have done 120 right lap donor nephrectomy. Indications were left kidney with three arteries, some benign pathology in right kidney, low GFR and small stone in right kidney. To maximize the length of right renal vein, one of three modifications is done. The first involves use of a TA 30 stapler which fires two staple lines without cutting. The vein is subsequently cut flush with the staple line to gain extra length. The parallel orientation of the stapler in relation to the vena cava results in preservation of additional right renal vein length. The second modification involves open surgical division of right renal vein. A 5-6 cm right sub costal incision is made. A third modification is used in case of a short renal vein and a graft of the recipient's saphenous vein can be used to reconstruct the renal vein. Following modification were done to increase renal arterial length. The dissection of interaortocaval space to allow the division of renal artery at its origin from aorta. This man oeuvre allows for the division of right renal artery at its origin, rather than at lateral border of vena cava. Another technique to gain arterial length involves mobilizing the vena cava by dividing the lumbar veins. This allows IVC to be rolled anteriorly and the kidney to be placed in a 'flipped' position, increasing exposure of the right renal artery at its origin from the aorta. These techniques are useful in early division of right renal artery patients.
Results: There were 120 patients. Transperitoneal procedure was done in 56 cases, retroperitoneal in 42 and lap assisted in initial 22 cases. First 22 cases, Satinsky clamp was used for venous control.TA 30 staplers were used after that in 78 cases. Due to non availability of TA 30 stapler in India, large Hem-o-lok clip was used in last 20 cases. Mean operative time was 115 ± 20 minutes, average blood loss 180 ± 20 ml.WIT was 6 ± 2 min.In 6 cases there was elective conversion to open surgery. Bleeding from Small twig arising from right renal vein seen in 3 cases. In one case there was duodenal injury (serosal tear) which was recognized immediately and sutured. Liver injury was in one case managed conservatively with Surgical and Floseal.There was no graft thrombosis,graft loss and delayed graft function .
Conclusions: These modifications have made RLDN is a safe procedure with equivalent results to LLDN (left laparoscopic donor nephrectomy).

P.1415

Renal transplant in patients with abnormal bladder: Is the outcome same in developing country?

Anant Kumar, Rahul Yadav, Adittya Sharma, Vimal Dassi, Gulia Anil.
Urology and Renal Transplant, Max Super Speciality Hospital, Saket, India.

Purpose: To study the outcomes and challenges faced in a developing country in patients who underwent renal transplant with reconstructed or rehabilitated bladder.
Material and Methods: 14 patients, between January 2001 to August 2014, underwent renal transplantation in reconstructed or rehabilitated bladder. We did an observational retrospective study.
Results: Out of 14 patients, 11 had reconstructed bladder and 3 were on clean intermittent self- catheterization(CISC). Mean age, gender ratio, mean donor glomerular filtration rate, median HLA match percentage, mean warm and cold ischemia time was 22.5 years, 12:2,47.9ml/min, 50%, 5.3 mins and 28.7 mins respectively. Post-transplant, all patients had repeated UTI and 6 patients developed pyelonephritis. Mean(±sd) Serum creatinine at 7 days, 3months, 1 year,3 years and at last follow up was 0.9(±0.20), 1.58(±0.65), 1.92(±1.02), 2.47(±1.17) and 2.75(±1.29) mg/dl respectively.
Conclusion: In the available literature so far from western world, though the incidence of post-transplant complications is higher but long term graft function and graft survival was comparable to patients with native bladder. Graft dysfunction in such group of patients is higher in our series due to frequent attacks of UTI & pyelonephritis. Higher serum creatinine is predictive of graft failure and poor outcome. So, our results are less than optimal due to overall less optimal cleanliness and personal hygiene in the developing country.

P.1416

TRANSFORM: Everolimus with reduced calcineurin inhibitors vs mycophenolate with standard calcineurin inhibitors in de novo kidney transplant recipients - baseline data according to donor type

Yoshihiko Watarai, Stefan P.Berger.
On behalf of the TRANSFORM Investigators, Novartis, Basel, Switzerland.

Introduction: Kidney transplantation (KTx) from living or deceased donor type may have different impact on the graft and patient outcome[1],[2]. TRANSFORM is the largest prospective *de novo* KTx study to date, that will evaluate the efficacy and safety of everolimus (EVR)+reduced calcineurin inhibitor (rCNI), in both living and deceased donor KTx recipients (R), using a novel composite endpoint of anti-rejection efficacy and renal preservation. Here, we present the baseline characteristics of KTxRs stratified by donor type.
Method: TRANSFORM (NCT01950819) is an ongoing 24-month, multicentre, open-label study, wherein adult *de novo* KTxRs are randomized (1:1) to receive EVR+rCNI or mycophenolic acid (MPA)+standard (s) CNI, with induction+steroids. Primary objective is to evaluate the effect of EVR+rCNI vs MPA+sCNI on the composite endpoint of treated biopsy-proven acute rejection (tBPAR) or estimated glomerular filtration rate <50 mL/min/1.73m^2 at M12 post-Tx. Key secondary objective is to evaluate the composite efficacy failure (tBPAR, graft loss or death) at M12 and M24 post-Tx. Subgroup analysis (living donor, standard criteria- and expanded criteria- deceased donor) will be performed for primary endpoint.
Results: Patients are being recruited from 218 study centers across 43 countries. As of 04 January 2016, a total of 1,978 KTxRs have been randomized; of which 949 and 972 received grafts from living and deceased donors, respectively. The majority of KTxRs were male and Caucasian for both donor groups, and Asians received a higher proportion of grafts from living donors. The mean age was lower for living vs deceased donor KTxRs. At baseline, mean BMI, cause of renal failure (except for IgA nephropathy), mean PRA and HLA mismatches, were comparable between both donor groups. The majority of both groups received induction with basiliximab. A higher proportion of KTxRs in the living donor group were transplanted pre-emptively compared with deceased donor group **(Table)**.
Conclusion: This is the largest study in *de novo* KTxRs; it is also the largest study in living donor KTxRs so far. The study results will provide insight into the efficacy and safety of EVR+rCNI regimen in this diversified population which is representative of the global transplant population by including living as well as deceased donors.

Table: Patients demographics and baseline characteristics stratified by living- vs deceased-donor as of 04-January-2016

Parameters	Living donor (N=949)	Deceased donor (N=972)
Sex, n (%)		
Male	674 (71.0)	671 (69.0)
Race, n (%)		
Caucasian	651 (68.6)	762 (78.4)
Asian	218 (23.0)	45 (4.6)
Others[a]	80 (8.4)	165 (17.0)
Age (years), mean ± SD	44.97 ± 14.10 (N=939)	52.67 ± 13.37 (N=969)
BMI (kg/m^2), mean ± SD	25.05 ± 4.28 (N=944)	26.10 ± 4.14 (N=966)
End-stage disease leading to transplantation, n (%)		
Glomerular disease	155 (16.3)	157 (16.2)
IgA nephropathy	122 (12.9)	57 (5.9)
Diabetes mellitus	120 (12.6)	119 (12.2)
Polycystic disease	117 (12.3)	158 (16.3)
Hypertension/nephrosclerosis	103 (10.9)	135 (13.9)
Unknown	145 (15.3)	120 (12.3)
Others[b]	187 (19.70)	226 (23.25)
PRA (most recent evaluation), mean ± SD	1.9 ± 7.27 (N= 821)	2.9 ± 11.07 (N=893)
Number of HLA mismatches, n (%)		
Loci A		
0-1	691 (72.8)	646 (66.4)
2	235 (24.8)	320 (32.9)
Loci B		
0-1	599 (63.12)	568 (58.44)
2	327 (34.5)	397 (40.8)
Loci DR		
0-1	651 (68.6)	735 (76)
2	275 (29.0)	231 (23.8)
Cold ischemia time (hr), mean ± SD	2.0 ± 2.78 (N=923)	14.8 ± 5.77 (N=960)
Induction, n (%)		
Basiliximab	699 (73.7)	748 (77.0)
rATG	121 (12.8)	153 (15.7)
Missing	129 (13.6)	71 (7.3)
Current Dialysis, n (%)		
Hemodialysis	512 (54.0)	746 (76.7)
Peritoneal dialysis	137 (14.4)	174 (17.9)
None	290 (30.6)	47 (4.8)
Missing	10 (1.1)	5 (0.5)

[a]Includes Native American, Black, Pacific Islander, unknown, and missing data
[b]Includes pyelonephritis, drug-induced toxicity, interstitial nephritis, vasculitis, obstructive disorder/reflux, renal hypoplasia/dysplasia, and missing data
BMI, body mass index; hr, hours; HLA, human leukocyte antigen; PRA, panel reactive antibodies; rATG, rabbit anti-thymocyte globulin; SD, standard deviation

References:
[1] Nemati E *et al*. Does kidney transplantation with deceased or living donor affect graft survival?. *Nephro-urology monthly*. 2014; 6: e12182.
[2] Naderi GH *et al*. Living or deceased donor kidney transplantation: a comparison of results and survival rates among Iranian patients. *Transplantation Proceedings*. 2009; 41: 2772-2774.

P.1417

Comparison of live donor pretransplant renal volume and posttransplant recipient renal volume

Masaaki Yanishi[1], Hidefumi Kinoshita[1], Kenji Yoshida[1], Takao Mishima[1], Hisanori Taniguchi[1], Yoshihiro Komai[1], Kaneki Yasuda[1], Masato Watanabe[1], Motohiko Sugi[1], Hiroyasu Tsukaguchi[2], Tadashi Matsuda[1].
[1]Urology and Andrology, Kansai Medical University, Hirakata, Japan; [2]2nd Department of Internal Medicine, Division of Nephrology, Kansai Medical University, Hirakata, Japan.

Background: In the present study, we examined that live donor kidney and posttransplant kidney volumetry obtained with the use of Synapse Vincent System had been associated with posttransplant renal function. And secondary objective was to examine whether the growth rate has been varied depending on the difference of gender between donor and recipient.
Methods: The thirty live donors who underwent 3DCT in pretransplantation and the thirty recipients who underwent 3DCT in posttransplantation after 1 year in our hospital were retrospectively reviewed. There were calculated renal volume of donor (Dvol) and recipient (Rvol) from 3DCT. It was observed how much the graft volume changed after transplantation (Vol-ratio). And it was divided Dvol or Rvol by the recipient weight (Dvol/RWgt, Rvol/RWgt). We compared the correlation of Vol-ratio, Dvol/RWgt or Rvol/RWgt and posttransplant renal function after 1 year. We examined the rate of change in kidney volume and renal function by gender differences with donor and recipient.
Results: The mean pretransplant kidney volume of donor(Dvol) and posttransplant kidney volume of recipients(Rvol) were 141.7 and 178.4mL. The mean Vol-ratio was 127.3%. Dvol/RWgt and Rvol/RWgt showed respectively significant linear correlations with remaining renal function after 1 year(r=0.6745 p<0.0001, r=0.6458 p<0.0001). The group of female donor and male recipient had the larger growth rate in kidney volume (p<0.001) and the higher proteinuria (p<0.001).
Conclusions: The growth rate was different by the body mismatch of the recipient and donor. Furthermore the donated kidney volume and posttransplant graft volume were correlations to graft function after 1 year.

Fig. 2a

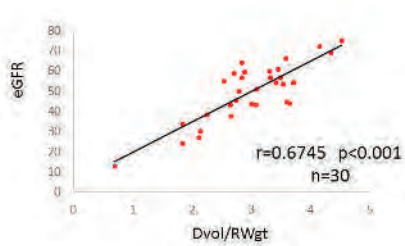
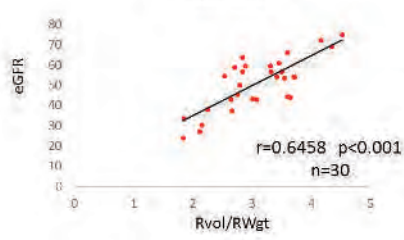


Fig. 2b



P.1418

Clinical outcomes in recipients of living donor kidney transplants from elderly donors

Takayoshi Yokoyama, Yu Kihara, Osamu Konno, Yuki Nakamura, Hitoshi Iwamoto, Shigeyuki Kawachi.
Digestive and Transplantation Surgery, Tokyo Medical University Hachioji Medical Center, Tokyo, Japan.

Introduction: Living donor kidney transplantation (LDKT) accounts for more than 90% of all kidney transplants in Japan. In accordance with the LDKT donor guidelines, donors are healthy individuals aged 20–70 years. However, LDKT from elderly donors is controversial because of uncertainty in graft function. We evaluated the clinical outcomes of LDKT recipients from elderly (≥60 years) donors.
Materials and Methods: Between January 2010 and December 2013, 69 patients underwent LDKT at our center. Patients were grouped into an elderly donor (ED; LDKT from donors ≥60 years old; n = 27) and a normal donor group (ND; LDKT from donors <60 years old; n = 42). We compared these 2 groups with respect to baseline data, graft function, immunosuppression, and pathological results using first year protocol biopsy.
Results: Patients in the ED and ND groups were 65.4 ± 4.1 years and 45.5 ± 10.0 years old, respectively. The donors' preoperative HbA1c (ED vs. ND: 5.9 ± 0.4 vs. 5.6 ± 0.4 mL/min, respectively; P = 0.012), the estimated glomerular filtration rates (ED vs. ND: 79.7 ± 13.3 vs. 93.6 ± 18.3 mL/min, respectively; P = 0.002), and the creatinine clearance (ED vs. ND: 91.8 ± 17.5 vs. 113.9 ± 19.9 mL/min, respectively; P < 0.001) were, however, significantly different between the 2 groups. In the immunosuppression data, the proportion of patients who were steroid-free within 1 year after the LDKT (ED vs. ND: 8/27 [29.6%] vs. 23/41 [56.1%], respectively; p ¯= 0.028) was significantly different between the 2 groups. There was no significant difference in the number of patients who required medication for acute rejection (ED vs. ND: 7/27 [25.9%] vs. 5/41 [12.2%], respectively; p = 0.130) and for cytomegalovirus infections (ED vs. ND: 8/27 [29.6%] vs. 5/41 [12.2%], respectively; p = 0.071) within 1 year after the LDKT. Although serum creatinine levels within 2 year after the LDKT were significantly higher in the ED than in the ND group, levels during the 3rd year were no longer significantly different (ED vs. ND at 2 years: 1.72 ± 0.42 vs. 1.23 ± 0.45, respectively; P = 0.001; 3 years: 1.61 ± 0.35 vs. 1.45 ± 0.57, respectively; P = 0.289). Banff classification scores were not significantly different between the 2 groups
Conclusion: In this study, LDKT from elderly donors was found to be relatively safe. As expected, initial lower renal function was found in the ED group; however, serum creatinine level at 3 years after LDKT was comparable to that in the ND group. Careful selection of elderly donors and delicate following up may result in successful transplant outcomes.

P.1419

Transplant glomerulopathy causes graft loss despite good early function

Ulkem Cakir[1], Gokcen Alis[1], Ozgur Cavdaroglu[2], Turker Erturk[2], Ibrahim Berber[2].
[1]Transplant Center, Nephrology, Acibadem University, Istanbul, Turkey; [2]Transplant Center, General Surgery, Acibadem University, Istanbul, Turkey.

Background: After the first year following kidney transplantation, 1–5% of grafts fail eachyear reaching to 20% graft loss at the end of the fifth year. This study aimed to analyze the results of biopsies of kidney transplants to identify the grafts with progressive decline in allograft function.
Methods: The study included 132 adult kidney transplant patients transplanted at Acibadem International Hospital between 10/2010 and 10/2015 with a mean graft survival and follow-up of 32 (14-51) months. The MDRD equation for estimating glomerular filtration rate (eGFRMDRD) was used to calculate the slope of renal function over time using all available serum creatinine values between 1 and 5 years post-transplant.
Results: The minority of transplants (43 patients) had a eGFRMDRD < 40 ml/min and majority of them (89 patients) had good function (eGFRMDRD ≥ 40 ml/min) at 1 year with positive eGFRMDRD slope between 1 and 5 years post-transplant. The diagnosis was 'Transplant Glomerulopathy' (TG) in 22 out of 132 biopsies; 18 of them were in the low GFR group while 4 of them were in the high GFR group. Among 18 patients with TG in the low GFR group 3 of them progressed to graft loss in the follow-up which may be acceptable. However, 4 patients with TG in the high GFR group surprisingly exhibited a strongly negative eGFRMDRD slope between 1 and 5-years suggestive of progressive loss of graft function.
Conclusion: This pattern of progressive decline even in eGFR despite good early function was thought to be associated with factors suggestive of enhanced anti-donor immunity. The strong link between TG and chronic antibody mediated rejection suggests a major role for HLA antibodies in TG formation leading to graft loss.

P.1420

Single port donor nephrectomy with vaginal extraction: A novel composite approach in living donor nephrectomy

Ozgur Cavdaroglu[1], Turker Erturk[1], Gokcen Alis[2], Ulkem Cakir[2], Ibrahim Berber[1].
[1]Transplant Center, General Surgery, Acibadem University, Istanbul, Turkey; [2]Transplant Center, Nephrology, Acibadem University, Istanbul, Turkey.

Background: Minimally invasive new laparoscopic techniques have recently been described as reducing morbidity by avoiding surgical incisions and external scars. Here we would like to present the technical aspects and outcomes of 'single port donor nephrectomy with vaginal extraction' which can be considered as a novel surgical approach for female living donors.
Methods: Six female living donors (mean age: 38.7 years, body-mass index: 26.6 kg/m²) with a history of at least one spontanous vaginal delivery were operated via this composite approach.Transumbilical laparoscopic donor nephrectomy was performed using an SILS™ port (Covidien, Inc., Norwalk, CT) and the kidney was removed transvaginally with an Endobag (Covidien, Mansfield, Massachusetts) inserted via a posterior colpotomy. We evaluated the following parameters: warm and cold ischemia time, duration of the operation, amount of blood loss during the operation, duration of hospitalization, creatinine levels of both donor and their recipients, and visual analogue scale score for pain at discharge.
Results: Left nephrectomy was performed for all of the donors. The means for the duration of the operation, warm ischemia time and hospital stay were 170 min, 155 s, 2 days, respectively. The mean length of external incision was 2,5 cm and low pain score was satisfying. No need for conversion to laparotomy or re-operation was observed. Besides the creatinine levels of all the donors and their recipients' were found in normal ranges.
Conclusion: 'Single port donor nephrectomy with vaginal extraction' can be recommended in selected women due to more desirable cosmetic results without jeopardizing the safety of the donors along with excellent recipient results.

P.1421

When to abort a live donor laparoscopic nephrectomy?

Linda Chen, Zhobin Moghadamyeghaneh, Gaetano Ciancio, George Burke, Adela Mattiazzi, Giselle Guerra.
Miami Transplant Institute, University of Miami/ Jackson Memorial Hospital, Miami, United States.

Laparoscopic nephrectomy is the preferred method for procuring kidneys from live kidney donors (LKD). It is safe and can be done with minimal long term risks to the LKD. The LKD undergoes a complete medical, psycho-social, radiological and laboratory evaluation as per UNOS guidelines. This includes evaluation by a transplant nephrologist and a transplant surgeon. Exclusion criteria at our center includes obesity (BMI>35) and existing liver disease. We present two cases of incidentally discovered liver disease, specifically hepatic fibrosis in LKD at the time of laparoscopic donor nephrectomy resulting in aborting the procedure.
Case 1: 55 yo Hispanic male, BMI 28.6, otherwise healthy with past medical history significant for hepatitis and jaundice at age 15. Past medical history was positive for gastric ulcer at age 30. Family history revealed a mother who died from cirrhosis in her seventies and brother who underwent a liver transplant at age 50. Social history: Alcohol < 8 times/year. Surgical history indicated past appendectomy. Review of systems was negative for liver disease. Physical exam demonstrated no evidence of portal hypertension or ascites. Medical work up revealed no evidence of hepatitis A, B or C, normal PT, PTT and INR, normal liver functions, albumin and platelet count. Iron studies were normal. Preoperative CT angiogram showed no splenomegaly or fibrosis (Fig 2). The patient was found to have visual evidence of liver disease intraoperatively (Fig 1) at the time of laparoscopic donor nephrectomy. Liver biopsy was performed. Frozen section showed 60% macrosteatosis and 80% microsteatosis. Portal and periportal fibrosis with bridging and early nodule formation, estimated at Stage 3 or higher, mild portal triaditis. The live kidney donor surgery was therefore aborted.
Case 2: 47yo Caucasian female, BMI 18.6, otherwise healthy with past medical history significant for constipation and dysmennorhea. No previous surgery. Medications -oral contraceptives. Social history: no alcohol or tobacco >3years. Patient previously worked as a bar tender. Review of systems was negative for liver disease. Physical exam demonstrated no evidence of portal hypertension or ascites. Medical work up revealed no evidence of hepatitis A, B or C, normal PT and INR, normal liver functions and platelet count. Preoperative CT angiogram showed no splenomegaly or fibrosis. The patient was found to have visual evidence of liver disease intraoperatively at the time of laparoscopic donor nephrectomy. Liver biopsy was performed. Frozen section showed fibrosis with bridging and possible early nodule formation, minimal portal triaditis. The live kidney donor surgery was therefore aborted.
Incidental findings during laparoscopic donor nephrectomy can pose significant medical and ethical dilemmas. Thus highlighting the importance of a thorough initial general inspection of the abdomen on introduction of the laparoscope to rule out unexpected donor pathology during laparoscopic donor nephrectomy prior to proceeding with dissection.

Figure 1: Laparoscopic image of liver

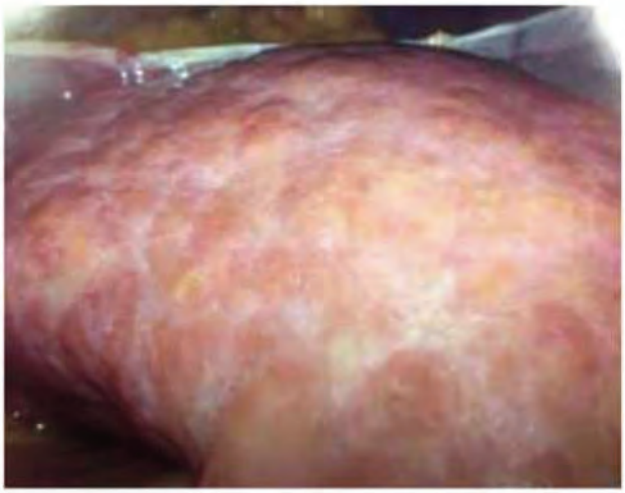
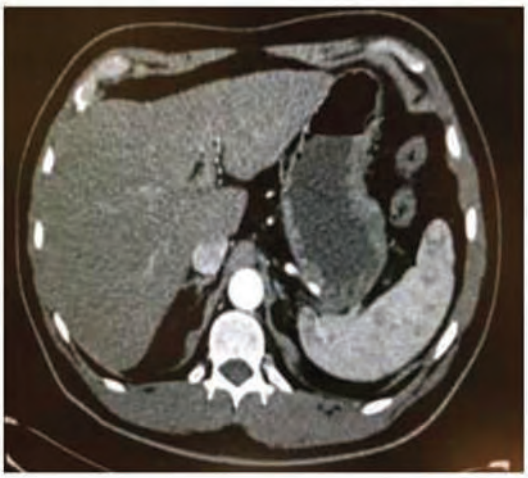


Figure 2: Preoperative CT scan



P.1422

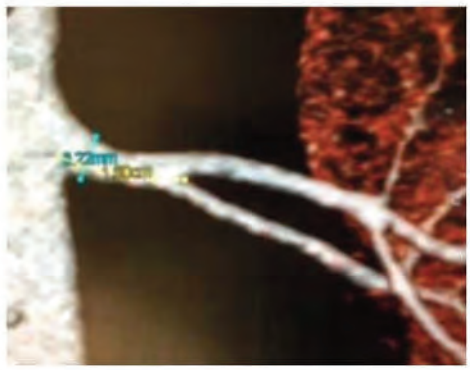
New application of powered vascular stapler for laparoscopic live kidney donor nephrectomy

Linda Chen, Zhobin Moghadamyeghaneh, Giselle Guerra, George Burke, Gaetano Ciancio.
Miami Transplant Institute, University of Miami/ Jackson Memorial Hospital, Miami, United States.

Laparoscopic Live Donor Nephrectomy technique has become the standard of care at our institution. Laparoscopic stapling technology has continued to advance over time. Our institution performed more than 500 laparoscopic live donor nephrectomies with no conversions to open operation, no blood transfusion, no reoperations, and an average hospital stay of 2 days using vascular staplers to staple the renal vessels. More than 100 of these laparoscopic nephrectomy cases were safely performed using the 9.6mm width Echelon Flex TM powered 35mm articulating vascular stapler which is an endoscopic linear vascular stapler which delivers six rows of staples and 430angle. However, a newer stapler- the Echelon Flex TM powered articulating vascular stapler with specific 2 row design is more specific when compared to the conventional 3 row stapler. The narrower shaft (7mm) and curved blunt tip anvil allows for improved maneuverability and greater flexibility offering 11% more articulation (500) in encircling the renal artery. This stapler has been used successfully in 10 patients at our center with no conversions to open operation, no blood transfusion, no reoperations, and an average hospital stay of 2 days at 6 months follow up.

Figure 1

Figure 2



P.1423

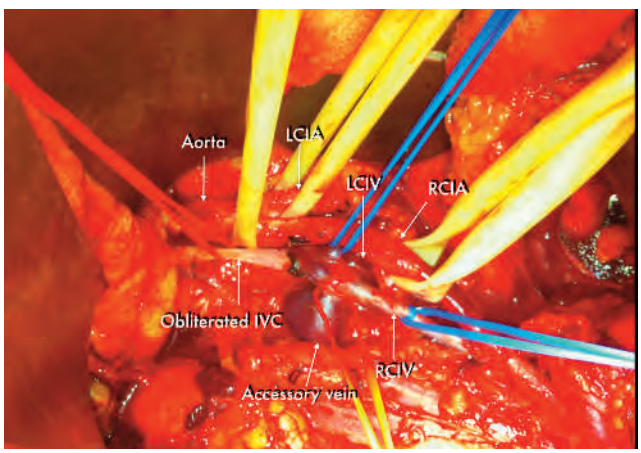
Successful use of a collateral vein in renal transplantation - First case report in an adult recipient

Vincent J.M. Chia, Linda J. Chen, Gaetano Ciancio, George Burke. Miami Transplant Institute, University of Miami/ Jackson Memorial Hospital, Miami, United States.

Introduction: Renal transplantation in the absence of a patent inferior vena cava (IVC) poses a formidable l challenge to the surgeon. It is an uncommon but known entity[1]. Various options have been reported in the pediatric population[2] .

Material: We present a 47 year old lady with ESRD from Adult polycystic kidney disease. She did not have any risk factors for IVC thrombosis. She had a cPRA of 88% and she was receiving IVIG before transplant. Intraoperatively, there was an obliterated IVC with prominent pelvic collaterals. We anastomosed the renal vein to a collateral vein at the level of the bifurcation of the common iliac veins. (Figure 1). The renal artery was anastomosed to the common iliac artery. Upon reperfusion, the kidney had good colour and turgour. The urinary drainage was accomplished with ureteroneocystostomy. She had thymoglobuin, basiliximab, and steroids for induction and was maintained on mycofenolate Mofetil and Tacrolimus. Her recovery was uneventful. Preoperative creatinine decreased from 6.73 mg/dl to 3.86 mg/dl on her discharge (POD5) .

Discussion: IVC thrombosis is associated with hypercoagulable state, nephrotic syndrome, eritoneal dialysis usage and previous catetherisation[3]. It can be asymptomatic and some recommend routinely screen in small children prior to transplantation[4]. Systemic venous drainage include the infra hepatic IVC, gondal vein, collateral vein and orthotopic transplant. In orthotopic transplant, the native nephrectomy is performed followed by anastomosis to the native renal vein or infrahepatic IVC[5]. Portal venous system drainage include using the portal vein, splenic vein(SV), inferior or superior mesenteric vein (IMV/SMV) . There may be an immunogenic benefit of using the portal venous system[6]. The IMV may be of small calibre and the vessel wall is thin. Rarely, when other sites are not available, the portal vein can be used with success[7]. In choosing a site for venous outflow, one should assess the adequacy of venous return, as this may lead to impaired function and even risk graft thrombosis[8] Two cases of using pelvic collaterals with success have been reported in pediatric recipients[2],[9]. In these cases, preoperative imaging showed adequate drainage of the pelvic veins. In our patient,the collateral vein was of adequate calibre and hence the anastomosis was performed at that level. Postoperatively, the patient had some slow graft function but she did not require dialysis. Her SCr was normal on followup in 2 weeks. This rare case adds to the growing notion that IVC thrombosis is not an absolute contraindication for renal transplantation.



Conclusion: The presence of unsuspected IVC thrombosis in renal transplantation is particularly challenging. Careful assessment of the various options for venous drainage helps in optimising the chance for a successful transplantation. The collateral system if suitable for anastomosis, is an option.

References:

[1] Eneriz-Wiemer M, Sarwal M, Donovan D, Costaglio C, Concepcion W, Salvatierra O, Jr. Successful renal transplantation in high-risk small children with a completely thrombosed inferior vena cava. Transplantation. 2006 Nov 15;82(9):1148-52. PubMed PMID: 17102765

[2] Stippel DL, Bangard C, Schleimer K, Koerber F, Beckurts KT, Hoppe B. Successful renal transplantation in a child with thrombosis of the inferior vena cava and both iliac veins. Transplantation proceedings. 2006 Apr;38(3):688-90. PubMed PMID: 16647445.

[3] Thomas SE, Hickman RO, Tapper D, Shaw DW, Fouser LS, McDonald RA. Asymptomatic inferior vena cava abnormalities in three children with end-stage renal disease: risk factors and screening guidelines for pretransplant diagnosis. Pediatric transplantation. 2000 Feb;4(1):28-34. PubMed PMID: 10731055.

[4] Yata N, Nakanishi K, Uemura S, Ishikura K, Hataya H, Ikeda M, et al. Evaluation of the inferior vena cava in potential pediatric renal transplant recipients. Pediatric nephrology. 2004 Sep;19(9):1062-4. PubMed PMID: 15206028

[5] Gil-Vernet JM, Gil-Vernet A, Caralps A, Carretero P, Talbot-Wright R, Andreu J, et al. Orthotopic renal transplant and results in 139 consecutive cases. The Journal of urology. 1989 Aug;142(2 Pt 1):248-52. PubMed PMID: 2664220

[6] Pirenne J, Benedetti E, Kashtan CE, Lledo-Garcia E, Hakim N, Schroeder CH, et al. Kidney transplantation in the absence of the infrarenal vena cava. Transplantation. 1995 Jun 27;59(12):1739-42. PubMed PMID: 7604445.

[7] Cauley RP, Potanos K, Fullington N, Lillehei C, Vakili K, Kim HB. Reno-portal anastomosis as an approach to pediatric kidney transplantation in the setting of inferior vena cava thrombosis. Pediatric transplantation. 2013 May;17(3):E88-92. PubMed PMID: 23461835.

[8] Patel P, Krishnamurthi V. Successful use of the inferior mesenteric vein for renal transplantation. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2003 Aug;3(8):1040-2. PubMed PMID: 12859544.

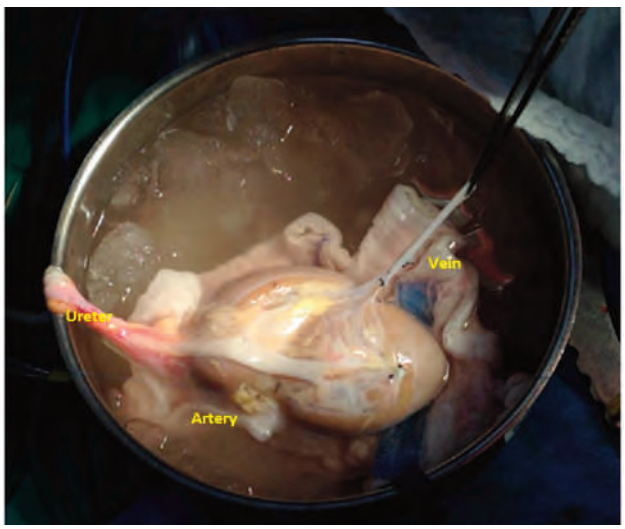
[9] Hajivassiliou CA, Wilkinson AG, Azmy A. Renal transplantation in a child with iliac vein thrombosis and absence of superior and inferior venae cavae. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 1997 Jun;12(6):1269-70. PubMed PMID: 9198068.

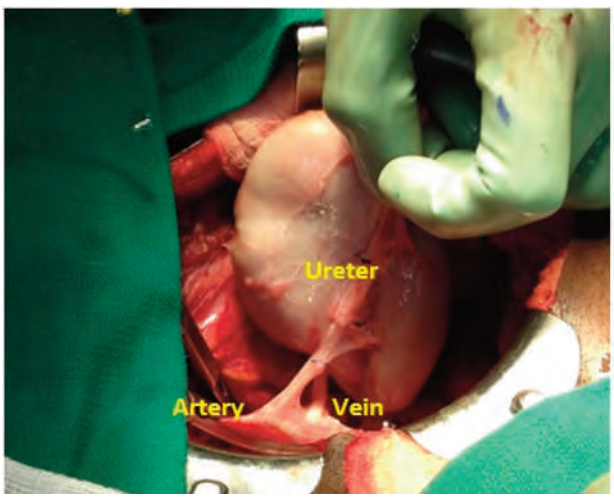
P.1424

Using kidneys with a congenital anomalies for transplantation: A judgement call

Vincent J.M.Chia, George Burke, Linda J. Chen, Gaetano Ciancio.
Miami Transplant Institute, University of Miami/ Jackson Memorial Hospital, Miami, United States.

Introduction: Shortage of organs remains a major obstacle in transplantation world wide. National registry reveals that up to 17.9% and 12% of kidneys are discarded in the US and UK respectively[1],[2]. We report the case of a kidney with congential abnormalities that was successfully transplanted.
Material: We present the case of a 30 year old unsensitised patient with ESRD secondary to HIV nephropathy. He was diagnosed with HIV in 2005 and was on HAART treatment. His CD4 count was 1034 and his viral load was undectable. He was on dialysis for 1 year prior to his transplant. The donor was a 17 year old man who died of head injury with a KDPI of25% and terminal creatinine of 1.4. The anatomy is shown in Figure 1/2. The ureter was on the anterior face of the kidney. Posteriorly there were 2 renal arteries on a patch. The left renal vein was originating from the opposite surface from the artery under the renal pelvis. The anastomosis was performed to the external ililac vessels. The allograft was making urine upoon reperfusion. Preoperative Scr was 17.7 mg/dL and was 12.14 mg/dL and 8.63mg/dL on subsequent 2 days.He made an uneventful recovery .





Discussion: In the US, 7.1% of discarded kidneys are due to anatomical abnormalities (1). For this category, the discard rate for kidneys with KDPI more and less than 85% is 8% and 6% respectively[3]. This implies a bias towards kidney with abnormal anatomy regardless of KDPI. These kidneys may require complex backtable reconstruction or novel ways to position the allografts to prevent technical failures[4]. This is examplified by successful transplants with horse shoe and even pelvic kidneys[5],[6]. However, not all such kidneys can be used as they may have abnormal collecting systems that predispose to urinary stasis and its inherent problems[7] .
Conclusion: This case illustrates that kidney with congenital anomalies can be technically transplanted with success. Judicious use of these kidneys will lead to more organs transplanted
References:
[1] Matas AJ, Smith JM, Skeans MA, Lamb KE, Gustafson SK, Samana CJ, et al. OPTN/SRTR 2011 Annual Data Report: kidney. Am J Transplant. 2013 Jan;13 Suppl 1:11-46. PubMed PMID: 23237695.
[2] Callaghan CJ, Harper SJ, Saeb-Parsy K, Hudson A, Gibbs P, Watson CJ, et al. The discard of deceased donor kidneys in the UK. Clin Transplant. 2014 Mar;28(3):345-53. PubMed PMID: 24506794
[3] Reese PP, Harhay MN, Abt PL, Levine MH, Halpern SD. New Solutions to Reduce Discard of Kidneys Donated for Transplantation. J Am Soc Nephrol. 2015 Sep 14. PubMed PMID: 26369343
[4] Santangelo M, Clemente M, De Rosa P, Zuccaro M, Pelosio L, Caggiano L, et al. The finding of vascular and urinary anomalies in the harvested kidney for transplantation. Transplant Proc. 2007 Jul-Aug;39(6):1797-9. PubMed PMID: 17692616.
[5] Corcoran AT, Shapiro R, Kayler LK. Transplantation of a horseshoe kidney. Transplantation. 2007 Mar 27;83(6):828-9. PubMed PMID: 17414722.
[6] Goldsmith PJ, Marco M, Hussain Z, Newstead C, Lodge JP, Ahmad N. Kidney transplantation using the inferior epigastric vessels for multiple anastomoses from a pelvic kidney. J Am Coll Surg. 2009 Jul;209(1):e1-3. PubMed PMID: 19651053.
[7] Luke PP, McAlister VC, Jevnikar AM, House AA, Muirhead N, Cross J, et al. Use of a pelvic kidney for living transplantation: case report and review of the literature. Am J Transplant. 2003 Feb;3(2):235-8. PubMed PMID: 12603220.

P.1425

Clinical utility of surveillance kidney transplant biopsies at one year post-transplantation: a long-term survival comparison among renal transplant recipients.

Grégoire Couvrat-Desvergnes[1,2], Angelina Dion[3], Christophe Legendre[4], Emmanuel Morelon[5], Nassim Kamar[6], Valerie Garrigue[7], Marc Ladriere [8], Magali Giral[2,9], Etienne Antan[10].
[1]Nephrology, CHD La Roche sur Yon, La Roche sur Yon, France; [2]INSERM UMR 1064, University Nantes, Nantes, France; [3]Biostatistics, University Nantes, Nantes, France; [4]Nephrology, CHU Paris Necker, Paris, France; [5]Nephrology, CHU Lyon, Lyon, France; [6]Nephrology, CHU Toulouse, Toulouse, France; [7]Nephrology, CHU Montpellier, Montpellier, France; [8]Nephrology, CHU Nancy, Nancy, France; [9]ITUN, CHU Nantes, Nantes, France; [10]EA-SPHERE Laboratory EA 4275 – Biostatistics, Clinical Research and Pharmaco-Epidemiology., University Nantes, Nantes, France.

Introduction: Kidney transplantation centers realized surveillance biopsies at one year post-transplantation, nevertheless clinical utility of practicing these biopsies does not appear an evidence and remains debated. The main objective of this study was to compare long-term patient and graft survival given between patients with or without a surveillance biopsy at one year post-transplantation.
Materials and Methods: From the French observational DIVAT cohort, we studied 1573 kidney recipients, between 2005 and 2014, from deceased donor, with a functioning and stable graft at one year post-transplantation. The main judgment criteria was the time to graft failure defined as a return-to-dialysis or a death with a functioning graft. We performed a multivariate Cox model to study graft failure risk given the one-year surveillance biopsy status.
Results: 804 recipients (51%) were patients from transplantation centers which systematically proposed surveillance biopsy while 769 patients (49%) had not one. The graft failure survival probabilities at 4 and 8 years were 92% (95% CI=[89;94%]) and 68% (95% CI=[62;75%]) among patients from transplantation centers using surveillance biopsy and 93% (95% CI=[90;95%]) and 74% (95% CI=[68;80%]) among patients without one year surveillance biopsy. From the multivariate survival model, patients from transplantation centers using surveillance biopsy had the same risk of graft failure than those who did not have one (HR=1.19; p=0.259), independently of adjustment variables (donor age, MDRD level, comorbidity or immunization).
Conclusion: Practicing one-year surveillance biopsies may possibly not provide benefit on patient/graft long-term survival and could be realized only in patients selected patients with high risk of graft failure.

P.1426

Borderline changes on dysfunctional renal allograft biopsies: Clinical relevance in a live related renal transplant setting

Muhammad Mubarak[1], Tahir Aziz[2], Mirza Naqi Zafar[1], Anwar Naqvi[3], Adib Rizvi[3].
[1]Pathology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [2]Nephrology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [3]Urology and Transplantation, Sindh Institute of Urology and Transplantation, Karachi, Pakistan.

Introduction: Borderline infiltrates on dysfunctional graft biopsies pose significant challenges to the transplant pathologists and physicians in the management.
Objective: To determine the clinical significance of borderline lymphocytic infiltrates on indicated renal allograft biopsies in a live related renal transplant setting.
Methods: The study was conducted at the histopathology department of Sindh Institute of Urology and Transplantation (SIUT). A retrospective review of case files of 421 renal transplant patients was carried out from October 2007 to September 2008. The biopsies were reported according to the Banff criteria.
Results: The mean age of recipients was 26.92 ± 9.14 years and of donors 38.46 ± 9.16 years. The males were predominant among the recipients (84.6 vs. 15.4%), and females among the donors (57.7 vs. 42.3%).
The best serum creatinine levels were 1.79±1.15 mg/dl. These were achieved after a median of 3 days (IQR: 2-7.25 days). The dysfunctional biopsies exhibiting borderline infiltrates were done at a median duration of 5.5 days (IQR: 3-14.25 days). The mean serum creatinine at the time of biopsies was 2.34± 1.43 mg/dl (range: 1.25-86.25 mg/dl). The biopsies showed borderline cellular infiltrates. All except one received antirejection treatment (five received ATG, one escalation of prograf dosage and rest pulse steroids) and all responded with a decline in serum creatinine toward baseline level, with the mean serum creatinine of 1.31±0.42 mg/dl (range: 0.40-2.71 mg/dl). This was achieved at a median duration of 9.73±5.32 days after starting treatment.
Conclusion: The borderline cellular infiltrates on dysfunctional renal allograft biopsies signify evolving phases of acute cellular rejection and respond favorably to antirejection treatment in our setting.

P.1427

Renal allograft biopsy findings in 475 pediatric live related renal transplant patients from a single center

Muhammad Mubarak[1], Tahir Aziz[2], Mirza Naqi Zafar[1], Anwar Naqvi[3], Adib Rizvi[3].
[1]Pathology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [2]Nephrology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [3]Urology and Transplantation, Sindh Institute of Urology and Transplantation, Karachi, Pakistan.

Introduction: There is scanty data in literature on the renal allograft biopsy findings in pediatric renal transplant patients.
Objective: This study was carried out to determine the pattern of renal allograft pathology in children with live related renal transplants at a single center in Pakistan.
Methods: A retrospective study of 511 renal allograft biopsies from 475 pediatric renal transplant patients (<18 years) was carried out from June 1986 till December 2011. The demographic, clinical, laboratory, and biopsy findings were collected and analyzed according to three eras of study period as: era 1 (1986-1995), era 2 (1996 to 2005) and era 3 (2006 to 2011).
Results: The mean age of recipients and donors was 14.82±2.9 and 37.18±9.1 years respectively. The male to female ratio of recipients and donors was 2.3:1 and 0.66:1 respectively.
Acute rejection was seen in 118 (23%) cases, cyclosporine toxicity in 107 (21%), and mild nonspecific changes in 116 (22.7%) cases. Acute rejection declined progressively from 48% in first era to 35% in 2nd to 17% in 3rd era. Interstitial fibrosis/tubular atrophy (IFTA) with variable degree of tubular atrophy was seen in 130 (25.4%) cases. Twenty one cases of acute pyelonephritis (4.1%) were detected in graft biopsy. A number of rare lesions included nine cases of infarction, six of recurrent/de novo glomerulopathy, three of oxalosis and one of polyoma virus nephropathy.
Conclusion: The study defines the causes of graft dysfunction on dysfunctional graft biopsies in a large cohort of live related pediatric renal transplant patients. The incidence of acute rejection declined progressively over the three eras of study.

P.1428

Renal allograft with calcium oxalate deposition: Its association with urinary tract infection and development of interstitial fibrosis

Handan Özdemir[1], Gökce Özdemir[1], Sebnem Ayva[1], Nurhan Özdemir[2], Pelin Börcek[1], Gökhan Moray[3], Mehmet Haberal[3].
[1]Pathology, Baskent University, Ankara, Turkey; [2]Nephrology, Baskent University, Ankara, Turkey; [3]Transplant Surgery, Baskent University, Ankara, Turkey.

Aim: The interaction between calcium oxalate (CaOx) deposition and urinary tract infection (UTI) is not well established. The aim of this study were two fold; first to identify the association between CaOx deposition and UTI and second to determine the role of CaOx deposition on the development of interstitial fibrosis (IF).
Material and Method: Renal allograft biopsies of 967 patients whom were transplanted between 1990 and 2010 were reviewed to identify those with CaOx deposition. Medical files of patients reviewed and all follow-up and indication biopsies of patients were evaluated for the degree of CaOx deposition and for the development of IF.±
Results: Among 962 patients, only 27 (2,8%) patients had CaOx deposits in their biopsies. Of 27 patients 7 had primary oxalosis (PO) and 20 had secondary oxalosis (SO). The time of CaOx deposition in allograft was found 1,1 ±0,37 months and 43,9±31 months in patients with PO and SO respectively. Significant difference was found between two groups ($p<0.01$). Among 27 patients 7 had tubulointerstitial nephritis (TIN) (25,9%), 4 had only UTI (14,8%) and 2 had both TIN and UTI (7,4%) at the time of CaOx deposition. The cause of TIN was secondary to bacterial infection in 4 cases and secondary to viral infection in 5 cases (Adenovirus: 2, CMV:1, Polyoma virus: 2). E. Coli was identified in all cases with UTI. Patients with UTI showed increased CaOx depositions in their follow-up biopsies. The time of the development of IF after CaOx deposition in allograft was 3,5±4,3 months and 10,2±4,2 months in patients with PO and SO respectively ($p=0.01$). Graft loss after CaOx deposition was 9,2±9,8 months in cases with PO and it was 21,8±12,2 months in cases with SO ($p<0.05$). Among PO patients, 1-, 2- and 5-year kidney graft survival was 43%, 28% and 0%. Whereas 1-, 2- and 5-year kidney graft survival was 100%, 100% and 67% in SO patients.
Conclusion: **We suggested that the presence of CaOx deposits increases the risk UTI and TIN. We also suggested that when both CaOx deposits and E.coli** were present, CaOx deposition has tendency to increase in allograft. In addition we also showed that CaOx deposition had a great influence on the development of IF and therefore negative impact on graft survival.

P.1429

Ultrastructural findings and capillary HLA-DR expression in renal allografts with humoral, vascular and tubulointerstitial rejection: Correlation with development of transplant glomerulopathy

Handan Özdemir[1], Nurhan Özdemir[2], Mehmet Haberal[3].
[1]Pathology, Baskent University, Ankara, Turkey; [2]Nephrology, Baskent University, Ankara, Turkey; [3]Transplant Surgery, Baskent University, Ankara, Turkey.

Introduction: The impact of early ultrastructural (US) changes of biopsies with the diagnosis of acute rejection that is causing transplant glomerulopathy (TG) was evaluated in very few studies. Relationship of capillary HLA-DR expression with the parameters that give rise to develop TG were not studied. With this purpose we evaluated the early and late US changes of 52 renal allografts and we compared these findings with the results of capillary HLA-DR expression.
Material and Method: Acute tubulointerstitial rejection (ATR), acute vascular rejection (AVR), acute humoral rejection (AHR), and chronic humoral rejection (CHR) were found in 12 (23%), 12 (23%), 14 (27%) and 14 (27%) patients, respectively. All biopsies (n:38) except cases with CHR (n:14) were taken within 3 months of Tx. Peritubular capillary HLA-DR (PTC-DR) and glomerular HLA-DR (GDR) expression was evaluated. Lower intensity of PTC-DR was considered to indicate more extensive PTC destruction. Follow-up biopsies of 38 cases with variable AR were evaluated for the development of TG.
Results: US changes including glomerular and PTC endothelial swelling and multilamellation, glomerular subendothelial widening and early GBM duplication was found to be highest in biopsies with AHR compared to biopsies with ATR and AVR ($p<0.001$). Biopsies with AVR and ATR were followed AHR respectively for the presence of these US changes. Biopsies with CHR showed highest degree of multilamellation and double counter. The loss of PTC-DR expression therefore the destruction of PTC was found highest in biopsies with AHR and CHR compared to biopsies ATR and ACR ($p<0.001$). TG was developed 8,3%, 33,3% and 57,1% in patients with ATR, AVR and AHR respectively ($p=0,01$). The development of TG was 45,5±9,2 months in ATR cases, 20,7±8 months in AVR cases and 7,2±3 in AHR cases ($p<0.001$). Glomerular and PTC endothelial swelling and multilamellation, glomerular subendothelial widening and early GBM duplication showed a great impact on the development of TG ($P<0.01$ for all). GDR showed positive relationship with development of TG ($p<0.001$). PTC-DR had a negative association with TG ($p<0.001$). The risk of TG development increases with decreasing expression of PTC-DR ($p<0.001$). Therefore the severity PTC destruction had a significant impact on the development of TG. In addition PTC-DR and GDR had significant association with all US findings ($p<0.01$ for all).
Conclusion: Analysis of early US changes and capillary HLA-DR expression are helpful for predicting the development of TG and graft prognosis. This type of assessment may be useful for determining the patients' with the risk of TG and chronic rejection and thus identifying the most appropriate treatment.

P.1430

What is the role of cytomegalovirus on interstitial fibrosis and graft survival?

Handan Özdemir[1], Nurhan Özdemir[2], Esra Esra Baskın[2], Gonca Özgün[1], Gökce Özdemir[1], Mehmet Haberal[3].
[1]Pathology, Baskent University, Ankara, Turkey; [2]Nephrology, Baskent University, Ankara, Turkey; [3]Transplant Surgery, Baskent University, Ankara, Turkey.

Aim: The aim of this study is twofold: first to clarify the influence of the cytomegalovirus (CMV) on the promotion of angiogenesis in renal allografts, second to show how CMV and angiogenesis association effects the development of interstitial fibrosis (IF) and graft survival.
Material and Methods: All indicated and follow-up biopsies of 85 patients with a mean age of 29.6±11 years at the time of transplantation were evaluated. Of 85 patients 29 had CMV infection (Group 1) and 56 had no CMV infection and used as a control group (Group 2).The microvessel density (MVD) was high lightened by CD34 immunostaining. Tubular and interstitial expressions of VEGF and density of macrophages in the interstitium were examined. Follow-up biopsies of all patients were evaluated for the development of IF.
Results: Total 26 (89.7%) patients in group 1 showed acute rejection (AR) episode at least one time, while only 36 (64.3%) patients in group 2 showed AR ($p=0.01$). Statistically significant more AR episodes were found to be occurred in group 1 (1.52±0.9) compared to group 2 (0.98±0.9) patients ($p=0.014$). MVD and macrophage infiltration was positively correlated with VEGF expressions ($p<0.001$). A significant difference in tubular and interstitial VEGF expressions were found between patients with group 1 and group 2 ($p<0.001$). MVD was highest in group 1 patients compared to group 2 patients ($p=0.01$). In follow-up biopsies development of IF and graft loss found earlier in group 1 patients compared to group 2 patients ($p<0.01$ and $p<0.05$ respectively).
Conclusion: Our results suggest that CMV infection play an important role in the induction of angiogenesis. In addition we point out that CMV may potentate interstitial fibrosis in vivo by stimulating angiogenesis and cause early interstitial fibrosis and poor outcome.

P.1431

Comparison of light and electron microscope renal biopsy findings with proteinuria levels of candidate living kidney donors

Handan Özdemir[1], Nurhan Özdemir[2], Esra Esra Baskın[2], Mehmet Haberal[3].
[1]Pathology, Baskent University, Ankara, Turkey; [2]Nephrology, Baskent University, Ankara, Turkey; [3]Transplant Surgery, Baskent University, Ankara, Turkey.

Introduction: Availability of cadaveric donors is limited and living donor transplantation (Tx) became the most important choice for having allograft. Donors with proteinuria were not accepted as donor in some centers or contrary some donors with proteinuria were accepted as donor without evaluating donor renal biopsy. Our aim is twofold; first to evaluate whether there is a correlation between proteinuria level with the light (LM) and electron microscopic (EM) findings of donor renal biopsy, second to correlate all these findings with graft survival of patients whom had taken renal allografts from these donors.
Material and Methods: Renal biopsies of 70 candidate donor whom had proteinuria were included in to study. LM and EM findings were scored. LM scoring was based on the presence and severity of glomerular sclerosis (GS), glomerular basement membrane (GBM) thickness, mesangial thickness (MT), interstitial inflammation, interstitial fibrosis (IF), glomerular magnitude (GM) and glomerular proliferation (GP). EM scoring was based on the presence and severity of glomerular subendothelial widening (SW), endothelial swelling (ES), foot process effacement (FPE), GBM thickness and lamellation. Donors with insignificant biopsy findings were separated into 2 groups according to proteinuria level. Group 1 donors had proteinuria lower than 300 mg/day and group 2 donors had proteinuria equal to or higher than 300 mg/day. Renal allograft survival was recorded from the files of patients whom had kidney Tx from these donors with insignificant biopsy findings.
Results: Among 70 cases with proteinuria, biopsy findings attributed to glomerulonephritis (GN) in 35 (50%) and tubulointerstitial nephritis (TIN) in 2 donors (2.8%). Remaining 33 donors had insignificant biopsy findings. Proteinuria was found 304±105 mg/day in donors with insignificant biopsy and it was found 557±363 mg/day in donors with GN and TIN ($p<0.001$). All LM and EM findings of 33 donor biopsies showed a statistically significant relationship with the level of proteinuria. The incidence of GBM thickness, MT, interstitial inflammation, IF, GP and wide GM in LM evaluation were found higher in group 2 donors compared to group 1. Also the incidence of SW, ES, FPE, GBM thickness and lamellation in EM evaluation were found higher in group 2 donors than group 1 ($p<0.005$ for all). LM and EM scores were 2±1,3 and 1,9±1,3 in group 1 respectively and they were 6±2,3 and 5±1,8 in group 2 respectively ($p<0.001$ for both). Among 33 donors, 21 donors had chance to gave their kidney to their relatives (Tx group). Remaining 12 donors were taken out of donor list because of their biopsy and/or clinical findings (Non-Tx group). Proteinuria level, LM score and EM score were higher in non-Tx group than Tx-group ($p<0.01$). Overall 1-, 3-, and 5-year graft survival rates were 90%, 80% and 80% respectively. During 5 years only 5 grafts lost and remaining 16 grafts were functioning well.
Conclusion: Although donors had proteinuria, kidneys with low LM and EM scores were appropriate for donation. Nevertheless we showed that proteinuria greater than 300 mg is a reliable marker of glomerular pathology and renal disease. Thus we must be cautious about donors with proteinuria greater than 300 mg and we recommend to take renal biopsy in every donor whom had proteinuria.

P.1432

The relation between the inflamatory cytokine expression with acute rejection episodes and hepatitis recurrence in liver allograft recipients

Gonca Özgün[1], Binnaz Handan Özdemir[1], Eda Yılmaz Akçay[1], Pelin Börcek[1], Gökhan Moray[2], Mehmet Haberal[2].
[1]Pathology, Baskent University, Ankara, Turkey; [2]Transplant surgery, Baskent University, Ankara, Turkey.

The effect of HBV and HCV positivity on liver transplant outcomes and its relationship with the incidence of acute rejection (AR) episodes are still controversial. Some studies reported higher rates of AR and allograft loss and, greater mortality rates among liver allograft recipients. In this study, we aimed to show the possible influence of HBV and HCV on the occurence of AR episodes and to find out helpful findings to differentiate AR morphology from hepatitis morphology.
We retrospectively evaluated data and biopsy findings of 338 liver transplant recipients who underwent transplantation at our hospital between 2001 and 2012. The mean age of the transplant recipients was 41,3±17. Selected 71 patients were categorized by their primary cause of cirrhosis. All biopsies were immunostained with TNF-α, TGF-β and α-Smooth muscle actin (SMA).
Primary HBV infection, HCV infection and cryptogenic cirrhosis (CC) were found in 37 (52,1%), 16 (22,5%) and, 18 (25,3%) patients, respectively. The mean survival time of the patients with HBV, HCV and, CC was 85,9±50,7, 56,8±47 and, 63,2±48 months, respectively. Survival of the patients with recurrence of the primary disease (RPD) was 60,7±45,7 months. Patients with RPD had an inferior survival compared to patients with AR ($p<0,05$).
The AR episodes and recurrences in patients with viral etiology were found higher than the patients with CC ($p<0,05$). Also patients with primary HBV infection showed higher incidence of AR episodes compared to patients with primary HCV infection or CC ($p<0,05$).
The rates of the TNF-α and TGF-β expression in inflamatory cells were found related with progressive fibrosis. The cytoplasmic SMA expression in hepatocytes were also found related with the fibrosis development ($p<0,05$). Although there were no diffence found in fibrosis between AR and RPD, TGF-β expression was significantly higher in RPD than AR ($p<0,05$). As a similar result, SMA expression was found higher in RPD than AR.
In conclusion HBV and HCV have a harmfulipact on allograft survival. Inflammatory cytokines have an important role in the differential diagnosis of AR episodes from hepatitis recurrences.

P.1433

Prevention of vascular stricture by using an expanded polytetrafluoroethylene (ePTFE) graft in a porcine model: preliminary study

Mehmet Haberal[1], Handan Ozdemir[2], Necdet Ozcay[1], Didem Bacanli[3], Zeynep Ersoy[4], Adnan Torgay[4].
[1]Transplantation Center, Baskent University, Ankara, Turkey; [2]Pathology, Baskent University, Ankara, Turkey; [3]Animal Laboratory, Baskent University, Ankara, Turkey; [4]Anesthesiology, Baskent University, Ankara, Turkey.

Introduction: Arterial complications continue to be a major source of morbidity, graft loss and mortality after liver transplantation. Therefore it is important to find out new techniques to improve the results. The aim of this study was twofold: first to show the usability of ePTFE for arterial anastomosis and second to show the role of ePTFE in the prevention of constriction at the site of anastomosis. We compared this technique with the traditional technique of arterial anastomosis.
Materials and Methods: A total of 11 pigs, weighing 22±2.3kg, were cared for according to institutional and published NIH guidelines. One of 11 pigs died on 3rd day and it was excluded from the study. In group I, 5 pigs underwent traditional end-to-end arterial anastomosis at the site of abdominal aorta. In group II, ePTFE (1 cm long, 6 mm in diameter) was used as a bridge at the site of arterial anastomosis in 5 pigs. After 8 days, 4 pigs (2 pigs from group I and 2 pigs from group II) and after 15 days, 6 pigs (3 pigs from group I and 3 pigs from group II) were sacrificed. Anastomosis were harvested en bloc and evaluated histopathologically.
Results: Animals from group I showed partial or circumferential stricture and calcification at the site of anastomosis both in the 8th and 15th days. Animals from group II produced smoother anastomoses without a constricted or pinched appearance at the PTFE-artery junction both in the 8th and 15th days. ePTFE was completely integrated to surrounding tissue in all animals in 15th day but the integration of the graft was incomplete on 8th day. In addition, the continuity of intima from artery to ePTFE was found in all pigs in day 15 but it was not noted in day 8.
Conclusion: Our results indicate that, using an1cm long ePTFE grafts for end-to-end anastomosis is preferable to provide the smoothest anastomosis avoiding constriction at the graft-artery junction.

P.1434

Bridging technique of bile duct anastomosis using an expanded polytetrafluoroethylene (ePTFE) graft in a porcine model

Mehmet Haberal[1], Handan Ozdemir[2], Didem Bacanli[3], Necdet Ozcay[1], Zeynep Ersoy[4], Adnan Torgay[4].
[1]Transplantation Center, Baskent University, Ankara, Turkey; [2]Pathology, Baskent University, Ankara, Turkey; [3]Animal Laboratory, Baskent University, Ankara, Turkey; [4]Anesthesiology, Baskent University, Ankara, Turkey.

Introduction: Biliary complications are common after orthotopic liver transplant. Biliary strictures and leaks represent 70% of all biliary complications and most of them are located at the site of the anastomoses. Our aim was to evaluate a method for biliary anastomoses using a patch of ePTFE in a porcine model.
Materials and Methods: A total of 14 pigs, weighing 45-120 kg, were cared for according to institutional and published NIH guidelines. In group 1, 5 pigs underwent end-to-end choledochoduonostomy (n=3) or choledochocholedochostomy (n=2). In group 2, 1 cm long ePTFE (6 mm in diameter) was used as a bridge between the end of two choledoch anastomoses in 9 pigs. After 30 days the animals were sacrificed and their abdomens were inspected for evidence of infection or biliary leak. Anastomoses were harvested en bloc and evaluated histopathologically.
Results: Bile leaks were detected at the bilioenteric anastomosis in 2 pigs from group 1, but none of anastomoses from group 2 showed bile leakage. Partial adhesion of the ePTFE patch to the bile duct as a free luminal foreign body was found in 3 pigs but ePTFE was completely integrated to surrounding tissue in the remaining 6 pigs. In addition, group 2 stented biliary anastomoses showed neither occlusion nor stenosis. Besides partial (20%) or circumferential stricture (60%) was found at the site of anastomoses in group 1. Statistically significant differences were found between the 2 groups with regard to bile leakage and stenosis ($p < .05$). Of the 3 circumferential strictures in group 1, 2 were also combined with bile leakage.
Conclusions: Biliary anastomoses using ePTFE graft between both choledoch is feasible with satisfactory anastomotic circumference and histological evidence of healing. This technique is promising for decreasing the incidence of bile duct leakage and stricture.

P.1435

Clinical and pathological analyses of interstitial fibrosis and tubular atrophy (IF/TA) cases after kidney transplantation

Tomokazu Shimizu[1], Kazuya Omoto[2], Shoichi Iida[2], Hiroshi Toma[2], Hideki Ishida[1], Kazunari Tanabe[1].
[1]Department of Urology, Tokyo Women's Medical University, Tokyo, Japan; [2]Department of Transplant Surgery, Toda Chuo General Hospital, Saitama, Japan.

Aim: We carried out a clinicopathological analysis of cases presenting with interstitial fibrosis and tubular atrophy (IF/TA) after renal transplantation in an attempt to clarify the mechanisms underlying the development and prognostic significance of IF/TA.
Patients: IF/TA was diagnosed in 35 renal allograft biopsy specimens (BS) obtained from 35 renal transplant recipients under follow up at the Department of Transplant Surgery, Toda Chuo General Hospital, between January 2014 and March 2015.
Results: IF/TA was diagnosed at a median of 39.9 months after the transplantation. Among the 35 patients with IF/TA, 19 (54%) had a history of acute rejection. Among the 35 BS showing evidence of IF/TA, the IF/TA was grade I in 25, grade II in 9, and grade III in 1. Arteriosclerosis of the middle-sized arteries was observed in 30 BS (86%). We then classified the 35 BS showing evidence of IF/TA according to their overall histopathological features, as follows; IF/TA alone (6 BS; 17%), IF/TA + medullary ray injury (12 BS; 34%), and IF/TA + rejection (12 BS; 34%).
Loss of the renal allograft occurred during the observation period in one of the patients (3%). Of the remaining patients with functioning grafts, deterioration of the renal allograft function after the biopsies occurred in 15 patients (43%).
Conclusions: About a half of the cases with IF/TA had the history of acute rejection; this suggests the likelihood of IF/TA occurring as a sequela of tissue injury associated with acute rejection. The presence of arteriosclerosis could cause IF/TA. The results of our study suggests that rejection contributes to IF/TA in 30%-40% of cases, medullary ray injury in 30%-40% of cases, and nonspecific injury in 20% of cases. Based on the observation that deterioration of the renal allograft function after the biopsies occurred in about a half of the cases with IF/TA, it appears that IF/TA contributes significantly to deterioration of renal allograft function.

P.1436

The Oxford classification as a predictor of outcome in patients with IgA nephropathy in the renal allograft

Sansanee Thotsiri[1], Vasant Sumethkul[1], Suchin Worawichawong[2], Atiporn Ingsathit[1], Chagriya Kitiyakara[1].
[1]Department of Medicine, Faculty of medicine Ramathibodi Hospital, Mahidol University, bangkok, Thailand; [2]Department of Pathology, Faculty of medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.

Background: IgA nephropathy (IgAN) in the transplanted kidney can lead to graft failure. The Oxford classification of renal histopathology may predict graft loss in IgAN in the native kidney, but its value in renal allograft IgAN is not known.
Methods: In this retrospective study, we investigated the outcomes of renal allograft IgAN and the predictive value of Oxford classification on graft failure (ESRD)
Results: Of patients with allograft IgAN (n=57), the most common presentation was proteinuria (35.1%). Mean baseline estimated glomerular filtration rate (eGFR) was 42.67 mL/min/1.73 m2. Median time to diagnosis after transplantation was 93 months. At 1 year after diagnosis: 17 (29.82%) reached to endpoint (ESRD or ΔGFR ≥50 %), 40 (70.18%) had stable renal function (ΔGFR < 50 %). Median Oxford scores were M1S1E0T1. Tubular atrophy (P< 0.001) were correlated with decreased estimated glomerular filtration rate (eGFR) at time of biopsy. Mean time to graft failed was 23.88 ± 25.59 months after renal biopsy. Endocapillary hypercellularity and tubulointerstitial fibrosis were predicted graft failure. Compared to stable patients, endpoint group had lower baseline eGFR and higher tubulointerstitial whereas systolic blood pressure, or type of immunosuppression were not different.
Conclusions: Oxford classification (E,T score) of IgAN may be useful in predicting graft failure in renal allograft IgAN.

Table: Histologic features affecting graft survival (dialysis and Δ GFR 50 %)

Variables	No. (%) of patients	P	Odd ratio	Confidence intervals (95%)	
				Lower	Upper
Mesangial hypercellularity					
M0	6 (10.53)	0.631	1.354	0.393	4.667
M1	51 (89.47)				
Endocapillary hypercellularity					
E0	45 (78.95)	0.022	2.673	1.153	6.192
E1	12 (21.05)				
Segmental glomerulosclerosis					
S0	25 (43.86)	0.080	2.073	0.918	4.683
S1	32 (56.14)				
Tubular atrophy/interstitial fibrosis					
T0	21 (36.84)	0.001	2.672	1.483	4.812
T1	26 (45.62)				
T2	10 (17.54)				

P.1437

Treatment and outcomes of recurrent focal segmental glomerulosclerosis post kidney transplantation in children

Anna Francis[1,2], Madeleine Didsbury[2], Tonya Kara[2,3].
[1]School of Medicine, University of Sydney, Sydney, Australia;
[2]Centre for Kidney Research, Westmead, Sydney, Australia;
[3]Paediatric Nephrology, Starship Children's Hospital, Auckland, New Zealand.

Introduction: Focal segmental glomerulosclerosis (FSGS) recurs post renal transplant in around 15-40% of cases[1]. Recurrence is difficult to treat and is associated with poor graft outcomes. There are numerous treatment options, including plasma exchange, cyclosporin and rituximab[2]. There is no clear optimal treatment regime. We aimed to report the experience of four paediatric transplant centres in the outcomes and treatment of recurrent FSGS.
Materials and Methods: We contacted the Australian and New Zealand Dialysis and Transplant Registry to obtain a list of all children with recurrent FSGS in the last 25 years in four centres across New South Wales, Queensland (Australia) and New Zealand. We then reviewed the charts of all patients to obtain demographic and clinical information. Ethics approval for this study was obtained from the relevant boards. Analysis of the data involved calculating the mean and 95% confidence intervals (95%CI) for descriptive statistics.
Results: From 1990 to 2015, there were 29 patients with recurrent FSGS in the four centres. Charts were available on 25 patients. There were 30 cases of recurrence in 27 transplants. Two children had recurrence in two transplants, one FSGS recur twice in the transplant and one had FSGS recur three times in the one transplant.
Of the cohort, 15/25 (60%) were female, 13/25 (52%) were Caucasian and 15/27 (55%) of transplants were from a deceased donor.
Recurrence was diagnosed on clinical grounds in 17/30 cases and biopsy plus clinical grounds in 13/30 cases. The average time to first recurrence was 5 days (95% CI 2-8 days). There were multiple therapies used to treat the recurrence, including plasma exchange, high dose cyclosporine, cyclophosphamide, rituximab, methylprednisone, IVIG and abatacept. The average patient had 2-3 different therapies. The most common treatment was plasma exchange (22/30 cases). Cyclosporin was the next most common (16/30 cases). IV cyclosporin was used in 4 of these cases, with all attaining full or partial remission. Rituximab was used in 8/30 cases, with 7 attaining full or partial remission.
18/30 cases of recurrent disease had full remission and 4/30 had partial remission. 7/30 episodes had no remission and all of these lost the graft due to the recurrent disease. Outcome data was missing on one patient. In this group, the average renal transplant survival time was 4.0 years (95% CI 2.2-5.8 years) with a 5-year graft survival rate of 32% (95% CI 14-50%).
Discussion: In this 25 year retrospective cohort of 25 children, recurrence of FSGS post transplant was rapid and difficult to treat. The plethora of treatment choices for recurrent FSGS reflects the fact that there is no clear best treatment approach. Outcomes of recurrence are poor, with inferior 5-year survival rates when compared to the general transplant population.
Conclusion: Randomised control trials are urgently needed to elucidate the optimal treatment of FSGS recurrence post renal transplant.
References:
[1] Nehus EJ et al: Focal segmental glomerulosclerosis in children: multivariate analysis indicates that donor type does not alter recurrence risk. Transplantation 2013, 96(6):550-554.
[2] Hickson LJ et al: Kidney transplantation for primary focal segmental glomerulosclerosis: outcomes and response to therapy for recurrence. Transplantation 2009, 87(8):1232-1239.

P.1438

Tonsillectomy and steroid pulse therapy for recurrence of IgA nephropathy after kidney transplantation

Kengo Horie, Tomohiro Tuchiya, Shigeaki Yokoi, Masahiro Nakano.
Urology, Gifu University School of Medicine, Gifu City, Japan.

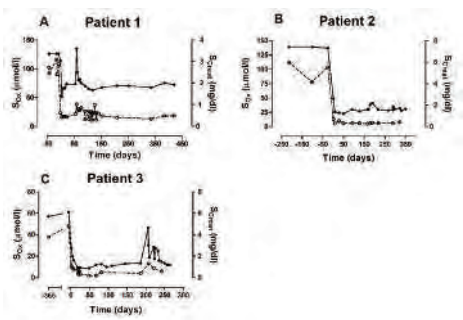
Introduction: Recurrent IgA nephropathy (IgAN) is a common recurrent glomerular disease after kidney transplantation. Recurrent IgAN, in particular, with crescent formation or endocapillary proliferation might result in kidney allograft loss. However, no specific therapy for recurrent IgAN is currently available. In this study, we evaluated the histological efficacy of tonsillectomy and steroid pulse therapy (TSP) for allograft IgAN.
Methods: Seven kidney recipients (male 5, female 2, mean age 29.0± 9.0 years), who received a diagnosis of IgA nephropathy by allograft biopsy, were enrolled in this study. TSP were performed 25.0 ± 44.9 months after the kidney transplantation and 4.0 ± 5.0 after diagnosis of recurrence IgAN. Six patients underwent a repeat graft biopsy at 18.0 ± 7.5 months after TSP
Results: All patients underwent TSP after they had microhematuria and proteinuria. At 12 months after treatment, the microhematuria disappeared in six of these patients and one patient had hematuria and proteinuria. This patient reached end-stage renal failure a year later after TSP. On histological analysis, four patients had acute lesions including cellular or fibrocellular crescents and mesangial proliferation. The acute lesions disappeared after these treatments in all patients. Six case reports suggest that the disease severity of recurrent IgAN with endocapillary proliferation regardless of crescent formation can be minimized by TSP. Graft loss case didn't undergo repeat biopsy.
Conclusions: TSP improves not only clinical findings but also ameliorates histological damage caused by recurrent IgAN after kidney transplantation. TSP is effective treatment for recurrent IgAN, but it is controvertial which tonsillectomy only or TSP for several type of recurrent IgAN.

P.1439

Successful approach to kidney transplantation in patients with secondary hyperoxaluria

Joke Roodnat[1], Anneke M.Evan Egmond[3], Wesley Visser[3], Stefan P.Berger[2], Wilbert van der Meijden[4], Felix Knauf[5], Madelon Agteren[1], Michiel G.H.Betjes[1], Ewout Hoorn[1].
[1]Nephrology and Transplantation, ErasmusMC, Rotterdam, Netherlands; [2]Nephrology and Transplantation, University Hospital Groningen, Groningen, Netherlands; [3]Dietics, ErasmusMC, Rotterdam, Netherlands; [4]Nephrology and Transplantation, Radboud University Hospital Nijmegen, Nijmegen, Netherlands; [5]Nephrology and Transplantation, Universitätsklinikum Erlangen, Erlangen, Germany.

Chronic oxalate nephropathy is observed secondary to enteric hyperoxaluria that is associated with malabsorption e.g. in short bowel syndrome. Kidney transplantation can be challenging given the risk of recurrence resulting in graft dysfunction. We studied whether a strategy to decrease serum oxalic acid prior to transplantation may allow successful kidney transplantation.
A protocol was developed to reduce serum oxalic acid levels pre and post kidney transplantation based on reduced intake and increased removal of oxalic acid via intensified hemodialysis (HD). Months before transplantation a low oxalic acid diet (40-50 mg/day), cholestyramine, potassium citrate, and calcium carbonate were started. One week before transplantation (living donor transplantation) or immediately after surgery (deceased donor transplantation), an oxalic-acid free drip-feed and daily 6h HD session were started. When a urine output>2L was reached HD was stopped and low oxalic acid diet was re-started. Patients were instructed to maintain diuresis > 2L/day.
We report three patients with short bowel syndrome following surgery for Crohn's disease who presented as potential kidney transplant recipients. All three patients had elevated serum oxalic acid levels and biopsy-proven calcium oxalate depositions in their native or transplanted kidney. The protocol reduced serum oxalic acid levels from 102, 112, and 48 to 23, 13, and 11 μmol/l. Patients 1 and 2 received a living donor kidney and had immediate functioning grafts. Patient 3 received a deceased donor kidney and achieved urine output >2L/day after 3 weeks. The post-transplantation periods were complicated by sepsis in Patient 1, symptomatic native kidney stones in Patient 2, and hydronephrosis in Patient 3, requiring temporary re-institution of the protocol. The patients are currently 14, 13, and 9 months after transplantation with stable eGFR of 38, 48, and 47 ml/min/1.73 m2, respectively.
We conclude that successful kidney transplantation in patients with secondary hyperoxaluria is feasible by implementing a strategy aimed at reducing serum oxalic acid levels in the peri-transplantation period.



References:
[1] Karaolanis G, Lionaki S, Moris D, Palla VV, Vernadakis S.Secondary hyperoxaluria: a risk factor for kidney stone formation and renal failure in native kidneys and renal grafts.Transplant Rev (Orlando). 2014;28(4):182-7.

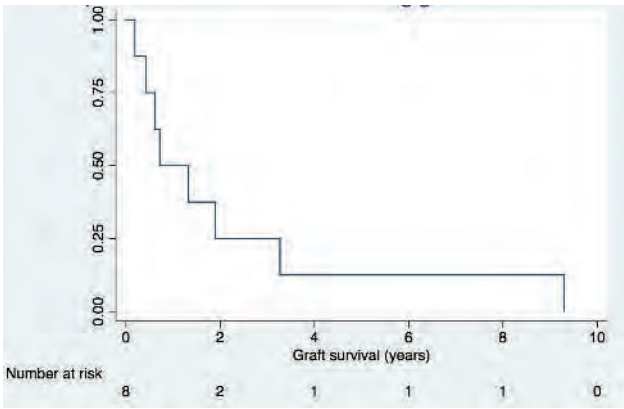
P.1440

Biopsy correlation of recurrent FSGS

Tareq Husein[1], Shafi Malik[2].
[1]Renal and Transplant, University Hospital of Wales, Cardiff, United Kingdom; [2]Renal and Transplant, University Hospitals of Leicester, Leicester, United Kingdom.

Background: Focal Segmental Glomerulosclerosis (FSGS) recurs in 20-30% of renal transplant recipients (KTR) and often leads to graft loss. The recurrence rate in second or subsequent grafts is up to 80%. A combination of plasmapheresis (PLEX), rituximab and ACE inhibitors are often used to achieve partial or full remission. It is not clear whether the same histological variant as defined by the Columbia classification seen in the native kidney recurs post transplant. The histological classification of FSGS is likely to have an impact on clinical outcomes.
Methods: We conducted a retrospective review of recurrent FSGS cases in our transplant centre between Jan 1988 to Jan 2015. In total, there were 10 cases, 2 patients underwent a second transplant and a third had 3 transplants in total. Native renal biopsy and post transplant biopsy reports were collected. Descriptive statistics were performed and a hypothesis that proteinuria and graft survival is different between deceased and living donor KTR's was tested using Mann Whitney test.
Results: 47 patients with biopsy proven FSGS have been transplanted; the recurrence rate is 12.7% for first grafts and 100% in subsequent grafts. The median age of patients was 39(20-51). Mean graft survival was 2.22 years(0.19-9.29). The median time from transplant to development of proteinuria(>1gm) was 9 days(1-186), median peak proteinuria was 9.95 gm(1.94-28). 6 cases were treated with PLEX, all but one patient transplanted post Jan 2000 were treated with PLEX. The median number of exchanges required to achieve control of proteinuria was 4(2-14), the frequency of PLEX depended on the clinical severity of the patient, all patients treated with PLEX achieved either partial or full remission. No difference was found between recurrent FSGS cases that underwent deceased or living donor transplant in the timing of proteinuria (p0.917), graft survival(p1.000) or peak proteinuria(p0.175).
Native renal biopsy FSGS histological variant correlated with post transplant FSGS histological variant. 3 patients had FSGS NOS and 1 had a tip lesion on their native kidney biopsy, native renal biopsy reposrts for other two patients were un obtainable. In patients whose native renal biopsy reports were available, the post transplant biospies showed the same histological variant. Compared to FSGS NOS, the patient with tip lesion on biopsy responded quicker to PLEX and only required 2 sessions.
Conclusion: Patients develop proteinuria very early post transplant, PLEX is effective in controlling proteinuria and most patients achieve partial if not full remission. The same histological variants recur post biopsy with tip lesion responding better to PLEX. The role of rituximab in combination with PLEX requires further study.

[fig. Kaplan-Meier survival curve showing graft failure for all cases]



References:
[1] Ponticelli C. Recurrence of focal segmental glomerular sclerosis (FSGS) after renal transplantation. Nephrol Dial Transplant. 2010;25(1):25-31.
[2] Wei C, El Hindi S, Li J, Fornoni A, Goes N, Sageshima J, et al. Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis. Nat Med. 2011;17(8):952-60.
[3] Franco Palacios CR, Lieske JC, Wadei HM, Rule AD, Fervenza FC, Voskoboev N, et al. Urine but not serum soluble urokinase receptor (suPAR) may identify cases of recurrent FSGS in kidney transplant candidates. Transplantation. 2013;96(4):394-9
[4] D'Agati VD et.al. Association of histologic variants in FSGS clinical trial with presenting features and outcomes.Clin J Am Soc Nephrol. 2013 Mar;8(3):399-406

P.1441

Urinary tract infections in kidney transplant recipients

Vinod Mathrani[1], Rebecca Bamber[1], Harriet Hughes[1], Shafi Malik[1,2]. [1]Nephrology and Transplant, University Hospital of Wales, Cardiff, United Kingdom; [2]Nephrology and Transplant, University Hospitals of Leicester, Leicester, United Kingdom.

Introduction: Urinary tract infections (UTI) are the most common infections in kidney transplant recipients (KTR) and are associated with morbidity and graft loss. This study was undertaken in a tertiary centre transplant follow up clinic to characterize the types of organisms, antibiotic sensitivity, associated symptoms and to identify any risk factors.
Methods: This was a prospective cohort study done over a one month period between February 2015 to March 2015, urine specimens were collected from patients that attended transplant follow up clinic, these specimens were then specially coded and sent for culture and sensitivity if they tested positive for either leucocytes or nitrites on dipstick. Samples were cultured irrespective of microscopy result. Data on baseline demographics, transplant factors including immunosuppression, inflammatory markers, symptoms, organisms and antibiotic sensitivity were collected. Parametric and non-parametric tests were used for descriptive statistics and logistic regression was used to adjust for measured confounders.
45 patients samples were collected of which 5 were not cultured due to incorrect labelling, however, the urine microscopy white count for these 5 samples were below the cut off used for non transplant urine cultures. Duplicate samples were excluded from the analysis. In 34% there were no growth, 28% E.coli, 21% mixed growth, 8% candida species, 3% Pseudomonas aeruginosa and 2% each of KESC enterococcus and K.pneumoniae. Of the E.coli 2 were likely ESBL. 11 organisms were resistant to trimethoprim, 1 was intermediate resistant, 10 were resistant to ciprofloxacin and 8 to amoxicillin.
Of the samples that would not normally have been cultured based on urine microscopy - 6 were no growth, 2 non specific growth, 3 E.coli, 2 candida species and 1 both E.coli and candida sp. Majority of patients who were asymptomatic and reported no symptoms had a positive urine culture - 3 candida, 4 E.coli, 1 E.coli and candida, 2 Pseudomonas, 1 KESC, 1 K.pneumoniae and 5 mixed growth.
Baseline Characteristics: Age (mean in years) 51.2. Sex 31 females, 14 males. Cause of ESRF25 GN, 8 APKD, 7 reflux nephropathy, 3 vasculitis, 2 unknown Co-morbidities23 none, 4 diabetes, 12 hypertension, 6 both diabetes and hypertension. Immunosuppression 25 (one agent), 17 (2 agents) and 3 (3 agents). Serum creatinine (mean) mmol/L162.6. Total white cell count (mean) x 109/L8.7CRP (mean) mg/dL10.5
In logistic regression models, there was no statistically significant association between the occurrence of infection and the measured risk factors after adjusting for measured confounders ($p>0.10$).
Conclusion: UTI's are common in KTR's, many asymptomatic patients have bacteriuria, E.coli is the commonest organism and incidence of ESBL was very low. No significant association was found between risk factors and infection; this may be due to the small sample size. Larger prospective cohort studies are needed to study graft outcomes.

References:
[1] Camargo L.F et al. Transplant Proc. 2014 Jul-Aug;46(6):1757-9
[2] Barbouch S et al. Saudi J Kidney Dis Transpl. 2012 Nov;23(6):1311-4

P.1442

Good patient and graft survival in recipients of kidney transplantation due to diabetic nephropathy

Amgad El-Agroudy[1], Sumaya Ghareeb[2], Eman Farid[2], Sadiq Abdulla[2].
[1]Arabian Gulf University, Medicine, Manama, Bahrain; [2]Nephrology and Transplant Department, Salmaniya Medical Complex, Manama, Bahrain.

Background: Compared with non-diabetic subjects, patients with type 2 diabetes and end-stage renal disease (ESRD) have seldom been selected for renal transplantation. The aim of this study is to compare patient and graft outcome in kidney transplant patients with diabetes mellitus.
Methods: We retrospectively studied 358 patients who underwent kidney transplantation between 1979 and 2014, including 88 with diabetic ESRD (DM group) (type 1, n = 8; type 2, n = 80) and 270 with non-diabetic ESRD (NDM group). Mean follow-up was 92±9 (0.1–389) months.
Results: Mean age was higher in the DM group (52.8 vs 44.6 years; P < .0001), and there was no significant difference in recipient gender, donor age or donor source. At the end of follow-up, there was no differences between the groups in terms of blood pressure control (DM 139.3±16.7/81.7±7.6 mmHg vs NDM 138.3±19.7/82.1±8.1 mmHg, P= 0.83/0.80) and renal function (creatinine, 115.4±47.1 vs 133.4±80.2 μmol/l, P= 0.18; calculated creatinine clearance, 66±24 vs 68.4±24 ml/min/1.73 m2, respectively, P = 0.9). In total, 26 patients had acute transplant rejections [8 patients with diabetes vs 18 patients without diabetes, P = 0.11]. There was no significant difference in post transplant surgical complications as wound dehiscence or infections. There was a significant high incidence of the urinary tract infection rate in DM group (17 vs 32 patients; P = .012). Four out of 22 patients died (18.2%) in the DM group and 10 out of 47 patients died (21.3%) in the NDM group died from cardiovascular disease (P = 0.17). The 1-, and 10-year patient survival rates in the DM and NDM groups were 97.5% vs 99% (ns), and 56.8% vs 58.8% (ns), respectively. The 1-, and 10-year graft survival rates were 97.5% vs 70.7% (ns) and 58.9% vs 66.1% (ns), respectively.
Conclusions: Renal transplantation in diabetic ESRD patients yields good results in terms of patient survival and complications, suggesting that renal transplantation can be performed in these patients and should become a more established treatment option.

P.1443

Mycophenolate mofetil dose reduction are associated with the risk of acute rejection and graft failure after renal transplantation

Amgad El-Agroudy[1], Ali Alaradhi[2], Eman Farid[2], Mohamed Nowrooz[2], Sadiq Abdulla[2].
[1]Arabian Gulf University, Medicine, Manama, Bahrain; [2]Nephrology and Transplant Department, Salmaniya Medical Complex, Manama, Bahrain.

Background: Mycophenolate mofetil (MMF) is a highly effective immunosuppressive agent in transplant therapy. Although MMF is generally well tolerated, optimal therapy may be limited by adverse effects, in particular gastrointestinal (GI) toxicity. MMF dose changes resulting from these adverse events may lead to sub-therapeutic dosing and impaired clinical outcomes. Aim of our study is to investigate the impact MMF dose reduction on the incidence of acute rejection and graft survival.
Methods: In this study, a cohort of 150 kidney transplant recipients who received immunosuppression using MMF in conjunction with cyclosporine and prednisone and Baziliximab induction were evaluated. We classified patients into 3 groups according to MMF dose per day in gm; group I with 2 gm/day, group II 1.5 gm/day and group III 1 gm or less per day. Clinical outcomes were compared and contrasted between patients with and without MMF dose changes within the first year post-transplantation. The study followed the Declaration of Istanbul (DOI) ethics' statement The study was undertaken in accordance with the Declaration of Helsinki, and all subsequent amendments, and was approved by the local ethics committees.
Results: The majority of patients (60%) had at least one dose change within the first post-transplant year. Compared with the 60 patients who did not have a dose change, these patients had a significantly higher incidence of acute rejection within the first post-transplant year (13% vs. 3%, p <0.01). This resulted in a significantly decreased 5-yr death-censored graft survival (77% vs. 68% and 57% in the groups I, II and III, respectively, p = 0.04). The incidence of acute rejection for patients who had a dose change was highest if the dose change occurred within the first post-transplant 6 months (38%). The duration to the first acute rejection was dose related (2.3±1.1 and 4.6±1.4 months in group II and III, respectively, p<0.05). Regarding other complications, there was no significant differences in terms of incidence of infections and malignancy within five year of follow-up.
Conclusion: Altering the dose of MMF within the first post-transplant year correlated with a significantly worse clinical outcome in renal transplant recipients. These data suggest that avoidance of MMF dose changes within the first year after renal transplantation would result in improved graft survival.

P.1444

Kidney transplantation tourism: high risk and bad outcome for the recipients

Amgad El-Agroudy[1], Basel Al-Hayky[2], Eman Farid[2], Salah El-Sharqawi[2], Abdulraqeeb Taher[2], Hamad Alhelow[2].
[1]Arabian Gulf University, Medicine, Manama, Bahrain; [2]Nephrology and Transplant Department, Salmaniya Medical Complex, Manama, Bahrain.

Background and Aim: While the ethical aspects of transplant tourism have received much attention recently, less has been written about the medical safety of this practice. We retrospectively evaluated the outcomes of patients who purchased organs internationally and presented to our center for follow-up care.
Methods: We report the outcome parameters of 270 local recipients of unrelated kidney (URT) vendor transplants presenting to our institute between 1986 and 2014. Their outcome was compared with 123 recipients of living-related donor transplants matched for age, gender and transplant duration done in our center as controls (RT).
Results: Age of unrelated recipients was 42.6 ± 13.4 years with Male % of 68. The country of transplant was mainly in Philippines (n = 85), Pakistan (n = 56), India (n = 57), Iran (n = 40) and Egypt (n= 25). Comparison of commercial recipients with controls showed high co morbidities (P = 0.01) with hepatitis-C (n=2 vs. 0) and hepatitis-B (n=2 vs. 0) and cytomegalovirus (n=4 vs. 1). Donor age was 25.9 ± 3.8 vs. 34.6 ± 8.6 years (P = 0.0001) and 90.4% were male. Biologic agents induction in 74 (27.4%) vs. 123 (100%) (P = 0.00001), acute rejections in 65 (24.1%) vs. 26 (21.1%) (P = 0.7), while recurrent rejection in 13 (4.8%) vs. 1 (0.8%) (P = 0.04), surgical complications including lymphocele 16 (5.9%) vs. 0 (0%) (P = 0.0001), ureteral obstruction 7 (2.6%) vs. 0 (0%) (P = 0.007), hematoma 4 (1.5%) vs. 1 (1.1%) (P = 0.06) and recurrent urinary tract infection 18 (9.9%) vs. 6 (6.8%) (P = 0.3). Overall 1- and 10-year for graft survival was 91% and 22% vs. 98% and 44% and for patient survival 96% and 70% vs. 98% and 78% in URT and RT, respectively (P = 0.001).
Conclusion: Although recent developments increased success in renal transplantation, receiving a kidney from a paid living donor at a commercial transplant center still carries great risks for the recipient.

P.1445

568 Living donor kidney transplants: A single transplant group experience

Issa Kawalit, Abbas Y. Elkhatib.
Nephrology Department, Arab Renal Care Group, Amman, Jordan.

Arab Renal Care Group /www.arabrenalcaregroup.com.

Background: Patient and graft survival after successful kidney transplantation (KT) have improved despite an increase in the number of challenging cases. Various factors have evolved during the long history of kidney transplantation.
Methods: Between 2005 and 2013, a total of 568 living donor KTs were performed by the Arab Renal Care Group covering the Hashemite kingdom of Jordan and the Gulf Cooperation Council region(GCC) . Long-term patient and graft survival during that period based on our standard protocol were retrospectively analyzed.
Results: Allograft survival rates at 1, 3, 5, and 8 years were 98.5, 96.4, 92.5, and 91.7 respectively. These survival rates have dramatically improved since 2000. The number of elderly recipients (older than 60 years) and the percentage of grafts donated from spouses have increased. The rejection rate decreased but the cytomegalovirus antigenemia-positive rate increased during the period assessed. The percentage of pre-emptive KTs progressively increased, with graft survival in this group tending to be better than non-preemptive KTs. The causes of graft loss were chronic allograft dysfunction (54.5%), acute rejection (0.5%) and vascular thrombosis (0.5%).
Conclusion: The 8-year graft survival rate of renal transplantation from living donor in our group is 91.7 %, and it is satisfactory and comparable with reports from large centers in the world. Important factors that played a major role in the graft survival was the age of the donor and the use of basiliximab-based induction treatment.
References:
[1] Kidney Int. 2000 Jan;57(1):307-13
[2] Am J Transplant. 2008 Nov;8(11):2410-9. doi: 10.1111/j.1600-6143.2008.02414

P.1475

Effect of conversion from cyclosporine to tacrolimus on cardiovascular risk profile in renal transplant recipients

Wonseok Do[1], Eugene Kwon[1], Geun-Hee Kim[2], Kyung Hee Lee[1], Kyu Yeun Kim[1], Minjung Kim[1], Youngae Yang[1], Taehoon Yim[1], Inryang Hwang[1], Sukyung Lee[1], Hee-Yeon Jung[1], Ji-Young Choi[1], Jang-Hee Cho[1], Sun-Hee Park[1], Yong-Lim Kim[1], Chan-Duck Kim[1]. [1]Department of Internal Medicine, Kyungpook National University Hospital, Daegu, Korea; [2]Transplantation Center, Kyungpook National University Hospital, Daegu, Korea.

Background: Cyclosporine is known to increase cardiovascular risk profiles, while tacrolimus is associated with more favorable effect on blood pressure and lipid levels. Little is known whether conversion form cyclosporine to tacrolimus could improve cardiovascular risk profiles. This study investigated the effect of conversion from cyclosporine to tacrolimus on cardiovascular risk in stable renal transplant recipients.
Methods: In this prospective, open label, single arm study, 32 renal transplant recipients were enrolled. Patients were evaluated for cardiovascular risk factors and renal function 3 and 6 months after conversion from cyclosporine to tacrolimus. Comparisons of before and after conversion to tacrolimus were made by paired t-test.
Results: The mean duration from transplantation was 12.6±4.1 years and chronic glomerulonephritis was the most common primary renal disease (59.4%) followed by diabetic nephropathy (15.6%). Six months after conversion from cyclosporine to tacrolimus, the serum creatinine level did not change (1.10±0.24 to 1.08±0.21 mg/dL, p= 0.35). After conversion, mean blood pressure did not significantly decrease (93.10±10.31 to 93.93±10.45 mmHg, p= 0.70). Fibrinogen level decreased from 306.4±63.3 at baseline to 271.3±55.8 mg/dL at 6 months (p= 0.001). During the study period, 2 patients (6.3%) reduced the dosage of statin. Serum total and LDL cholesterol tended to be lower (180±28.40 to 168.53±25.01 mg/dL, p= 0.05, 97.90±25.95 to 91.43±25.25 mg/dL, p= 0.22, respectively). The level of HbA1C increased from 5.7±0.8 to 6.0±1.3 % (p= 0.025) and 4 patients (12.5%) elevated the dosage of insulin or oral hypoglycemic agent at 6 months. However, new onset diabetes did not develop.
Conclusions: Conversion from cyclosporine to tacrolimus has beneficial effects on lipid profiles and fibrinogen in renal transplant recipients. However conversion to tacrolimus seems to have negative influence on glucose metabolism.

P.1476

Variation of induced tolerance practices and outcomes across countries: A scoping review

Jathurong Kittrakulrat[1], Krit Pongpirul[1,2,3]. [1]Department of Preventive and Social Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand; [2]Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States; [3]Bumrungrad Research Center, Bumrungrad International Hospital, Bangkok, Thailand.

Introduction: Immunologic tolerance could be better option for long-term graft outcome without toxicity from immunosuppressive drugs; however, various protocols for induced tolerance have been introduced with varying outcomes. This study was aimed to systematically synthesize the current induced tolerance practices and outcomes of induced tolerance in kidney transplantation (KT) across countries.
Materials and Methods: We search the Medline and Scopus databases using the term "transplantation tolerance" and "kidney" in order to retrieve relevant literature published during the past five years. The retrieved articles were screened by the investigators to include only relevant articles. The systematic review protocol complied with A Measurement Tool to Assess Systematic Reviews (AMSTAR). An induced tolerance was considered successful if the immunosuppressive drugs for kidney transplant are withdrawn.
Results: Of 177 articles identified from the literature search, 158 articles with abstracts were included. Seventy irrelevant articles were excluded and data from 88 articles were extracted. Induced tolerance was mainly performed in USA at three major institutions—Stanford, North Western (NW), and Massachusetts General Hospital (MGH) whereas only three cases from Chile, India, and Thailand were reported. Conditioning chemotherapy was performed in some institutions using either Cyclophosphamide or Fludarabine. Alemtuzumab was used for antibody therapy in NW and Thailand; MGH preferred anti-CD 2 mAb and Chile chose Basiliximab; whereas anti-thymocyte globulin was used in India and Stanford, with and without Rituximab, respectively. Total body irradiation was done at NW and MGH and total lymphocyte irradiation was done at Stanford and Thailand; only MGH irradiated thymus gland. The success rates were considered fair—MGH 67% (8/12), Stanford 50% (19/38), and NW 44% (17/39). While two cases from Thailand and India were successful, the case from Chile who received both bone marrow and kidney transplant still needed immunosuppressive drugs for bone marrow transplant.
Conclusion: Induced tolerance practices vary across institutions/countries with fair success rates.

P.1477

Study of serum BKV load in patients prior to renal transplantation

Ho Gabriella Wong[1], Brandon Dow Chan[2], Magnolia Muk-Lan Lee[2], Wing-Yan Wong[2], Francis Kim-Ming Wong[3], William Chi-Shing Tai[2].
[1]Medicine, Nursing and Health Sciences, Monash University, Melbourne, Australia; [2]Department of Applied Biology & Chemical Technology, The Hong Kong Polytechnic University, Hong Kong, Hong Kong; [3]Medical and Renal Center, Hong Kong, Hong Kong.

Objectives: BK virus associated nephropathy (BKVN), linked to reactivation of latent BK virus (BKV) in the immunocompromised conditions following renal transplantation, is the cause of renal dysfunction and allograft loss in renal transplantation patients[1]-[3]. Based on previous observations by our group (manuscript in preparation), patients with BK viremia before transplantation may be at higher risk for developing high BK viremia and possibly BKVN after renal transplantation. We conducted a study of the serum BKV DNA loads of renal transplantation patients prior to surgery.
Methods: 66 renal allograft recipients (Mean age, 52.7 ± 11.8 years; 40/66 (60.6%) male, 26/66 (39.4%) female) were enrolled in this single centre study. From April 2014 to November 2015, we collected serum samples from patients prior to renal transplantation. Serum BKV DNA load was assessed using quantitative real-time polymerase chain assay.
Results and Discussion: We collected and analysed 70 samples from the 66 patients enrolled in this study. Serum BKV DNA levels ranged from undetectable to 1.2×105 copies/ml. Of the 70 samples, BKV DNA was undetectable in 38 samples (49.4%). Of the 70 samples, 15 samples (18.6%), from 14 patients, had high levels of serum BKV DNA (>1,000 copies/ml). Prevalence of BK viremia in patients prior to renal transplantation was 14/66 (21.2%). Our findings show that a significant proportion of patients preparing to undergo renal transplantation have readily detectable levels of serum BKV DNA.
Conclusion: We have seen that patients with detectable BK viremia before transplantation may be at higher risk for developing high BK viremia and therefore BKVN after renal transplantation in our previous studies. The results of this study suggest the importance of screening patients for serum BKV DNA before surgery, to allow for the deployment of an optimal strategy for immunosuppressive therapy in order to reduce the risk of post-transplantation development of BK viremia and BKVN.
References:
[1] Reploeg MD et al. Clin Infect Dis. 2001, 33(2):191-202.
[2] Beimler J et al. Nephrol. Dial. Transplant.2007, 22(suppl8):viii66-viii71.
[3] Hariharan S. Kidney Int. 2006, 69(4):655-62.

P.1478

Longitudinal study of serum BKV load in patients before and after renal transplantation

Brandon Dow Chan[1], Wing-Yan Wong[1], Magnolia Muk-Lan Lee[1], Ho Gabriella Wong[2], Francis Kim-Ming Wong [3], William Chi-Shing Tai[1].
[1]Department of Applied Biology & Chemical Technology, The Hong Kong Polytechnic University, Hong Kong, Hong Kong; [2]Medicine, Nursing and Health Sciences, Monash University, Hong Kong, Hong Kong; [3]Medical and Renal Center, Hong Kong, Hong Kong.

Objectives: Development of BK virus associated nephropathy (BKVN) has been linked to reactivation of latent BK virus (BKV) in the immunocompromised conditions following renal transplantation. BKVN can lead to renal dysfunction and renal allograft loss in transplantation patients[1]-[3]. Monitoring serum BKV load in renal transplantation patients can potentially be used to identify those at risk for development of BKVN. We conducted a longitudinal study to observe the changes in BKV load in renal transplantation recipients. We measured serum BKV loads and investigated the relationship between BKV load before and after renal transplantation.
Methods: 52 renal allograft recipients (Mean age, 51.7 ± 12.5 years; 33/52 (63.5%) male, 19/52 (36.5%) female) were enrolled in this single centre study. All patients were under an immunosuppressive regime comprised of prednisolone, tacrolimus and mycophenolate at standard doses. From April 2014 to January 2016, we collected serum samples from patients before transplantation, and at 1-2 month intervals for up to 12 months after transplantation. Patients with 6 months or more of follow up samples were included in the study. Serum BKV load was assessed using quantitative real-time polymerase chain assay. High BK viremia was defined as two consecutive readings of serum BKV DNA >10,000 copies/ml.
Results and Discussion: The 52 patients in this study were stratified into two groups according to their pre-transplantation serum BKV levels. 40/52 patients (76.9%), who had pre-transplantation serum BKV levels less than 1,000 copies/ml were placed in the Pre-BKVL group. The remaining 12/52 patients (23.1%), who had pre-transplantation serum BKV levels greater than 1,000 copies/ml were placed in the Pre-BKVH group. Of the Pre-BKVH group, 8/12 patients (66.7%) developed high BK viremia (>10,000 copies/ml) after transplantation. Of the Pre-BKVL group, 37/40 (92.5%) patients did not develop high BK viremia, while 3/40 patients (7.5%) developed high BK viremia after transplantation.
Operating characteristics of a pre-transplantation serum BKV load of 1,000 copies/ml for the post-transplantation development of high BK viremia were as follows: sensitivity, 66.7%; specificity, 92.5%; negative predictive value, 90.2%; and positive predictive value, 72.7%.
Based our observations, patients with a pre-transplantation BKV load of <1,000 copies/ml were at low risk for developing high BKV viremia, while patients with a pre-transplantation BKV load of >1,000 copies/ml would develop post-transplantation high BK viremia.
Conclusion: Our findings suggest that post-transplantation BKV load may be linked to the pre-transplantation BKV levels of the patient. The results of our study provide a potential guide for the identification of patients at high risk of developing BKVN, allowing for development of an optimal strategy for post-transplantation immunosuppressive therapy.
References:
[1] Dall A et al. Clin J Am Soc Nephrol. 2008, 3: S68 –S75
[2] Beimler J et al. Nephrol. Dial. Transplant. 2007, 22(suppl8):viii66-viii71.
[3] Hariharan S. Kidney Int. 2006, 69(4):655-62.

P.1479

A comparison of early postoperative results of living-donor kidney transplantations using calcineurin inhibitors (tacrolimus extended-release formulation vs. cyclosporine)

Osamu Konno, Takayoshi Yokoyama, Yu Kihara, Yuki Nakamura, Takuya Ueno, Hitoshi Iwamoto, Shigeyuki Kawachi.
Digestive and Transplantation Surgery, Tokyo Medical University, Hachioji Medical Center, Tokyo, Japan.

Introduction: Tacrolimus extended-release formulation (TACER) can now be administered once daily, potentially reducing side effect onset and improving user compliance. Few studies have compared cases involving early postoperative use of cyclosporine after kidney transplantation.
Objectives: This study targeted 57 living-donor kidney transplant cases at our facility. Thirty-two cases in which TACER was used (TACER group) were compared with 25 cases where cyclosporine was used (CyA group).
Methods: Function of transplanted kidney, rejection response, onset of infection, dyslipidemia, and glucose tolerance were compared at 1 and 3 months after transplantation. A protocol renal biopsy for additional histopathological examination was taken after 3 months. Drugs used other than calcineurin inhibitors included methylprednisolone, mycophenolate mofetil, and basiliximab; doses for these drugs in all cases were determined based on our department protocols.
Results: No significant differences were observed between the study groups regarding subject background (age, HLA match number, eGFR, 24-hour creatinine clearance). Recipient serum creatinine values during the two measurement periods were as follows: after postoperative month 1: TACER group, 1.20±0.39 mg/dL; CyA group, 1.13±0.40 mg/dL; after postoperative month 3: TACER group, 1.36±0.70 mg/dL; CyA group, 1.21±0.40 mg/dL. eGFR (ml/min./1.73 m2) values were as follows: after postoperative month 1: TACER group, 53.9±18.2; CyA group, 54.18±16.5; after postoperative month 3: TACER group, 51.8±20.6; CyA group, 50.2±16.5. No significant differences in transplanted kidney function were observed between the groups. During the 3-month observation period, acute rejection response was observed in 1 case in the TACER group and 5 cases in the CyA group. The number of dyslipidemia cases increased from 9.4% preoperatively to 31.3% postoperatively in the TACER group, and from 16% to 44.0% in the CyA group. No differences in infections were observed between the groups. HbA1c levels in TACER group subjects changed from 6.6±1.0% preoperatively to 7.5±0.9% after postoperative month 3. HbA1c levels in CyA group subjects changed from 6.0±0.8% preoperatively to 7.3±1.4% after postoperative month 3. No differences between the groups were observed with regard to onset of arteriolar hyalinosis based on histopathological examination.
Discussions: Scattered reports have suggested that TACER improves user compliance and mitigates the appearance of side effects. Although an inhibitory effect on acute rejection responses and a reduction in the number of cases exhibiting dyslipidemia were observed in this study, no differences were observed in transplanted kidney function, glucose tolerance, or infection onset based on histopathological examinations of transplanted kidney biopsies.
Conclusions: Thus, TACER may be effective in the early postoperative phase as a calcineurin inhibitor.

P.1480

Cost of kidney transplant in Colombia

Eyner Lozano.
Surgery, Universidad Nacional de Colombia, Bogota, Colombia.

Background: Kidney transplant improves quality of life and survival when compared to dialysis. Despite advances in immunosuppressant regimens and prevention and treatment of acute rejection, graft survival rates have not improved significantly in the past decade. Although the clinical effectiveness of these regimens has been studied, the impact of changes over time on cost has not.
Methods: Costs of kidney transplant were compared between 2 periods demarcated by a scheduled change from cyclosporine (early) to tacrolimus or m-TOR (late) and from azatioprina to mychophenolate (late) therapy in adult patients receiving a first kidney-only transplant in Bogota Colombia, over a 6-year period.
Results: Recorded complete costs for 6 years after transplant were available for 120 patients, in the period (June 30, 2009 – July 1 2015).70% was male 30% female. Body mass index was less than 18.5 in 5% of patients, more than 18.5 and less than 25 in 60% and, more than 25 in 35%. The age range was from 18 to 70 year, younger than 18 years were excluded. 5% of surgical complications occurred in the immediate post-operative period. 10 % of them was acute rejection diagnosed by kidney biopsy. Graft loss rate was of 20% at five years post-transplant. 30% of patients were admitted to hospital at least once. 5% of patients presented with post-transplant diabetes.
The mean total 3-year cost for recipients of transplant was US$12.100 and $ 7510 in the next 3 year period, this reduction largely attributed to decrease in the cost of immunosuppressant (P< .001). The reason for this reduction was government policy aimed at cost control and rationing of immunosuppressant.
Conclusions: Kidney transplant remains the best therapeutic option for kidney failure patients. Government support on cost control over immunosuppressant resulted in decreased costs. Therefore, kidney transplant continue to be the most cost effective option available for patients.

P.1481

Everolimus/low tacrolimus(TAC) compared to MPA/regularTAC for renal transplantation in the elderly recipient - preliminary analysis of the nEverOld trial

Elias David-Neto[1], Fabiana Agena[1], Fernanda Ramos[1], Ana H.K. Triboni[1], Marcelo Altona[2], Venceslau Coelho[2], Nelson Z.Galante[1], Francine B.C.Lemos[1].
[1]Kidney Transplant Service, Hospital das Clínicas - University of São Paulo School of Medicine, Sao Paulo, Brazil; [2]Department of Geriatrics, Hospital das Clínicas - University of São Paulo School of Medicine, Sao Paulo, Brazil.

In the last decade, elderly patients (≥60y) have been more frequently listed for renal transplantation. From 2007 to 2014 the percentage of elderly renal transplant recipients increased from 11% to 21%, at our center. Elderly recipients are more prone to develop diabetes, Infection, cardio-vascular disease and cancer. The nEverOld trial is an ongoing, single center, prospective randomized trial, comparing conversion to low TAC/Everolimus (EVL) at 1st month with TAC/enteric coated - sodium mycophenolate (EC-MPS) in elderly renal transplant recipients (≥60y). All received induction therapy with a single dose of ATG (2mg/kg). Patients received oral valGCV prohylaxis up to 90 days that was withdrawn when EVL started. So far, 72 (80% of total) patients with a mean age of 65±4 (60-78) years, have been enrolled:39 in the lowTAC/EVL arm and 33 in the TAC/EM-MPS arm. Demographics and RTx features were very similar.TAC trough levels were lower ($p<0.046$) in the EVL arm at 3, 6 and 12 months, as per protocol. At the mean f-up of 425±352 days patient survival (76±8vs77±9%) death-censored graft survival (92±4vs90±7%) and eGFR (MDRD4) (48±19 vs 47±16ml/min/ 1.73m2 did not differ for EVL and MPS arm, respectively. 6/10 patients died before conversion In the EVL arm and 4/6 (before day 30), in the MPS arm. 50% due to infection. Although not statistically significant yet, there were 2 cancers, 2 BK nephropathy and 3 CMV disease in group MPA but only 1 CMV and less patients re-admitted to the hospital in the EVL arm.

	EVL/lowTAC n= 39	MPS/TAC n = 33
Deaths (before conversion or day 30)	10 (6)	6 (4)
Graft loss	3	2
IS change	3	4
Acute Rejection	9 (23%)	6 (18%)
Cancer	0	2
BK nephropathy	0	2
CMV disease	1	3
DM pre/post Tx	14/2	19/3
Number of Patients without Hospitalizations	21(54%)	14 (42%)

P.1482

Is 90-days CMV prophylaxis effective in CMV-seropositive patients receiving thymoglobulin as induction therapy?

Elias David-Neto, Emanoela B.Feitosa, Fabiana Agena, José Otto Reusing, Francine B.C.Lemos.
Renal Transplantation Service, Hospital das Clinicas - University of São Paulo School of Medicine, Sao Paulo, Brazil.

Thymoglobulin (ATG) used as induction therapy is considered a risk factor for CMV disease in renal transplantation (RTx) and prophylaxis is recommended. Our center policy is ganciclovir and/or valGCV prophylaxis for 90 days. However, the outcome of this policy it is not clear. All RTx in CMV-seropositive adults (≥18y) (Jan07-Dec14) with ATG (6mg/kg) as induction therapy (n=544) were retrospectively evaluated using our electronic database. 65 patients were discarded because there were no clear data on prophylaxis duration. Graft losses/death before day 19 (n=7) were also excluded. 472 patients, receiving tacrolimus/MPA and steroids but not mTORi, were analyzed. Prophylaxis was defined as started up to the 7th POday with at least 14 days of medication. CMV disease (CMVd) was considered present if it occurred up to the 2nd year after TX. Two cases with very late episodes (1220 and 2341 days) were not considered CMVd. CMVd was diagnosed when clinically suspected along with a positive PCR/DNAemia or biopsy. 73 patients developed CMVd (17% by Kaplan Meier) and 399 did not. CMVd occurred after pro[hylaxis was finished (160±85 days) and in 95% (69 cases) before day 300. 60 CMVd (82%) were syndrome and 13 (18%) invasive disease (12 G-I and 1 encephalitis). There was no difference in the length of prophylaxis in patients with and without CMVd (78±29 vs74±33 days, respectively). These groups also did not differ in terms of demographics, transplant features and immunosuppressive drugs. At a mean follow-up of 945 days, patient and graft survival did not differ between patients with and without CMVd but eGFR (MDRD4) at last f-up was lower in the CMVd group (44±20 vs 51±21 ml/min/1.73m2, $p=0.013$). Acute rejection (AR) occurred more frequently in patients with CMVd (45% vs 25%, $p<0.001$). 13 patients developed AR after CMVd (median +186 days (64-675) and 22 before CMVd(-117±87days) being 10 AMR (3 treated with ATG and 1 with rituximab. IVIG and plasmapheresis were the main treatments) and 12 ACR (treated with methylprednisolone). These data indicate that CMV prophylaxis seems to reduce CMV disease rate in seropositive patients using ATG as induction therapy. Nevertheless, CMVd develops in 17% of these cases after prophylaxis is withdrawn. Therefore, either the extension of the prophylaxis period or CMV monitoring up to 300 days, for preemptive treatment, should be performed in order to avoid late CMV disease.

P.1483

Outcomes of patients on non-standard immunosuppression after renal transplantation

Asha Zimmerman[1], Reginald Gohh [2], George Bayliss [2], Reza Saidi[1,2], Paul Morrissey[1,2].
[1]Department of Surgery, Alpert Medical School of Brown University, Providence, RI, United States; [2]Division of Organ Transplantation, Rhode Island Hospital, Providence, RI, United States.

Introduction: Protocol regimens for immunosuppression are modified for a variety of reasons including infection, medication intolerance, and malignancy. Decreasing immune suppression is often weighed against the risks of acute rejection and the concern for inferior graft survival. We compared the outcomes of patients on non-standard therapy to controls.
Materials and Methods: We performed a retrospective review of patients undergoing a renal transplant from 2005-2014 who were initiated on tacrolimus, mycophenolic acid and prednisone (TMP). Exclusion criteria were: graft failure within 90 days of transplant, patients not on TMP initially, SPK transplants and those who had a previous transplant. We compared patients on TMP to those who required a change to non-standard immunosuppression (NSI). Student's t-test and Chi-square analysis were used to compare non-categorical and categorical variables, respectively. Kaplan–Meier curves were used to compare graft and recipient survival. Logistic regression was performed to compare the rates of infectious complications, medication intolerance and malignancy. A p value of < 0.05 was considered significant.
Results: We evaluated 426 patients including 111 (26%) on non-standard immunosuppression (NSI). Of these, 54% remained on a three-drug regimen (CAP, CMP, SMP, SAP, TAP; C-cyclosporine, A-azathioprine, S-sirolimus); the remainder were maintained on a two-drug therapy (AP, MP, SM, SP, TM, TP). The rationale for modifying therapy included infectious complications (58%), medication intolerance (50%) and malignancy (21%). Three hundred and fifteen patients in the same era were maintained on TMP. Mean follow-up for the two groups was 63 months for TMP and 73 months for NSI. Patient characteristics were similar between TMP and NSI groups in regards to age (49 vs 52, NS), sex (65% male vs 57% male, NS), incidence of diabetes (27% vs 23%, NS), proportion live donor recipient (43% vs 43%, NS), and nadir creatinine (1.36 vs 1.30, NS). The rate of acute rejection (13% vs 16%, NS) did not differ. There was no difference in graft survival (Figure 1) or patient survival (Figure 2) between the groups. Renal function did not differ between TPM and NSI (Cr 1.32 vs 1.43, NS).
Discussion: Changes in initial immunosuppression are frequent after renal transplantation; however, any change or reduction in immunosuppression carries a risk of new complications or acute rejection. We demonstrated in a cohort of 426 first renal transplant recipients initiated on TMP that changes in the regimen are well tolerated and did not affect intermediate-term outcomes.
Conclusion: In certain clinical scenarios where patients cannot tolerate TMP, altering immunosuppression appears to be a safe alternative that does not increased risk of graft failure.

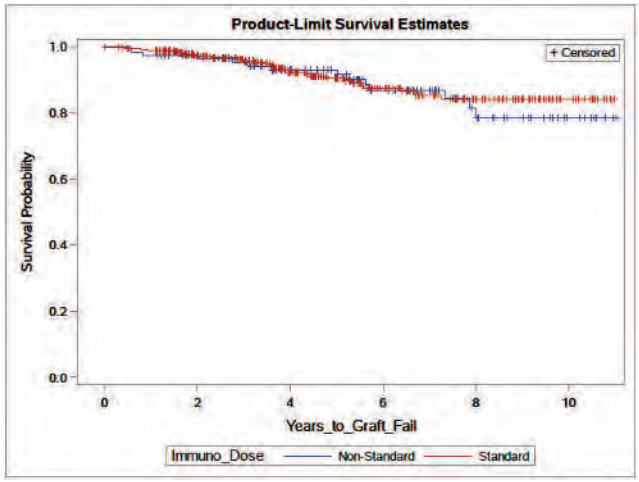


Figure 1. Kaplan–Meier Curve for Graft Survival
p = 0.88

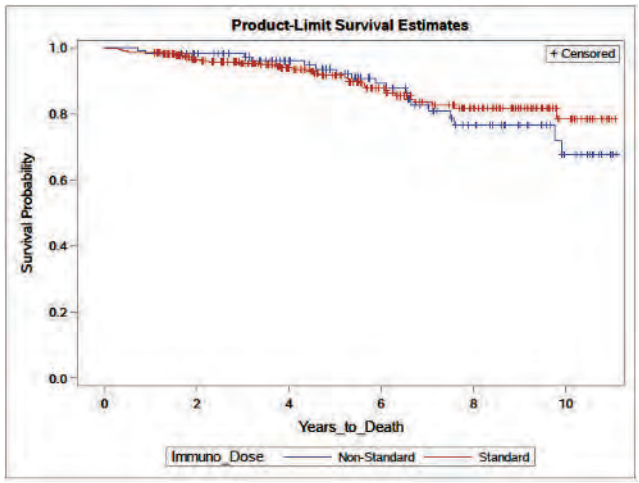


Figure 2. Kaplan–Meier Curve for Patient Survival
p = 0.82

P.1484

Impact of conversion from original MMF to generic MMF in kidney transplant recipients: a single-center experience

Hajime Hirano[1], Tomohisa Matsunaga[1], Hayahito Nomi[1], Ryouichi Maenosono[1], Takuya Tsujino[1], Yuki Yoshikawa[1], Taizo Uchimoto[1], Kenkichi Saito[1], Naoki Tanda[1], Hirosato Takai[1], Kiyoshi Takahara[1], Teruo Inamoto[1], Satoshi Kiyama[1], Hideki Matsumura[2], Akira Ashida[2], Haruhito Azuma[1].
[1]Department of Urology, Osaka Medical College, Takatsuki, Japan; [2]Department of Pediatrics, Osaka Medical College, Takatsuki, Japan.

Background: Recently, more and more generic drugs have been used for immunosuppressive drugs in the field of organ transplantation. Some reports have indicated that blood concentration of most generic drugs is difficult to be stable, and it may cause the difference in graft survival of transplanted organs between original drugs and generic drugs. In this article, we report the cases which could not maintain blood concentration of generic drugs of mycophenolate mofetil (MMF).
Results: In 4 cases out of 5 cases that we had to change original MMF to generic MMF, there were cases that blood concentration level was not stabilized. There were possibility that the lowered blood concentration level of MMF caused a rejection, in two cases. Mean MMF trough level wase decreased from 3.6±1.9μg/ml to 0.6±0.4μg/m. Due to the early detection, it did not become severe or failure of graft function, however, we cannot deny the possibilities that side effects were increased and rejection rose. In these cases, we discontinued to use the generic drugs thereafter due to unstable plasma concentration of MMF.
Discussion: Some reports have indicated that failure to maintain plasma concentration of MMF leads to rejection. Therefore, maintenance of effective plasma concentration and prevention of rejection are essential to long-term graft survival in kidney transplant.
Conclusion: Conversion to the generic drug,it may cause differences in effects and absorption. If the generic drug should be used, patients should be closely monitored.

P.1485

What has changed in renal transplantation in some small islands in Japan? Strategy of our center

Hajime Hirano, Hayahito Nomi, Tomohisa Matsunaga, Ryouici Maenosono, Yuki Yoshikawa, Takuya Tsujino, Taizo Uchimoto, Kenkichi Saito, Hirosato Takai, Naoki Tanda, Kiyoshi Takahara, Teruo Inamoto, Satoshi Kiyama, Haruhito Azuma.
Department of Urology, Osaka Medical College, Takatsuki, Japan.

Background: Okinoerabu Island and Tokunoshima Island, lying in the sea to the south of Japan, about 100 km north of Okinawa and about 500 km south of Kyushu.
There were no facilities that specialize in kidney transplants, so the patients needed to leave the island to undergo the procedure, and up to a few years ago, there were only 2 or 3 kidney transplant patients on the island.
Objective: We report the status of transplant medicine on remote islands, including concrete methods for periodic examinations and how to handle emergencies.
Methods: Mean recipient age was 60.0±8.9 years(mean ±SD); 15 were males and 10 were females. Mean donor age was 57.9 ± 8.48 years(mean ±SD); 14 were males and 11 were females. The commonest recipient diseases leading to ESRD were diabetes (36.0%), chronic glomerulonephritis (28.0%), and ADPKD (12.0%). Mean dialysis duration pre-transplantation was 382.6 ± 233.2 days(mean ±SD).
Results: We physicians specializing in kidney transplants formed an alliance with local facilities a few years back to create specialized outpatient facilities, the number of transplant patients has gradually increased. Delayed graft function was observed in only one patient. and biopsy-proven acute rejection in 8 cases, and chronic allograft nephropathy in 2cases. In these cases, the local doctor perform the treatment in their facilities under the direction of our treatment.It is usually done safely and successfully. The mean follow-up period is 1208±1809 days, and not a one of the patients has had extinction of graft loss, with mean SCr (serum Cr level) of 1.35 ± 0.85 mg/dl.
Conclusions: To coordinate medical care with their primary care physician,we physicians specializing in kidney transplants no longer need to force to travel a long distance to receive a follow-up outpatient.Nowadays, likelihood of kidney transplantation has been much higher among these islands. The number of transplant patients has gradually increased.

P.1486

Excellent antirejection prophylaxis and low incidence of infections after kidney transplant with low dose rabbit antithymocyte globulin induction therapy in high immunologic risk patients

PedroW. Baron[1], Sergio Infante[2], Jill Weissman[3], Marina Karmanova[1], Arputharaj Kore[1], Philip Wai[1], Lawrence Beeson[4], Michael de Vera[1].
[1]Surgery/Transplantation Institute, Loma Linda University Medical Center, Loma Linda, CA, United States; [2]Medicine, Loma Linda University Medical Center, Loma Linda, CA, United States; [3]Pharmacy, Loma Linda University Medical Center, Loma Linda, CA, United States; [4]Center for Nutrition, Healthy Lifestyle and Disease Prevention, Loma Linda University, Loma Linda, CA, United States.

Introduction: Rabbit antithymocyte globulin (rATG) is the most common prophylactic therapy for acute rejection used in kidney transplant with excellent results[1]. Infections post-transplant are noted to increase after the use of more than 7 mg/kg[2],[3]. Basiliximab is also used to prevent acute rejection with inferior results to rATG[1].
Material Methods: We retrospectively reviewed the data of patients who underwent first-kidney transplant and received rATG or basiliximab between January 2008 and December 2012. They were divided into 3 groups, r ATG dose < 3 mg/kg (n = 79), rATG dose > 3 mg/kg (n = 223) and basiliximab (n = 37). The following variables were analyzed: age, sex, ethnicity, cold ischemia time (CIT), delayed graft function (DGF), type of donor, panel reactive antibody (PRA), acute cellular rejection (ACR), bacterial, viral and fungal infections, and serum creatinine level, graft survival (GS) and patient survival (PS) at 1 year. High immunologic risk patients received rATG including African Americans, PRA> 20 %, cold ischemia time > 24 hours, recipients of donation after cardiac death and expanded criteria donors. Low immunologic risk patients, received basiliximab. rATG was given at a dose of 1.5 mg/kg in the operative room and a day later. Patients whose their CD3 T cell absolute count was > 20 cells/mm3 after the second dose received a third dose. All patients received tacrolimus, mycophenolate mofetil and prednisone. Comparisons of means for continuous variables were done via ANOVA and for categorical variables using the chi-square statistic. Analyses were performed using IBM SPSS 23 (IBM, Armonk, NY).
Results: CIT was significantly different between groups (p = 0.009). More than one third of the patients in the rATG groups had PRA > 20 % (35.5% and 42 %, respectively, p = 0.013) in contrast to the Basiliximab group, 88.6% had PRA < 20 %. Patients with high risk for DGF received rATG (p = 0.03). No significant differences were found in infection rates, ACR, GS and PS at 1 year (Table 1).
Discussion: These data showed the use of low dose thymoglobulin or basiliximab in patients with high or low immunologic risk, respectively, achieved low ACR and infectious complication rates as well as excellent GS and PS. Brennan et al. compared rATG to basiliximab in patients at high risk for ACR or DGF. The rATG group as compared with the basiliximab group had lower incidence of ACR episodes and greater incidence of infections. Patient and graft survival rates were similar[1]. Clesca et al. reported the incidence of infectious complications increased when the total dose of rATG was > 7 mg/kg[2].
Conclusion: Low dose rATG or basiliximab did not increase the incidence of infections, achieved excellent acute rejection prophylaxis and short-term outcomes in high or low immunologic risk patients, respectively.

Table 1.

1-year (%)	rATG dose < 3 mg/kg (n = 79)	rATG dose 3 > mg/kg (n = 223)	Basiliximab (n = 37)	P-value
CMV infection	11.4	13.0	5.4	0.41
GS	91.5	87.6	100	0.12
PS	95.8	90.4	96.8	0.31

References:
[1] Wong W. Comparison of two dosages of thymoglobulin used as a short-course for induction in kidney transplantation. Transplant International. 2006; 19: 629-635
[2] Clesca P. Thymoglobulin and rate of infectious complications after transplantation. Transplant Proc. 2007; 39; 463
[3] Brennan DC. Rabbit antithymocyte globulin versus basiliximab in renal transplantation. N Engl J Med. 2006; 355: 1967-1977

P.1487

Outcome of deceased donor renal transplantation patients who underwent induction therapy with basiliximab and rabbit anti-thymocyte globulin at National Kidney and Transplant Institute from January 2007 to December 2011

Joel Benavidez, Adolfo C.Parayno, Marc Anter E.Mejes, Benito V.C.Purugganan.
Organ Transplantation and Vascular Surgery, National Kidney and Transplant Institute, Quezon, Philippines.

Introduction: This study aimed to review and compare immediate vs. delayed graft function in patients recipients undergoing deceased donors renal transplantation given with Basiliximab and rabbit Anti-thymocyte globulin (rATG) induction therapy at the National Kidney and Transplant Institute (NKTI) from January 2007 to December 2011.
Materials and Methods: This is a retrospective cross-sectional study of deceased donor renal transplantation done within 5-year duration at NKTI. Chart review was done for data collection pertinent for this study.
Results and Discussion: A retrospective study of **165** deceased donor renal transplantation transplanted recipients were studied at NKTI, **116 (70.30%)** patients were given Basiliximab and **49 (29.70%)** had rATG induction therapy. In patients given Basiliximab the incidence of DGF was **15.6%** as compared to **22.5%** for rATG *p value of 0.370.*

IMMEDIATE VERSUS DELAYED GRAFT FUNCTION ACCORDING TO INDUCTION THERAPY

Induction Therapy	Graft Functioning		Total	P-value
	Immediate	Delayed		
Basiliximab	98 (84.5)	18 (15.5)	116 (70.3)	0.370
rATG	38 (77.6)	11 (22.5)	49 (29.7)	
Total	136 (82.4)	29 (17.6)	165 (100.0)	

In the early post-transplant period, there was only 1 graft loss in the rATG group. After 6 months, 4 and in 1 year 7 had developed graft loss. After 3 years, patients with graft loss increased to 12. In 3 months time 1 patient died. After 6 months, 4 patients had expired. The number of deaths had increased to 5 after 1 year and 9 after 3 years. In 3 years time interval, the proportion of deaths receiving rATG was higher than those given Basiliximab **(10.34% vs 8.33%).** Out of **103** patients observed, **9 (8.74%)** deaths were recorded. Majority of the patients who died **(n=87;84.47%)** had a functioning graft. The proportion of deaths in Basiliximab was higher than those in rATG **(10.00% vs 8.22%).** The proportions of those having graft loss in both therapies were approximately the same **Basiliximab (6.67%)** vs **rATG (6.85%).**

Incidence of graft outcomes between Basiliximab and rATG (*Anti-thymocyte globulin*) induction therapy at 3 months, 6 months, 1 year and 3 years

Induction Therapy	Time Interval	Graft Outcome		Total	OR (90%-CI)	P-value
		Graft Functioning	Graft Loss			
Basiliximab	3 months	103 (100.00)		103 (69.13)		0.309
rATG		45 (97.83)	1 (2.17)	46 (30.87		
Total		148 (99.33)	1 (0.67)	149 (100.00)		
Basiliximab	6 months	89 (97.80)	2 (2.20)	91 (68.42)	1.0	0.421
rATG		40 (95.24)	2 (4.76)	42 (31.58)	2.2 (0.4, 11.9)	
Total		129 (96.99)	4 (3.01)	133 (100.00)		
Basiliximab	1 year	85 (96.59)	3 (3.41)	88 (69.29)	1.0	0.119
rATG		35 (89.74)	4 (10.26)	39 (30.71)	3.2 (0.9, 11.9)	
Total		120 (94.49)	7 (5.51)	127 (100.00)		
Basiliximab	3 years	60 (90.90)	6 (9.10)	66 (69.50)	1.0	0.488
rATG		25 (86.20)	4 (13.13)	29 (30.50)	1.6 (0.5, 5.0)	
Total		85 (89.5)	10 (10.5)	95 (100.00)		

Conclusion: In conclusion, study showed no significant difference in short and long term graft and patient outcomes between induction therapies with rATG vs Basiliximab in recipients with deceased donors. These observations may be confounded by the small number of patients in each cohort and overshadowed by the excellent graft and patient survival outcomes in the institution regardless of the induction therapy used.

Keywords: *Basiliximab, rATG, Anti-thymocyte globulin, Deceased Donor Renal, Transplantation.*
Department of Organ Transplantation and Vascular Surgery- NKTI; I would like to show my gratitude to the National Kidney and Transplant Institute and Dr. Benito VC. Purugganan,Jr. for their full support throughout the study and for providing insight and expertise that greatly assisted the research.
We are also immensely grateful to Dr. Servando Simangan, Dr. Leo Baloloy and Dr. Arwin Ronsayro for comments that greatly improved the manuscript.
Special thanks to Dr. Marc Anter Mejes and Dr. Dennis Serrano for your patience and guidance in correcting our paper.
Mr. Aristotle Magallenes for guiding us with the statistical aspect of the study.

References:

[1] Kahan BD, " Individuality: The Barrier to Optimal Immunosuppression", *Nat Rev Immunol*, 2003;3(10):831
[2] Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group, "KDIGO clinical practice guideline for the care of kidney transplant recipients", *Am J Transplant.* 2009;9Suppl 3:S1
[3] Webster AC, Playford EG, Higgins G, Chapman JR, Craig JC, "Interleukin 2 Receptor Antagonists for Renal Transplant Recipients: a Meta-analysis of Randomized Trials", *Transplantation*. 2004;77(2):166
[4] Szczech LA, Berlin JA, Aradhye S, Grossman RA, Feldman HI, "Effect of anti-lymphocyte Induction Therapy on Renal Allograft Survival: a Meta-analysis", *vJAm Soc Nephrol.* 1997;8(11):177
[5] Szczech LA, Berlin JA, Feldman HI, " The Effect of Anti-lymphocyte Induction Therapy on Renal Allograft Survival: A Meta-analysis of Individual Patient-level Data. Anti-Lymphocyte Antibody Induction Therapy Study Group", *Ann Intern Med.* 1998;128(10):817.
[6] Bourdage JS, Hamlin DM. Comparative polyclonal antithymocyte globulin and antilymphocyte/antilymphoblast globulin anti-CD antigen analysis by flow cytometry.*Transplantation*1995;59:1194-1200
[7] Zand MS, Vo T, Huggins J, et al. Polyclonal rabbit antithymocyte globulin triggers B-cell and plasma cell apoptosis by multiple pathways.*Transplantation* 2005;79:1507-1515
[8] Michallet MC, Preville X, Flacher M, Fournel S, Genestier L, Revillard JP. Functional antibodies to leukocyte adhesion molecules

in antithymocyte globulins. *Transplantation* 2003;75:657-662
[9] Bonnefoy-Berard N, Revillard JP. Mechanisms of immunosuppression induced by antithymocyte globulins and OKT3. *J Heart Lung Transplant* 1996;15:435-442
[10] Woodside KJ, Hu M, Meng T, Hunter GC,Sower LE, Daller JA. Differential effects of interleukin-2 blockade on apoptosis in naive and activated human lymphocytes. *Transplantation* 2003;75:1631-1635
[11] Kahan, B.D., Rajagopalan P.R. and Hall M., *Transplantation*, 67, 276-284 (1999)
[12] Bacigalupo A, Lamparelli T, Bruzzi P, et al. "Antithymocyte Globulin for Graft-versus-host Disease Prophylaxis in Transplants from unrelated Donors: 2 randomized studies from GruppoItalianoTrapiantiMidollo Osseo (GITMO)", *Blood98* (10): 2942–7. DOI:10.1182/blood.V98.10.2942. PMID 11698275 (November 2001)
[13] Y.Lui, P. Zhou, M. Han, C-B, Xue, X.P. Hu, C. Li "Basiliximab or Antithymocyte Globulin for Induction Therapy in Kidney Transplantation: A Meta-analysis", *Transplantation Proceedings*, (June 2010), Vol.42 (5): 1667-1670
[14] D. Brenan, J. Daller, K. Lake, D. Cibrik, D. Del Castillo, "Rabbit Antithymocyte Globulin versus Basiliximab in Renal Transplantation", *N Engl J Med 2006*; 355: 1967-1977 (November 9, 2006)
[15] Moore R., Amlot P., Schmidt A.-G., Abeywickrama K. and Soulillou J.-P., Lancet. "Basiliximab versus placebo for control of acute cellular rejection in renal allograft recipients" *Transplantation* 350, 1193-1198 (1997)

P.1488

Non induction therapy in low risk living related kidney transplantation in India

Sudhir G.Kulkarni[1], Kshitija G. Gadekar[1], Nilrohit B.Paike[1], Nilesh Bhange[1], Abhay Mahajan[2], Dhruti Amlani[2].
[1]Nephrology, MGM Medical College, Aurangabad, India; [2]Urology, MGM Medical college, Aurangabad, India.

Introduction: The optimal prophylactic induction immunosuppressive therapy to prevent kidney transplant rejection remains controversial[1],[2]. Induction therapy increases cost of transplantation in low risk individuals, more so in economically constrained population in developing countries. The safety and efficacy of non induction therapy in low risk living related kidney transplantation was analysed retrospectively in the present study.
Material and Method: 25 patients who underwent living related kidney transplant without induction were analysed for the outcome of kidney transplant. All patients had 50% or more HLA match with negative lymphocyte crossmatch. DSA was not performed. According to TTS Protocol patients recieved Tacrolimus and mycophenolic acid 1 day prior to transplant in the dose of 0.06 mg/kg and 720 mg repectively as a single dose in evening.
From the day of Transplant patients recieved Tacrolimus 0.06 mg/kg & Mycophenalic acid 720 mg both twice a day.
Patients received methylprednisolone 500 mg during the surgery and 100 mg 6 hours after the surgery. From 1st day of surgery patients recieved 20 mg of oral prednisolone in divided doses upto 15 days and then Prednisolonoe was tapered to 15 mg per day upto 3 months.
Patients recieved prophylactic IV antibiotic cefuroxime 750 mg twice a day for 7 days. Routine antihypertensives, antidiabetics, antacids were given. CMV prophylaxis was given to the patients who were D+R-.
Patients recieved normal saline & 5% dextrose as replacement fluid depending on central venous pressure.
Results: All patients had good urine output immidiately post operative period. There was no patient with delayed graft function. 3(12%) patients had rejection. 1 patient had AMR who had marginal donor(old age) and 2 had T cell mediated rejection within 15 days. 4(16%) patients had ATN but didnt need dialysis. 4(16%) patients had infective episodes, 1 had CMV diarrhoea, 1 had bacterial pneumonia and 2 had diarrhoea due to Isospora Belli. 2(8%)patients died,one bacause of bacterial pneumonia after one month and other because of post transplant diarrhea after 3 months. No patient had tacrolimus toxicity. 3(12%)patients had NODAT within one month. 6(24%) patients had thrombocytopenia not requiring platelet transfusion.
Conclusion: Non induction in low risk living kidney transplant is safe and effective and is associated with low rate of rejection and infection. Thus non induction policy may be followed in economically constrained patients in developing country. The cost of kidney Transplant without induction is $3000, out of which $2000 is supported by State Government. The cost of Kidney Transplantation with indcution increases by $2000 which patient has to pay from his own expenses. Thus although our study is limited to a small number of patients, this should encourage Indian nephrologists to use non induction therapy for kidney transplant in poor patients. A larger study is desirable for drawing definite conclusion.

TABLE SHOWING EVENTS

SR. NO.	EVENTS		NO. OF PATIENTS	PERCENTAGE
1	ANTIBODY MEDIATED REJECTION		1	4%
2	ACUTE T CELL MEDIATED REJECTION		2	8%
3	ACUTE TUBULAR NECROSIS		4	16%
4	INFECTIONS	CMV INFECTIONS	1	4%
		BACTERIAL PNEUMONITIS	1	4%
		DIARRHEA- ISOSPORA BELLI	2	8%
5	TACROLIMUS TOXICITY		0	NIL
6	THROMBOCYTOPENIA NOT REQUIRING TRANSFUSION		6	24%
7	NODAT		3	12%

Dr. Ajit Shroff, Dean, MGM Medical College, Aurangabad

References:

[1] Mourad, Georges; Garrigue, Valérie; Squifflet, Jean-Paul; et al. Induction Versus Non Induction in Renal Transplant Recipients with Tacrolimus-Based Immunosuppression; *Transplantation*: 27 September 2001 - Volume 72 - Issue 6 - pp 1050-1055

[2] Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group. KDIGO clinical practice guideline for the care of kidney transplant recipients. *Am J Transplan*t 2009; 9 Suppl 3:S1.

P.1489

After alemtuzumab induction, Tac+MMF is superior to CsA+MMF in pediatric kidney transplantation

Michael Kaabak[1], Nadeen Babenko[1], Allan Zokoyev[1], Ron Shapiro[2]. [1]Kidney Transplant, Boris Petrovsky Scientific Center of Surgery, Moscow, Russian Federation; [2]Kidney Transplant, Mount Sinai Hospital, New York, NY, United States.

Introduction: Recipient parenchymal lymphatic cells are crucial for direct and indirect pathways of allorecognition. We proposed that administering alemtuzumab several weeks pretransplantation could eradicate peripheral lymphatic cells and promote donor-specific acceptance.

Method: This was a single center, retrospective review of 101 consecutive living-donor kidney transplantations in pediatric patients age seven month to 18 years, performed between September 2006 and April 2010 and described previously in details[1].

Immunosuppression (IS) protocol included two 30 mg doses of alemtuzumab, the first dose administered 12–29 days prior to transplantation and second at the time of transplantation. Maintenance IS was based on combination of a CNI and mycophenolate. Maintenance steroids were tapered off in 96% of patients 7±6·4 days after transplantation. Patients were followed for 7·2±1·0 years, and 412 protocol biopsies were taken one month, and one, three, and five years post transplant.

The choice of CNI (cyclosporine or tacrolimus) was initially dependent on drug availability; beginning in April 2009, we began a randomized trial of cyclosporine vs. tacrolimus (clinicaltrials.gov identifier: NCT01346397). The trough levels of tacrolimus were adjusted to 2-8 ng/ml starting from day 11. The levels of cyclosporine were measured as a 12 hour trough and at one and three hours after administration of the dose and adjusted for the target area under the curve (AUC) 1500-2000 ng/ml/hours and C0 50-100 from day 31.

Results: The 7 year Kaplan-Meier graft survival and graft function was statistically significantly better in tacrolimus group. There were trend to better patient survival and lower blood pressure and proteinuria in tacrolimus group (table).

	7 year survival, %		Proteinuria, mg/24 h	Blood creatinine, mg%	GFR, Schwartz	Blood pressure	
	graft	patient				systolic	diastolic
cyclosporine, n=63	71.9±4.8	88.1±4.1	414±801	1,6±1	62±29	116±20	73±10
p	0.04	0.06	0.05	0.02	0.01	0.3	0.06
tacrolimus, n=36	85.2±8	97.2±5.3	127±99	1,2±0.4	82±33	112±9	69±8

Conclusion: Alemtuzumab pretreatment prior to LRD kidney transplantation, followed by maintenance immunosuppression with tacrolimus and MMF, is associated with reasonable long-term results in pediatric patients.

References:

[1] Kaabak M. Alemtuzumab induction in pediatric kidney transplantation. Pediatric Transplantation. 2013; 17: 168-178

P.1490

Antibody induction therapy for kidney transplant before the introduction of Thai transplant care

Krit Pongpirul[1,2], Wannarat Pongpirul[3], Adisorn Lumpaopong[4], Yingyos Avihingsanon[5].
[1]Department of Preventive and Social Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand; [2]Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States; [3]Department of Medicine, Bamrasnaradura Infectious Diseases Institute, Nonthaburi, Thailand; [4]Department of Pediatrics, Phramongkutklao Hospital, Bangkok, Thailand; [5]Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

Background: Antibody induction therapy has based partly on varying level of evidence but mostly on clinical judgment and/or financial concern. Under the Thai Transplant Care (TTC) launched in 2014 based on international standards then systematically revised by clinical experts and representatives of national health insurers; risk categorization was introduced for scenario-based estimation of resource need and utilization. To better estimate potential clinician compliance, this study explored antibody induction therapy prescribed before the launch of TTC.
Methods: All kidney transplant (KT) data in the Thai Transplant Registry were analyzed. Each record was categorized into Very Low (VLR), Low (LR), Moderate (MR), High (HR), and Very High Risk (VHR), based on the TTC criteria (Table 1). The number of VHR could be underestimated, as positive crossmatch was not allowed in the current transplant policy. As part of the HR criteria, expanded criteria donor (ECD) included (1) donor age at least 60 years or (2) donor age 50-59 years with two of the followings: donor creatinine > 1.5 mg/dl, donor deceased from cerebrovascular accident, and hypertensive donor.
Results: A total of 5,140 KT were performed during 1987-2015. Of 4,257 KT with supporting data, VLR, LR, MR, HR, and VHR contributed 10.25%, 10.57%, 47.45%, 27.14%, and 4.6%, respectively. Overall cold ischemic times (CIT) for LRKT and CDKT were 2.36 and 19.04 hours, respectively. CIT significantly varied across TTC risk groups ($p<0.001$): VLR 1.04, LR 14.80, MR 7.69, HR 14.66, and VHR 6.41 hours. Delay graft function (DGF) was found in 20.72%, with variation across risk groups: VLR 5.44%, LR 29.04%, MR 20.06%, HR 25.41%, and VHR 11.18%. Mean serum creatinine at 12 months post transplant were 1.48 (95%CI: 1.44-1.52), with no differences between risk groups ($p=0.67$).
Although not recommended, 19.21% of VLR cases received induction therapy (Table 3). IL-2RA was used in only 22.52% of LR cases. While induction therapy was recommended in patients with at least moderate risk, 49.78% MR, 43.92% HR, and 60.94% VHR cases received no induction therapy.
Conclusion: Compliance to TTC's recommendation for antibody induction therapy seems promising. While short term graft function varied by risk groups, mean serum creatinine at one year post transplant were comparable, reflecting successful risk-specific management.

Table 1 TTC 2014 Risk Categorization & Antibody Induction Therapy Regimen

Risk Group	Detail	IL-2RA	ATG	Precondition
Very Low Risk (VLR)	• 1st LRKT 0 mismatch and PRA 0%	N*	N*	N*
Low Risk (LR)	• 1st LRKT > 0 mismatch (except HLA-DR, HLA-DQ, HLA-DP mismatch) and PRA 0-10% or • 1st CDKT 0 mismatch and PRA 0-10%	Y [2B]	N*	N*
Moderate Risk (MR)	• 1st LRKT >= 0 mismatch and PRA 10-50% or • 1st CDKT >= 0 mismatch and PRA 10-50% or • HLA-DR mismatch or • HLA-DQ or HLA-DP mismatch	Y [1B]	Y [2B]	N*
High Risk (HR)	• Retransplantation • 1st LRKT >= 0 mismatch and PRA >50% or • 1st CDKT >= 0 mismatch and PRA >50% • 1st CDKT (Expanded Criteria Donor) or • Recipient Age <= 20 years or • Positive Specific Antibody from Luminex PRA and Negative CDC-AHG Cross Match	Y [2B]	Y [2B]	Y [2D]
Very High Risk (VHR)	• Positive Cross Match or • ABO Blood Group Incompatibility	Y [2C]	Y [2B]	Y [2A]

Source: Thai Transplant Care, 2014

Table 2 Actual Practice of Antibody Induction Therapy by TTC Risk Groups

Risk Group	N	None	IL-2RA	ATG	Others
VLR	432	349	67	15	1
LR	453	308	102	39	4
Living	138	92	37	7	2
Cadaveric	315	216	65	32	2
MR	2,021	1,006	867	130	18
Living	1,227	640	509	66	12
HLA-DR,DQ,DP	1,208	629	502	65	12
Deceased	697	325	303	63	6
HLA-DR,DQ,DP	659	307	288	58	6
HR	1,159	509	507	131	12
Prior KT	177	31	83	62	1
Living, PRA>50	217	152	58	7	0
Deceased, PRA>50	161	50	95	15	1
Deceased, ECD	283	101	130	49	3
Age<20	256	130	108	11	7
VHR	192	117	68	5	2

P.1491

Antithymocyte globulin induction prescription heterogeneity. Results of an observational study on the French DIVAT kidney transplant network

Chapal Marion[1,2], Magali Giral[2], Yohann Foucher[3], Sandra Attard[3].
[1]Service de Néphrologie et Hémodialyse, CHD Vendée, La Roche sur Yon, France; [2]ITUN and Inserm U1064, Nantes University, Nantes, France; [3]SPHERE-EA4275, Nantes University, Nantes, France.

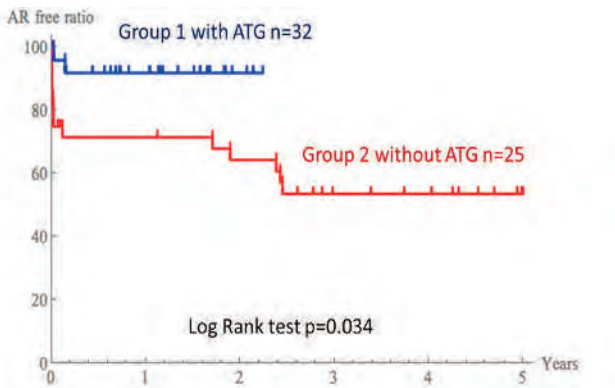
Background: In kidney transplantation, while it is mainly admitted to prescribe depleting induction therapy such AntiThymocyte Globulins (ATG) in high immunological risk patients[1],[2], there is currently no consensus in low immunological risk patients[3].
Methods: Our aim was to analyse habits of induction prescriptions by analysing 2601 recipients of kidney transplant from deceased donor of 6 different French transplantation centres belonging to the DIVAT cohort (www.divat.fr) and transplanted between 2007 and 2014. We performed a logistic regression to study parameters associated with depleting (ATG) or non-depleting (anti IL2R receptor or no induction) in each of the six transplantation centers.
Results: Depleting induction was prescribed in 56% of the recipients, ranging from 29% to 85% among the 6 centres. Ten parameters significantly associated with ATG induction therapy: recipient age, gender and hypertension history, anti HLA class I and II immunization, initial kidney disease, donor age and creatininemia, HLA-A-B-DR incompatibilities and cold ischemia time. But the associations between these parameters and the induction choice were highly different between centers. Within each center, we also underlined high differences in the standardisation of the induction protocol with a concordance index (AUC) ranging from 0.64 (difficult prediction of the induction choice according to the patient characteristics) to 0.98 (quasi-perfect prediction of the induction choice according to the patient characteristics)
Conclusion: ATG as induction therapy in kidney transplantation is highly heterogeneous inter- and intra-centres. Regarding the possible serious adverse events of patients wrongly treated by ATG and the loss of chance for patients wrongly indicated for non-depleting therapy, it appears urgent to provide clear recommendations related to the induction therapy choice.
References:
[1] Noel, C., et al., Daclizumab versus antithymocyte globulin in high-immunological-risk renal transplant recipients. J Am Soc Nephrol, 2009. 20(6): p. 1385-92.
[2] Brennan, D.C., et al., Rabbit antithymocyte globulin versus basiliximab in renal transplantation. N Engl J Med, 2006. 355(19): p. 1967-77.
[3] Mourad, G., et al., The role of Thymoglobulin induction in kidney transplantation: an update. Clin Transplant, 2012. 26(5): p. E450-64.

P.1492

Efficacy of administration of rabbit anti thymocyte globulin as induction therapy in living-donor kidney transplant sensitized patients

Toru Murakami, Kazuhiro Iwadoh, Yuichi Ogawa, Katsuyuki Miki, Kotaro Kai, Akihito Sannomiya, Kumiko Kitajima, Takeshi Hachisuka, Ichiro Koyama, Ichiro Nakajima, Shohei Fuchinoue.
Kidney Center Surgery, Tokyo Women's Medical University Hospital, Tokyo, Japan.

Objective: The aim of this study was to evaluate the efficacy of immunosuppression induction therapy using rabbit anti thymocyte globulin (ATG) for living-donor kidney transplant (LDKT) sensitized patients.
Methods: Retrospectively, 57 recipients of LDKT, who had preformed donor-specific HLA antibodies (DSA), with average age of 50.5±12.4 (24 males and 33 females), were included in the study. All patients received desensitization protocol which contains double filtration plasma pheresis (DFPP) for the purpose of the antibody removal and/or a ritximub (100mg) at four days before transplantation. Recipients were divided into 2 groups, Group 1 (n=32): with ATG administration (1.5 mg/kg/day 4days) and Group 2 (n=25): without ATG administration. Occurrence of acute antibody mediated rejection (AMR), graft-survival rates (GSR) and eGFR at 2 years were compared between them.
Results: 2-year AMR free ratio was 92.0% in Group 1 and 64.4% in Group 2 (p=0.034). 2-year GSR in Group 1 and 2 were 92.9% and 93.7%, respectively (p=0.83), and 2-year eGFR values were 48.9±0 and 46.2±11.4 mL/min (p=0.21).
All AMRs responded to treatment consisted of DFPP, intravenous immunoglobulin, and ATG. There was no serious side effect which requires discontinuation of ATG.



Conclusinon: In LDKT patients with preformed DSA ATG administration as an induction therapy contribute to control the onset of the AMR at early period after transplant. However in terms of GSR at 2 years, there was no difference between both groups, further studies requires to evaluate efficacy long term period.

P.1493

Experience of 120 cases using antithymocyte globulin as an induction immunosuppressant in kidney transplantation in Korea

Mi-Hyeong Kim, Kang-Woong Jun, Hyun-Kyu Kim, Young-Hwa Kim, Kyungjai Ko, Jeong-Kye Hwang, Sang-Dong Kim, Sun-Cheol Park, In-Sung Moon, Ji-Il Kim.
Department of Surgery, College of Medicine, The Catholic University of Korea, Seoul, Korea.

Introduction: Antithymocyte globulin (ATG) is used as induction immunosuppressant in kidney transplantation. We have 120cases of ATG induction cases and plan to report our experience.
Methods: ATG was used in three types of indication; first, for sensitized recipient of living donor KT (LDKT), second, recipients for deceased donor KT (CDKT) with high value of panel reactive antibody test (PRA ≥ 50%) and third, KT from extended criteria donor (ECD). CMV prophylaxis was done with gancyclovir for 5days between August, 2012 to December, 2013 (period 1, 39 cases, 33.6%). And after January 2014, we used ganciclovir during admission (for 14 days) and switched valganciclovir for 14 days at discharge. (period 2, 77 cases, 66.3%).
Results: Incidence of delayed graft function and rejection was acceptable. (Table 1) Cytomegalovirus (CMV) infection was common especially in period 1. Insufficient CMV prophylaxis during period 1 was analyzed as a risk factor of CMV infection in multivariate regression test ($p < 0.001$) Overall graft survival was 97.3% and according to subtypes of indication, 97.9% in ECD KT, 95.7% in high risk LDKT and 100% in CDKT in ECD with high PRA. (log rank test, p=0.723)
Discussion: This study has limitation due to small number of and retrospective, non-comparative study. Rejection and delayed graft function incidence was not able to analyzebecause of small number of events. We used insufficient CMV prophylaxis period 1 and suboptimal in period 2 for high cost of valgancyclovir, but we need to extend the duration of prophylaxis.
Conclusion: ATG can be effective immunosuppressant in immunologically high risk KT and KT from ECD, but sufficient period of prophylaxis for CMV infection is mandatory.

Table 1. Characteristics of enrolled 116 patients

Male	63 (45.6%)
Age	50±9.66
ATG indication	
Living, immunologic high risk (HR)	27 (23.2%)
Cadaver, HR	20 (17.2%)
Cadaver, extended criteria donor (ECD)	51 (43.9%)
Cadaver, combined (HR + ECD)	16 (13.7%)
Mean Follow-up (day)	443 ± 245.6
ATG dose (mg/kg/day)	1.12±0.16
ATG total dose (mg/kg, for 5days)	5.62±1.06
Delayed graft function	7 (10.4% of ECD cases)
Rejection within 30days	7 (6%)
30-180days	6 (5.1%)
CMV prophylaxis, period 1	39
CMV infection within 30days	14 (35.8% of period 1 cases)
CMV infection between 30-180days	25 (64.1% of period 1 cases)
CMV infection total	28 (71.7% of period 1 cases)
CMV prophylaxis, period 2	77
CMV infection within 30days	2 (2.5% of period 2 cases)
CMV infection between 30-180days	14 (18.1% of period 2 cases)
CMV infection total	15 (19.4% of period 2 cases)
Graft loss	3 (2.5%)
Patient loss (with functioning graft)	3 (2.5%)

P.1494

Lymphocyte subset test as a determinant of cytomegalovirus prophylaxis in renal transplant recipients with antithytocyte globulin induction therapy: A pilot study

Mi-Hyeong Kim, Kang-Woong Jun, Hyun-Kyu Kim, Young-Hwa Kim, Kyungjai Ko, Sang-Dong Kim, Jeong-Kye Hwang, Sun-Cheol Park, In-Sung Moon, Ji-Il Kim.
Department of Surgery, College of Medicine, The Catholic University of Korea, Seoul, Korea.

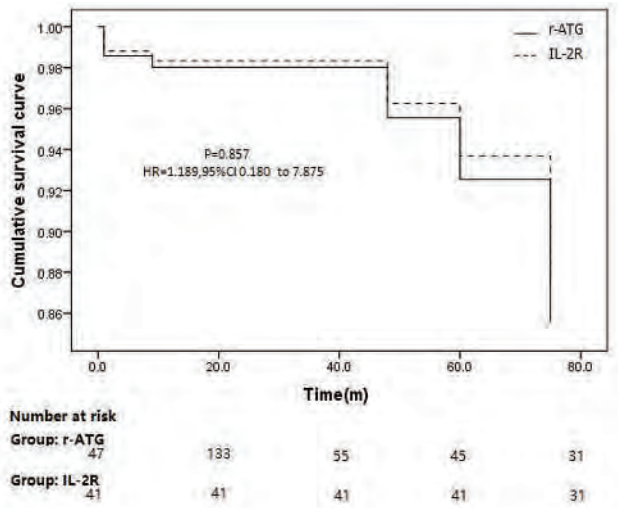
Introduction: Cytomegalovirus (CMV) infection is an important complication after kidney transplantation (KT). The American society of transplantation (AST) recommended CMV prophylaxis or preemptive therapy according to donor/recipient seropositivity status. In a high risk group, CMV prophylaxis is preferred and AST told to take valgancyclovir 900mg per day during 3 to 6 months. Korea is one of the high rate of CMV seropositive area, therefore, we preferred preemptive therapy. However, the recipients with ATG induction need prophylaxis therapy. Unfortunately, in Korea, the price of valganciclovir is too expensive; one capsule of 450mg costs 22 US dollars, so totally 7,920 US dollars are needed for the prophylaxis therapy for three months. We altered our prophylaxis regimen as intravenous ganciclovir during the admission (for 14 days, 4.6 US dollar per one ample) and valganciclovir for two weeks. After that, we altered our strategy to preemptive therapy on the basis of weekly CMV PRC. Our results of CMV prevention, however, were poor with high incidence of CMV infection (19.4%). We felt keenly a need to reduce this rate, we planned to do optimal prophylaxis to selective high risk recipients. This study was aimed to figure out the high risk recipients.
Methods: We hypothesized that a difference of lymphocyte suppression and recovery may affect the CMV reactivation, so we focused on the lymphocyte subset results. We experienced 116 cases of ATG induction from August, 2012, and lymphocyte subset test was checked from April, 2014 at preoperative day, postoperative day (POD) #1, 8 and 14. Total 63 cases of serial lymphocyte subset test data were collected. We investigated a failure of complete recovery of CD3, CD8, CD19 and CD56 subsets at POD #14. Patients were divided into two groups according to a presence of CMV infection within 6 months.
Results: There was no difference in general characteristics and dose of ATG. The failure of complete recovery at POD #14 of CD3, CD8 and CD56 was frequent in CMV infection group with statistical significance. (p=0.028, 0.033, 0.031, respectively) All of the CMV infection recipients were failed to get complete recovery of CD3 subset.
Discussion: It is uncertain that these results are applicable in clinical situation, because of high rate of false positive. We need more precise marker. This study is limited due to small number of patients and retrospective design. We are going to extend the duration of prophylaxis in the patients who failed to get complete recovery of CD3 subset at POD #14. And we plan to recheck the lymphocyte subset test at 1 month after transplantation, and to select the patients who need further extension of prophylaxis on a setting of prospective study.
Conclusions: The recipients with ATG induction need CMV prophylaxis. Further studies are needed to determine the applicability of lymphocyte subset test as a marker for high risk patient selection.

P.1495

Efficacy and safety of rabbit antithymocyte globulin or interleukin-2 receptor antagonist for induction therapy in Chinese living donor renal transplantation recipients: A retrospective study

Jiang Qiu, Gang Huang, Lizhong Chen, Changxi Wang, Guodong Chen, Jun Li, Qian Fu, Jiguang Fei, Suxiong Deng.
Division of Organ Transplantation, The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, People's Republic of China.

Introduction: KDIGO 2009 guidelines recommend interleukin-2 receptor antagonist (IL2-RA) as a 1st-line induction therapy for living donor renal transplantation. The relatively-more-potent immunosuppressant rabbit antithymocyte globulin (rATG) has been associated with a lower risk of acute rejection and higher rate of infection compared with IL2-RA, and is recommended for high-risk donor recipients. This retrospective study compared the efficacy and safety of rATG with IL-2RA in Chinese patients who underwent living donor renal transplant over a 10-year period.
Materials and Methods: This retrospective analysis included 188 adult (≥18 years) patients who received induction immunosuppression and underwent living donor renal transplantation at our institution from Feb 2004–Dec 2013. Patients were stratified based on induction therapy received; rATG (thymoglobulin) 1 mg/kg/day at Day 0–Day 2 after transplantation (n=41), or IL2-RA (basiliximab) 20 mg/day at Day 0–Day 3 after transplantation (n=147). The main outcome was incidence of acute rejection. Other outcomes included detection of de novo donor-specific antigen (dn-DSA), graft survival, and rate of infection.
Results and Discussion: The rATG group had a higher mean panel reactive antigen (PRA) score, and more complete human leukocyte antigen (HLA) mismatches, compared with the IL2-RA group (Table). There was no significant difference in the incidence of acute rejection between the rATG and IL2-RA groups (9.8% vs. 8.8%), although patients in the rATG group had a significantly lower rate of biopsy-proven acute rejection (BPAR) (p=0.01) (Table). There was no significant difference in 1, 3 or 5-year graft (Figure 1) or recipient (data not shown) survival between the two groups. The safety profile in both treatment groups was comparable, which is contrary to current treatment guidelines and previous research that showed a relatively lower rate of infection with IL-2RA versus rATG (Table). Given that dn-DSAs directed against HLA are one of the major risk factors for allograft failure, it is interesting that this study found the incidence of dn-DSA was comparable in the rATG (two cases [4.9%]) and IL2-RA (seven cases [5.4%]) groups.
Conclusion: It may be beneficial to stratify high-risk renal donor recipients to induction with rATG; similar efficacy and safety outcomes were achieved for higher-risk patients following rATG, with a lower rate of BPAR, compared with relatively lower-risk patients treated with IL-2RA.



	rATG n=41	IL2-RA n=147	P-value
Baseline characteristics			
Age, years	31.0 ± 12.2	33.5 ± 8.3	0.11
Gender (m/f), n	26/15	123/24	0.005
Dialysis time, months	11.2 ± 8.3	12.2 ± 15.4	0.7
PRA score, %	30.0	4.9	<0.001
Complete HLA mismatch, n	13	1	<0.001
Median follow-up time, weeks (range)	45 (17-96)	37(17-73)	—
Efficacy			
Acute rejection, % (n/n)	9.8 (4/41)	8.8 (13/147)	0.832
Graft lost because of rejection, n/n*	0/4	4/13	—
Biopsy-proven acute rejection, n	1	6	0.01
Safety, n (%)			
Liver injury	2 (4.9)	15 (10.2)	0.31
Myelosuppression	0 (0)	3 (2.0)	0.36
Infection	8 (19.5)	24 (16.3)	0.96
Pneumonia	8 (19.5)	24 (16.3)	0.82
Dn-DSA	2 (4.9)	8 (5.4)	0.44

All values are mean ± standard deviation unless specified.
Dn-DSA = de novo donor-specific antigen; HLA = human leukocyte antigen; IL2-RA = IL-2 receptor antagonist; PRA = panel reactive antibody; rATG = rabbit antithymocyte globulin
*Denominator is the number of patients with acute rejection

This study was supported by the National Natural Science Fund project (81470976), the National Natural Science Fund Youth Science project (81302549)

References:

[1] Guodong Chen, Xingqiang Lai, Dicken Shiu-Chung KO, et al. Comparison of efficacy and safety between rabbit anti-thymocyte globulin and anti-T lymphocyte globulin in kidney transplantation from donation after cardiac death: A retrospective cohort study. Nephrology. 2015; 20: 539–543.
[2] Brokhof MM, Sollinger HW, Hager DR, et al. Antithymocyte globulin is associated with a lower incidence of de novo donor-specific antibodies in moderately sensitized renal transplant recipients. Transplantation. 2014; 6: 612-617.
[3] Everly MJ, Rebellato LM, Haisch CE, et al. Incidence and impact of de novo donor-specific alloantibody in primary renal allografts. Transplantation. 2013; 3:410-417.

P.1496

MICA positive status and immunosuppression

Vijaya Rajakumari.
Apollo Hospital, Apollo Hospital, New Delhi, India.

MICA positive status was studied in 50 living related kidney transplants in 2015.
The first five cases were traed with a combo of rituximab injection plus plasma exchange and kidney transplant. these patients had mixed rejection and neede rATG and plasma exchange and IVIg for treatment.
The second group with MICA positive status and with no DSA treated with just ontable rATG and triple immunosuppression resulted in hyperacute rejection of the graft.
The remaining patients were treated with one week of triple immunosuppression with low tacrolimus plus plasma exchange, rATG plus IVIg pre kidney transplant with excellent results

P.1497

Optimal dose of thymoglobulin for induction therapy in high risk kidney transplant recipients

Yaerim Kim[1,3], Seong Sik Kang[1,3], Woo Yeong Park[1,3], Kyubok Jin[1,3], Sung Bae Park[1,3], Won Hyun Cho[2,3], Hyoung Tae Kim[2,3], Seungyeup Han[1,3].
[1]Department of Internal Medicine, Keimyung University School of Medicine, Daegu, Korea; [2]Department of Vascular Surgery, Keimyung University School of Medicine, Daegu, Korea; [3]Keimyung University Kidney Institute, Daegu, Korea.

Introduction: Thymoglobulin has been used for induction therapy to prevent acute rejection and delayed graft function (DGF) in kidney transplant patients. However, the usual dose of thymoglobulin is considered to be related with frequent infection.
Objectives: We compared the efficacy and safety of low dose thymoglobulin to high dose in high risk recipients with kidney transplantation.
Materials and Methods: Twenty-one kidney transplant patients underwent induction treatment with thymoglobulin in Keimyung university Dongsan hospital and they were divided into two groups : the patients who treated with low dose thymoglobulin(< 6.0 g/kg) and high dose(≥ 6.0 g/kg). All patients who included in our study have one or more risk factors for acute rejection or DGF. The risk factors were re-transplantation, recipient or donor age over 60, human leukocyte antigen (HLA) full mismatch and panel-reactive antibody (PRA) more than 50%. We compared incidence of acute rejection, infection, hematologic complications and graft survival between two groups.
Results: The demographic characteristics of two groups were comparable. Mean follow up duration was 11.9 ± 4.3 months, cumulative thymoglobulin dosage was 6.3 ± 1.6 g/kg. The incidence rates of acute antibody mediated rejection (AMR), DGF and infectious events as cytomegalovirus disease or urinary tract infection were not significantly different between two groups. Neutropenia occurred more frequently in high dose thymoglobulin group, but there was no statistically significant difference. The rate of graft loss were similar between two groups.
Conclusions: There were no differences in graft survival, infectious disease and hematologic problem between two groups. We can suggest to lower the dose of thymoglobulin less than 6 g/kg for prevent acute AMR and DGF in high risk patients.

P.1498

A case of coexistence of intracranial and intramedullary tuberculomas in kidney transplantation recipient

Yaerim Kim[1,3], Seong Sik Kang[1,3], Woo Yeong Park[1,3], Kyubok Jin[1,3], Sung Bae Park[1,3], Hyoung Tae Kim[2,3], Won Hyun Cho[2,3], Seungyeup Han[1,3].
[1]Department of Internal Medicine, Keimyung University School of Medicine, Daegu, Korea; [2]Department of Vascular Surgery, Keimyung University School of Medicine, Daegu, Korea; [3]Keimyung University Kidney Institute, Daegu, Korea.

Tuberculosis infection is known to be unusually high in Korea. Especially, infection rate in transplantation recipients is reported about 50 times higher than in general public. Most of them are pulmonary tuberculoses, but central nervous system tuberculosis such as spinal intramedullary tuberculoma or intracranial tuberculoma is uncommon. Herein, we report a case of kidney transplantation recipients diagnosed with coexistence of spinal intramedullary tuberculoma and intracranial tuberculoma.
Sixty five-year-old woman who underwent kidney transplantation 6 months ago, visited our emergency department, presenting progressive paraplegia. Although she demonstrated positive to Quantiferon test before transplantation, INH prophylaxis was not performed because she has no past history of tuberculosis. Five months after transplantation, she diagnosed as miliary tuberculosis and started anti-tuberculosis medication. The magnetic resonance imaging showed a spinal intramedullary mass and multiple intracranial nodules. Surgical resection of spinal mass was performed and the lesion was confirmed as Mycobacterium tuberculosis histopathologically.
We report the rare case of coexistence of spinal intramedullary and intracranial tuberculomas in kidney transplantation recipients with Quantiferon positive result before transplantation who did not performed INH prophylaxis.

P.1499

Cost-effectiveness of single daily-dose of Sirolimus and Tacrolimus-XR for kidney transplantation

Yingyos Avihingsanon[1,2], Rosarin Sruamsiri,[2], Natavudh Townamchai [1,2], Salin Wattanatorn[2], Bunthoon Nonthasoot[1], Supanit Nivatvongs[1].
[1]Excellent Center of Organ Transplantation, King Chulalongkorn Memorial Hospital and Chulalongkorn University, Bangkok, Thailand; [2]Medicine/Nephrology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

Currently, the Thailand Reimbursement Schemes have long been supported all Thai kidney transplant patients for their life-long use of immunosuppressive drugs. This policy is based on the results of cost-effectiveness studies which compared cost-utility of kidney transplant and long-term dialysis (1). However, costs of immunosuppressive regimens vary widely from 15,480 to 27,870 THB per month. It remains a question which regimen is the most cost-effective. On the other hand, quality of life is much gained among ESRD patients who receive kidney allograft. While drug compliance is one of the most important factors of allograft survival, it is believed that single daily dose could improve patients' compliance, graft outcomes, as well as quality of life. In this study, a Markov model was used to estimate the relevant costs and health outcomes over the patients' lifetimes using a societal perspective. Efficacy of single daily immunosuppressive drugs (SDD) (Rapamune plus Advagraf) and standard treatment (Prograf plus CellCept) were collected from a cohort of kidney transplant at King Chulalongkorn Memorial Hospital. Occurrence of acute rejection in both groups was taken into the model. Dialysis or death events were collected during the observation period (year 2011-2016). Health-related quality of life of kidney transplantation patients were measured using EQ5D (EuroQoL EQ-5D) and converted to utility score using Thai preference score. Significant different of quality of life can be observed between patients receiving SDD and standard treatment. The mean utility score was 0.85 (0.173) for SDD group and 0.61 (0.108) for comparator (p-value = 0.0008). The incremental cost per QALY gain shows cost-effectiveness for the SDD over the control group.
In conclusion, in Thai kidney transplant patients, the single daily immunosuppressive drugs may provide a good efficacy, could improve quality of life and was a cost-effectiveness regimen. A larger cohort with a longer observation is required to guide the National policy of reimbursement schemes.

References:

[1] Teerawattananon Y, Mugford M, Tangcharoensathien V.*Value Health*. 2007;10:61-72.

P.1500

Therapeutically equivalent results to caucasians in black kidney transplant recipients using Astagraf XL despite a higher degree of HLA mismatch: A post-hoc analysis of Phase III data from over 2000 patients

Jason Schwartz[1], Sam Wilson[1], Fei Shi[1], Nasrullah A.Undre[2], Mysore S.A.Kumar[3].
[1]Medical Affairs, Americas, Astellas Pharma Global Development, Northbrook, IL, United States; [2]Astellas Pharma Global Development, Medial Affairs, EMEA, Chertsey, United Kingdom; [3]Medical Affairs, Global, Astellas Pharma Global Development, Northbrook, IL, United States.

Introduction: One of the many challenges faced by Black kidney transplant recipients in the U.S. can be a higher rate of intrapatient variability with respect to tacrolimus whole blood concentrations. This has been attributed, in part, to a greater incidence of CYP polymorphisms as well as poorer rates of medication adherence overall[1],[2]. Thus, when contemplating the use of a new immunosuppressant agent in kidney transplantation, measuring outcomes in this potentially vulnerable population is of paramount concern. As such, we report the first large-scale clinical efficacy data of extended-release tacrolimus (ER-T) in Black kidney transplant recipients, a group historically underrepresented in clinical trials and with generally inferior outcomes when compared to other ethnic groups[3].

Materials and Methods: Results were pooled from three, large Phase III RCTs [02-0-158, FG-506E-12-03, PMR-EC-1210 (OSAKA)] as part of an ER-T (Astagraf XL®) clinical development program (n=2162). Logistic regression and proportionate hazards modelling were used to ascertain differences between treatment groups over 24 weeks. To account for study heterogeneity, both fixed and random effects were incorporated into the modelling.

Table 1. Patient Demographics

Parameter	Black IR-T (n = 77)	Caucasian IR-T (n= 721)	Black ER-T (n = 87)	Caucasian ER-T (n=1227)	p-value
Pre-op DM (%)	26.0	13.5	12.6	13.8	0.286
HgbA1c (%)	5.79	5.40	5.58	5.38	0.009
Pre-op HTN (%)	89.6	86.8	95.4	87.1	0.827
Living Donor (%)	26.0	25.8	17.2	19.0	<0.001
Avg. HLA mismatch	4.1	3.1	3.8	3.1	<0.001
Avg. PRA	3.3	1.8	2.6	1.8	0.124

Results and Discussion: Despite a higher degree of HLA-mismatch (P<0.001) in Black *de novo* kidney transplant recipients treated with ER-T, clinical efficacy was statistically non-inferior to Caucasians treated with *either* ER-T *or* immediate-release tacrolimus (IR-T, Prograf®). Likewise, in Blacks treated with ER-T, therapeutic equivalence was observed vs. IR-T and eGFR was noted to be statistically similar.

Table 2. Black ER-T (Astagraf XL) vs. Caucasian Cohorts

	Black / ER-T (N=87)	Caucasian / IR-T (N=721)	Caucasian / ER-T (N=1277)
Efficacy Failure:	14 (16.1%)	128 (17.8%)	266 (20.8%)
Death	0	15	22
Graft Failure (including death)	5	40	89
BPAR	8	85	179
Lost to follow-up	3	11	32
Efficacy Failure Non-inferiority			
Difference (Caucasian minus Black)		0.0166	0.0474
95% CI of the Difference		(-0.0648, 0.0980)	(-0.0356, 0.1303)
P-value of Noninferiority		0.0092	0.0017
Margin of Equivalence: - 0.10			

Table 3. Black IR-T vs. Black ER-T (Astagraf XL)

	Black / IR-T (N=77)	Black / ER-T (N=87)
Efficacy Failure:	13 (16.9%)	14 (16.1%)
Efficacy Success:	64 - 0.8312	73 - 0.8391
Death	1	0
Graft Failure (including death)	6	5
BPAR	7	8
Lost to follow-up	1	3
Efficacy Failure Non-inferiority		
Difference (IR-TAC minus ER-TAC)		0.0079
95% CI of the Difference		(-0.882, 0.1040)
P-value of Noninferiority		0.0323
Margin of Equivalence - 0.10		

Conclusions: While higher rates of acute rejection and graft loss have typically been observed in Black kidney transplant recipients, Blacks treated with ER-T demonstrated results comparable to that of Caucasians in this pooled analysis of Phase III data.

References:

[1] Jacobson PA, Oetting WS, Brearley AM et al. Novel polymorphisms associated with tacrolimus trough concentrations: results from a multicenter kidney transplant consortium. Transplantation. 2011; 91: 300-8.
[2] Tsapepeas D, Langone A, Chang L et al. A longitudinal assessment of adherence with immunosuppressive therapy following kidney transplantation from the Mycophenolic Acid Observational REnal Transplant (MORE) Study. *Ann Transplant*. 2014; 19: 174-81.
[3] Narayan M, Pankewycz O, Shihab F et al. Long-term outcomes in African American kidney transplant recipients under contemporary immunosuppression: a four-yr analysis of the Mycophenolic acid Observational REnal transplant (MORE) study. *Clin Transplant*. 2014; 28: 184-91.

P.1501

Comparative performance of apparent oral midazolam clearance and plasma 4β-hydroxycholesterol to predict tacrolimus disposition in renal recipients

Thomas Vanhove[1], Hylke de Jonge[1], Henriëtte de Loor[1], Pieter Annaert[2], Ulf Diczfalusy[3], Dirk R.J. Kuypers[1].
[1]Nephrology and Renal Transplantation, University Hospitals Leuven, Leuven, Belgium; [2]Pharmaceutical and Pharmacological Sciences, KU Leuven – University of Leuven, Leuven, Belgium; [3]Laboratory Medicine, Karolinska University Hospital, Stockholm, Sweden.

Aims: We compared the relationship between the CYP3A4 metrics weight-corrected midazolam apparent oral clearance (MDZ Cl/F/W) and plasma 4β-hydroxycholesterol/cholesterol (4β-OHC/C) as predictors of tacrolimus (Tac) disposition in renal transplant recipients.
Methods: A cohort of 147 patients underwent calculation of 8 hour area under the curve (AUC) for tacrolimus and oral MDZ as well as measurement of 4β-OHC/C. A subgroup of 70 patients additionally underwent intravenous erythromycin breath test (EBT) and the intravenous MDZ probe. All patients were genotyped for common polymorphisms in CYP3A4, CYP3A5 and P450 oxidoreductase, among others.
Results: MDZ Cl/F/W, 4β-OHC/C/W, EBT and Tac Cl/F/W were all moderately correlated (r=0.262-0.505). In the MDZ-based model, predictors of Tac Cl/F/W were CYP3A5 genotype (R^2=0.267), Tac formulation (R^2=0.131), MDZ Cl/F/W (R^2=0.077), hematocrit (R^2=0.081) and age (R^2=0.017; total R^2=0.573). In the 4β-OHC/C/W-based model, predictors were CYP3A5 genotype (R^2=0.265), 4β-OHC/C/W (R^2=0.103), Tac formulation (R^2=0.050), hematocrit (R^2=0.057) and age (R^2=0.025; total R^2=0.500). In CYP3A5 non-expressors, this difference in model fit was accentuated (R^2=0.446 vs. 0.327). When genotype information was ignored, predictors of Tac Cl/F/W were 4β-OHC/C/W (R^2=0.158), Tac QD formulation (R^2=0.070), MDZ Cl/F/W (R^2=0.053) and hematocrit (R^2=0.037; total R^2=0.318). 4β-OHC/C/W, but not MDZ Cl/F/W, was higher in CYP3A5 expressors because it was higher in CYP3A4*1b carriers, which were almost all CYP3A5 expressors.
Conclusions: MDZ Cl/F/W seemed the superior intrinsic CYP3A4 probe. Because 4β-OHC/C/W was influenced by the CYP3A4*1b polymorphism, however, it was better at predicting Tac disposition in a model in which no genotype information was available.

P.1502

The P-glycoprotein probe fexofenadine does not predict tacrolimus disposition in renal recipients

Thomas Vanhove[1], Pieter Annaert[2], Dirk R.J. Kuypers[1].
[1]Nephrology and Renal Transplantation, University Hospitals Leuven, Leuven, Belgium; [2]Pharmaceutical and Pharmacological Sciences, KU Leuven – University of Leuven, Leuven, Belgium.

Introduction: The combination of CYP3A5 genotype, in vivo CYP3A4 activity assessed by calculating apparent oral midazolam clearance (MDZ Cl/F) and hematocrit have been shown to explain 60-72% of interindividual differences in tacrolimus disposition among renal recipients. Because tacrolimus is a dual substrate for CYP3A and P-glycoprotein (P-gp, ABCB1), we explored whether quantifying P-gp activity using the probe drug fexofenadine (FEX) could further refine prediction of tacrolimus disposition.
Methods: A cohort of 70 adult renal recipients > 1 year after transplantation underwent simultaneous 8-hour pharmacokinetic profiles for once-daily tacrolimus, MDZ and FEX. All patients were genotyped for 32 polymorphisms in genes with a proven or possible relevance to tacrolimus or FEX disposition including CYP3A5, CYP3A4, ABCB1, POR, COMT, FMO, PPARA, APOA5, MRP2, SIM1, GAN, OATP2B1, -1B1 and -1B3.
Results: FEX Cl/F0-24 did not correlate with tacrolimus Cl/F0-24 (correlation coefficient 0.057, p=0.642). In CYP3A5 non-expressers (n=54), independent predictors of tacrolimus Cl/F0-24 were MDZ Cl/F0-inf, hematocrit and the NR1I2 8055C>T polymorphism (semipartial R^2 0.368, 0.153 and 0.063, respectively; p<0.01 for all), which together explained 58.4% of interpatient variability in tacrolimus Cl/F0-24. In CYP3A5 expressers (n=16), no predictors of tacrolimus Cl/F0-24 were identified. FEX Cl/F0-24 was not predicted by any of the genetic polymorphisms.
Conclusion: The probe drug FEX did not predict tacrolimus disposition in renal recipients, which could be related to intrinsic limitations of the probe's specificity for P-gp activity (it is also a substrate for several other drug transporters) and/or lack of a significant effect of variability in P-gp activity on interindividual differences in tacrolimus disposition. In renal recipients, none of the previously described genetic polymorphisms affecting FEX disposition could be confirmed.

P.1503

Evaluation and inter-laboratory comparison of a real-time PCR-based DNA demethylation assay to assess the frequency of naturally occurring regulatory T cells (nTregs) in peripheral blood

Maria Metzker[1], Maria Shipkova[1], Nicolas von Ahsen[2], Reiner Andag[2], Manabu Abe[1], Ortrun Canzler[1], Corinne Klett[1], Simone Leicht[1], Christoph Olbricht[3], Eberhard Wieland[1].
[1]Central Insitute for Clinical Chemistry and Laboratory Medicine, Klinikum Stuttgart, Stuttgart, Germany; [2]Department of Clinical Chemistry, University Medicine Goettingen, Goettingen, Germany; [3]Department of Nephrology and Transplant Center, Klinikum Stuttgart, Stuttgart, Germany.

Introduction: Life long immunosuppression is required in solid organ transplantation to prevent rejection but is accompanied by side effects. Differences in the response to therapy are recognized and some patients are over-immunosupressed by standard treatment. Reducing immunosuppression in those patients is therefore desirable. A promising biomarker to recognize patients who may benefit from dose reductions are naturally occurring Tregs (nTregs) that can be quantified based on the Treg cell Specific Demethylated Region (TSDR) by quantitative PCR (qPCR)[1]. However, to establish the clinical utility of a biomarker its analytical performance must be robust and reproducible. Therefore, the analytical performance of a qPCR assay for nTregs was evaluated. In addition, results were compared between two **laboratories and to flow cytometry (FACS).**
Material and Methods: qPCR amplifications were performed independently in two laboratories using the same protocol[1]. The demethylation index (DI) was calculated as the ratio of demethylated divided by demethylated + methylated TSDR copies and expressed in percent. Method precision, limit of detection (LOD), analyte stability, and the reference interval were established with EDTA blood from healthy blood donors (HBD). In addition, blood from immunosuppressed (tacrolimus, mycophenolate sodium, steroids) kidney transplant recipients (KTR) was used for inter-laboratory comparison. CD4+CD25highCD127low Tregs were determined by FACS
Results and Discussion: The qPCR assay showed a satisfactory within series and between series precision (CV<20%, n=6). For the methylated and demethylated qPCR the LOD was 100 copies/mL. Both whole blood and DNA samples can be stored for 24h at room temperature and for 12 months at -80°C. The DI was significantly higher ($p<0.05$) in female HBD (1.0%±0.27%, n=30) than in male HBD (0.45%±0.23%, n=27) and generally higher in HBD (0.73%±0.23%, n=60) than in KTR (0.45%±0.21%, n=60). The DI in HBD spread from 0.43% to 1.57% in females and from nil to 0.93% in males. There was in KTR no correlation between tacrolimus concentrations and nTregs.
Results correlated well between laboratories (r=0.645; p=0.0002, n=29) but had an inter-laboratory bias of 76% (Passing&Bablok regression: y=-0.046+1.76x). This may be due to the use different reagents, standards, and PCR instruments. Results between qPCR and FACS did not correlate at all (r=0.215; p=0.1, n=60). The discrepancies suggest that qPCR and FACS reflect different Treg populations.
Conclusion: The qPCR PCR assay for nTregs is analytically reliable but a lack of standardization hampers exchange of results between laboratories. Data generated by FACS and qPCR can also not be interchangeably used. For the assessment of nTregs gender specific reference intervals have to be considered.
Supported by Foundation for Pathobiochemistry and Molecular Diagnostics
References:
[1] Wieczorek G. et al. Cancer Res. 2009;69:599-608.

P.1504

Influence of CYP3A4*22 and CYP3A5*3 genetic polymorphisms on tacrolimus pharmacokinetics in adult renal transplant patients

Pilar Salvador[1], María Outeda[1], Isaura Pedreira[1], Constantino Fernández[2], Angel Alonso[2], Isabel Martín[1].
[1]Pharmacy Department, A Coruña University Hospital, A Coruña, Spain; [2]Neprology Department, A Coruña University Hospital, A Coruña, Spain.

Background: The CYP3A4*22 variant has been associated with a significantly lower tacrolimus (TAC) dose.
Aim: To assess the impact of CYP3A4*22 polymorphisms, either nominally or in combination with the CYP3A5 genotype (expressers and nonexpressers) on dose requirements and trough concentration/dose (C/D) ratio of TAC in adult renal transplant patients at different post-transplant moments.
Patients and Methods: 51 Caucasian renal transplant patients treated with a TAC-based immunosuppressive regimen, which included mycophenolate and corticoids. TAC whole blood trough concentrations (Ctrough) were measured by a chemiluminescent immunoassay on the ARCHITECTTM platform. A genetic test was used for the genotyping of CYP3A4*22 and CYP3A5*3, and the genetic variants obtained were correlated to TAC dose requirements (daily dose, weight-adjusted daily dose), trough concentrations and C/D ratio at 3 and 15 days after commencing the TAC treatment and 1, 6 and 12 months after transplantation. A statistical analysis was carried out using SPSS 19.0. The Mann-Whitney test was used. Values were considered statistically significant at $p<0.05$.
Results: The study included 51 patients (30 men/21 women), aged 51 years and weighing 72 kg.
The genetic variants identified were CYP3A4*22 CC in 46 patients (90%) and CT in 5 (10%). This distribution was in accordance with the Hardy-Weinberg equilibrium.
When patients were grouped according to CYP3A4*22 genotype, at 1 month post-transplant, there was a significant increase in TAC daily dose in CYP3A4*22 CC carriers in comparison with the CYP3A4*22 CT variant carriers (4.86±2.40 vs. 2.40±0.55 mg/day, p=0.027); however the weight-adjusted daily was not significantly different (0.07±0.05 vs. 0.03±0.01 mg/kg, p=0.064), in order to achieve similar levels (11.85±4.53 vs. 10.96±4.21 ng/mL). The trend towards a higher increase in daily dose in CYP3A4*22 CC carriers was also present at 15 days post-transplant although it was not significant (5.16±2.68 vs. 3.20±0.84 mg/day, p=0.092). At 12 months following transplantation, TAC levels were significantly lower in CYP3A4*22 CC in comparison with the CYP3A4*22 CT variant (7.57±2.19 vs. 10.36±2.45610.55 mg/day, p=0.027), but not the dose requirements.
When patients were grouped according to CYP3A5*3 and CYP3A4*22, at 12 months post-transplant, TAC levels were significantly different (7.92±2.14 vs. 11.33±1.89 ng/mL, p=0.019) between CYP3A4*22 CC (30 patients) and CYP3A4*22 CT carriers (3 patients) in CYP3A5 non-expressers. When patients were expressers, no difference was found.
As for the other pharmacokinetic parameters assessed and other post-transplant moments, no significant changes were found.
Conclusion: Based on our results, CYP3A4*22 CC seems to require increased daily doses, and CYP3A4*22 CT showed higher trough levels. In clinical practice, CYP3A4*22 could be of value in personalizing TAC dose requirements.

P.1505

Effect of MDR1 C3435T, C1236T and G2677T/A polymorphisms on 5-year tacrolimus pharmacokinetics and renal function in renal transplant recipients

Pilar Salvador[1], María Outeda[1], Isaura Pedreira[1], Constantino Fernández[2], Angel Alonso[2], Isabel Martín[1].
[1]Pharmacy Department, A Coruña University Hospital, A Coruña, Spain; [2]Nephrology Department, A Coruña University Hospital, A Coruña, Spain.

Background: MDR1 C3435T, C1236T and G2677T/A polymorphisms could affect tacrolimus (TAC) pharmacokinetics and could be related to renal injury induced by this agent.
Aim: To analyze the influence of MDR1 C3435T, C1236T and G2677T/A polymorphisms on TAC pharmacokinetics and renal function over 1-5 years in adult renal transplant patients.
Patients and Methods: A retrospective study of 51 patients who underwent a cadaveric renal transplantation treated with a TAC-based immunosuppressive regimen and with a follow-up period of at least 5 years. The TAC pharmacokinetic variables (daily dose, weight-adjusted daily dose, trough concentration and trough concentration/dose ratio) and renal function (serum creatinine level and glomerular filtration rate –GFR: MDRD-4: mL/min/1.73m2-) were obtained at 1, 2, 3, 4, and 5 years. Blood levels were determined using CMIA on an Architect™ C8000 analyzer. All patients were genotyped for C3435T, C1236T and G2677T/A with DNA chip technology using genomic DNA extracted from a peripheral blood sample. Patients were stratified according to genotype.
A statistical analysis was performed using SPSS version 19.0. At the five previously defined time points post-transplant, the influence of the genetic polymorphisms on the pharmacokinetic variables studied and renal function were analyzed, using the Mann-Whitney test. $P<0.05$ was considered statistically significant.
All patients provided their consent.
Results: The study included 51 patients (30 men/21 women), aged 51 years and weighing 72 kg.
The genetic variants identified for C3435T were as follows: CC in 12 patients (24%) and CT+TT in 39 (76%); for C1236T: CC in 14 patients (27%) and CT+TT in 37 (73%); and for G2677T/A: GG in 16 patients (31%), GT+TT in 35 (69%). This distribution was in accordance with the Hardy-Weinberg equilibrium.
None of the MDR1 polymorphisms studied (C3435T, C1236T and G2677T/A) were individually associated with dose requirements, trough concentrations or trough concentration/dose ratio at any moment. Likewise, there was no significant difference between the major wild type (CCG) and mutant (TTT) haplotypes. However, a non-statistical trend was observed towards lower dose-corrected exposure and higher dose requirements in those patients who are MDR1 3435CC, 1236CC and 2677GG or major (CCG) wild type carriers compared with T-allele carriers. At no time was renal function statistically significantly different among the MDR1 genotypes studied.
Conclusions: Based on our results, we found no evidence of a significant association of MDR1 C3435T, C1236T and G2677T/A genetic polymorphisms on tacrolimus pharmacokinetics (dose requirements, trough concentrations and trough concentration/dose ratio) and renal function over a period of 1-5 years after transplantation.

P.1506

Influence of CYP3A5 polymorphisms on 5-year tacrolimus pharmacokinetics and renal function in renal transplant recipients

Pilar Salvador[1], María Outeda[1], Isaura Pedreira[1], Constantino Fernández[2], Angel Alonso[2], Isabel Martín[1].
[1]Pharmacy Department, A Coruña University Hospital, A Coruña, Spain; [2]Nephrology Department, A Coruña University Hospital, A Coruña, Spain.

Background: CYP3A5 polymorphisms affect tacrolimus (TAC) pharmacokinetics and could be related to renal injury induced by this agent.
Aim: To analyze the influence of CYP3A5 polymorphisms on TAC pharmacokinetics and renal function over 1-5 years in adult renal transplant patients.
Patients and Methods: A retrospective study of 51 patients who underwent a cadaveric renal transplantation treated with a TAC-based immunosuppressive regimen and with a follow-up period of at least 5 years. The TAC pharmacokinetic variables (daily dose, weight-adjusted daily dose, trough concentration and trough concentration/dose ratio) and renal function (serum creatinine level and glomerular filtration rate –GFR: MDRD-4: mL/min/1.73m2-) were obtained at 1, 2, 3, 4, and 5 years. Blood levels were determined using CMIA on an Architect™ C8000 analyzer. All patients were genotyped for CYP3A5 with DNA chip technology using genomic DNA extracted from a peripheral blood sample. Patients were stratified according to genotype.
A statistical analysis was performed using SPSS version 19.0. At the five previously defined time points post-transplant, the influence of the genetic polymorphisms on the pharmacokinetic variables studied and renal function were analyzed using the Mann-Whitney test. $P<0.05$ was considered statistically significant.
All patients provided their consent.
Results: The study included 51 patients (30 men/21 women), aged 51 years and weighing 72 kg.
The genetic variants identified were *3/*3 in 33 patients (65%) and *1/*3 + *1/*1 in 18 (35%). This distribution was in accordance with the Hardy-Weinberg equilibrium.
The mean trough levels of tacrolimus in patients carrying CYP3A5*1 allele (expressors) were comparable with patients carrying CYP3A5*3/*3 (non-expressors) from 1 to 5 years after renal transplant. However the required daily doses and weight-adjusted daily doses were significantly higher in CYP3A5*1 carriers compared with those in CYP3A5*3/*3 during that period ($p<0.05$). The trough concentration/dose ratio was nearly 1.7-fold higher for CYP3A5*3/*3 than CYP3A5*1 carriers ($p<0.001$) for all studied moments.
With respect to renal function, the expressors were associated with lower GFR at 1 year compared to non-expressors: 44.67±13.67 vs. 54.15±16.43 mL/min/1.73m2; however this difference was not significant for the other years or for serum creatinine at any time.
Conclusions: Based on our results, CYP3A5 genetic polymorphisms affected dose requirements (daily dose and weight-adjusted daily dose), which were higher for expressors compared to non-expressors, and a lower concentration/dose ratio of tacrolimus 1-5 years after transplantation. The CYP3A5*1 carriers were associated with lower glomerular filtration rate at 1 year post-transplant, but not with serum creatinine.

P.1507

Influence of CYP3A4*1 and MDR1 C3435T, C1236T and G2677T/A genetic polymorphisms on itraconazole-tacrolimus interaction in adult renal transplant patients

Maria Macias[1], Pilar Salvador[1], Isaura Pedreira[1], Constantino Fernández[2], Angel Alonso[2], Isabel Martín[1].
[1]Pharmacy Department, A Coruña University Hospital, A Coruña, Spain; [2]Nephrology Department, A Coruña University Hospital, A Coruña, Spain.

Background: Itraconazole (ITRA) is a substrate and an inhibitor of CYP3A4 and P-glycoprotein.
Aim: To determine the influence of CYP3A4*1 and MDR1-C3435T, C1236T and G2677T/A polymorphisms on changes to TAC dosage requirements, trough concentrations (Ctrough) and concentration/dose (C/D) ratio during and after ITRA administration in adult renal transplant patients(RTPs).
Patients and Methods: A retrospective study of 49 Caucasian RTPs receiving TAC and prophylactic ITRA (200 mg/day) for the first 3 months post-transplant. At stable moments, closest in time to azole discontinuation and after its withdrawal, TAC blood concentrations were measured using CMIA on an Architect™-C8000 analyzer.
DNA chip technology was used for the genotyping of CYP3A4*1 and MDR1 C3435T, C1236T and G2677T/A polymorphisms, which were associated with daily dose-D-, weight-adjusted daily dose-D/kg-, Ctrough and C/D ratio during cotreatment and after ITRA withdrawal using the Mann-Whitney Test. Statistical analysis: SPSS 19.0. P<0.05: statistically significant.
All patients provided their informed written consent.
Results: 49 patients (30 men/19 women) were included, age: 51 years, weight: 73 kg.
The genetic variants identified were: for CYP3A4*1: *1/*1 in 45 patients (92%) and *1/*1B +*1B/*1B in 4 (8%); for C3435T: CC in 12 (24%) and CT+TT in 37 (76%); for C1236T: CC in 14 (29%) and CT+TT in 35 (71%) and for G2677T/A: GG in 16 (33%), GT+TT in 33 (67%). This distribution was in accordance with the Hardy-Weinberg equilibrium.
A significant increase in dosage requirements occurred after ITRA withdrawal (D:from 3.28±2.70 to 4.91±2.97 mg/day; D/kg:from 0.05±0.04 to 0.07±0.05 mg/kg/day), by a mean of 80%, in order to achieve levels of 11.09±2.75 and 8.66±2.64 ng/mL, respectively (p<0.001). These requirements were always higher in CYP3A4*1B and 3435CC, 1236CC and 2677GG carriers, but the MDR1 wild-type carriers showed significant lower increases than T-allele carriers (35 vs. 94%, p= 0.003; 48 vs. 90%, p= 0.059; 46 vs. 93%, p= 0.023, respectively).
Mean C/D ratio showed a significant 2.38-fold decrease (from 5.23±3.01 to 2.35±1.42 ng/mL per mg/day) after co-administration. Although this parameter did not differ significantly between MDR1 wild-type and allele carriers, 3435 CT+TT carriers experienced a higher decrease (2.52 vs. 1.97) (p=0.085).
No significant differences in the pharmacokinetic parameters evaluated were observed for CYP3A4*1 and MDR1 C3435T, C1236T, G2677T/A polymorphisms with and without ITRA.
Conclusions: This study in RTPs revealed that mean TAC daily doses increased significantly by around 80% after ITRA withdrawal.
Although the polymorphisms studied did not significantly influence the pharmacokinetic parameters considered, 3435CC, 1236CC and 2677GG carriers seem to require lower increases in doses, experiencing 3435 CT+TT a higher decrease in C/D ratio after ITRA withdrawal.
In clinical practice, close monitoring of TAC levels is required during and after ITRA co-administration.

P.1508

Levetiracetam treatment of epileptic seizures in transplant patients

Maria Macias, Pilar Salvador, Cristina Mondelo, Isaura Pedreira, Isabel Martín.
Pharmacy Department, A Coruña University Hospital, A Coruña, Spain.

Background: Transplant patients may have or develop seizures or epilepsy.
Aims: To evaluate the effectiveness and safety of levetiracetam(LEV) in transplant patients (TPs), and the usefulness of its therapeutic drug monitoring (TDM) in our clinical practice.
Patients and Methods: Retrospective study in all TPs who were or are being treated with LEV where the levels were available, between January 2015-January 2016.Variables recorded for each patient:anthropometric characteristics (age,weight,height);clinical situation (type of transplant,liver and kidney functions,etiology of the seizures);controlled/uncontrolled seizures and therapeutic regimen (LEV and immunosuppressive therapy). With respect to LEV monitoring 3 time points were established: 1st) First measurement of LEV level(Visit 1);2nd)10-15 days after the first dosage adjustment(Visit 2);and 3rd)the following visit with a new dosage adjustment (Visit 3). At these times the following data were registered:LEV administered doses (D) and obtained plasma trough concentrations (Ctrough) and the doses recommended by the pharmacy service, as well as the immunosuppressive doses and the blood levels obtained during cotreatment with LEV and after its discontinuation if it occurred.
LEV plasma Ctrough were measured by spectrophotometry on the ARCHITECT-C8000 using the ARKTM LEV Assay.Therapeutic range(TR):12-42 mcg/mL,standard doses:500 and 3,000 mg/day.
Statistical analysis: SPSS 19.0.
Results: 18 TPs (6:kidney,3:kidney-pancreatic,4:liver,2:heart,3:lung) treated with oral LEV in monotherapy for seizure control (2:with a previous history of epilepsy, 16:developed seizures after transplantation) were included.
Visit 1: D:2,138±723mg/day,Ctrough:48±20mcg/mL:33% of the patients were in TR (mean D:1,583±801 mg/day,MDRD-4: 67±25mL/min/1.73 m2); 67% of them were above TR (D: 2,416 ±514 mg/day,MDRD-4: 57±20mL/min/1.73 m2). In these patients,LEV monitoring made it possible to adjust the dose:23% continued with the same dose and 77% required a lower dose.Therefore, during visits 2 and 3 all patients showed LEV levels in TR (visit 2: 1,357±534mg/day, Ctrough:30±9mcg/mL;visit3:977±467mg/day, Ctrough:20±6mcg/mL, respectively).
All patients with supratherapeutic levels presented adverse reactions(somnolence,asthenia, headache irritability,personality change (5.5%), delusional disorder(5.5%)) which were resolved when therapeutic levels were achieved.
Seizures were controlled in all patients. LEV was well-tolerated when plasma concentrations were therapeutic.
LEV was withdrawn in 7 when they were seizure-free. In these patients the immunosuppressive doses and blood levels did not experiment any significant change.
Conclusions: Based on our results, LEV TDM proved highly useful in improving the management of TPs treated with this drug, allowing us to individualize the antiepileptic treatment, and therefore LEV may be a suitable antiepileptic drug in transplantation due to its lower level of interactions and favorable safety profile.

P.1509

The association between trough blood level and systemic exposure of tacrolimus: Comparison between once-daily (Advagraf®) and twice-daily (Prograf®) formulation

Sayamon Sukkha[1], Atiporn Ingsathit[2], Busba Chindavijak[1], Preecha Montakantikul[1], Wichit Nosoongnoen[1], Vasant Sumethkul[2].
[1]Department of Pharmacy, Faculty of Pharmacy Mahidol University, Bangkok, Thailand; [2]Department of Medicine, Faculty of Medicine Ramathibodi Hospital, Bangkok, Thailand.

Introduction: Available data concerning the pharmacokinetic (PK) parameters of TAC-OD in early conversion from TAC-BID are limited. This study aimed to compare the PK profiles conducting at steady state of TAC-OD after early conversion from TAC-BID and the association between the trough blood level (C_{min}) and the area under the blood concentration-time curve (AUC_{0-24}) in *de novo* kidney transplant recipients.
Materials and Methods: A single center, open label, prospective and single arm conducted in *de novo* kidney transplant recipients. Eligible patients who received TAC-BID (Prograf®) were converted to TAC-OD (Advagraf®) by 1:1 mg of total daily dose at 7 days post transplantation. Pharmacokinetic studies were performed before and 7-14 days after the conversion.
Results: Fifteen patients were included and provided AUC_{0-24} of 202.9 ± 44.4 ng h/mL for TAC-BID (pre conversion) and 193.0 ± 63.4 ng h/mL for TAC-OD (post conversion) (p = 0.41). Mean trough blood level of TAC-BID and TAC-OD was 6.4 ± 1.4 ng/mL and 4.9 ± 1.6 ng/mL, respectively (p = 0.01). Mean maximum blood level (C_{max}) of TAC-BID and TAC-OD was 18.4 ± 5.7 ng/mL and 15.1 ± 5.2 ng/mL, respectively (p < 0.01). Time to reach maximum blood level (T_{max}) was longer in TAC-OD (p = 0.73). There was no significant trend toward lower fluctuation of both formula (% fluctuation of TAC-BID and TAC-OD was 139.1 ± 52.2 and 126.9 ± 41.4, respectively; p = 0.41).Trough blood level of TAC-OD was associated with higher correlation coefficient between C_{min} and AUC_{0-24} (r = 0.875, p = 0.01) than TAC-BID (r = 0.620, p = 0.01).

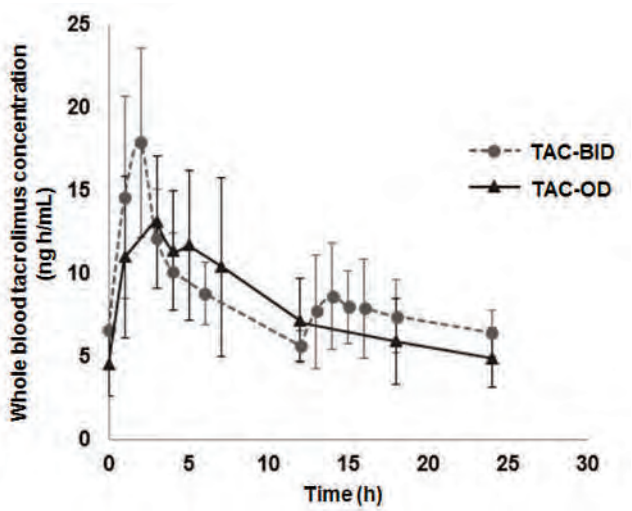


FIGURE 1 Whole blood tacrolimus concentration-time curve in kidney transplant recipients taking TAC-BID (circle) and TAC-OD (triangle). Each point and bar represents the mean ± SD.

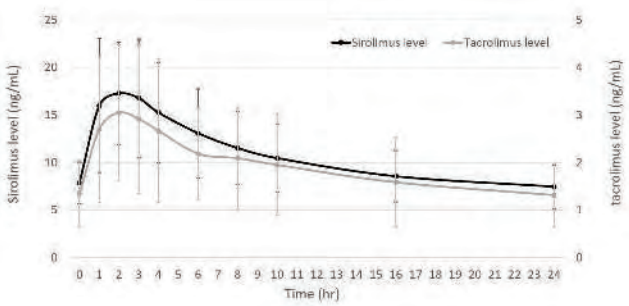
Renal function was similar between two formulations. At the time of pre conversion, mean ± SD of eGFR was 88.40 ± 24.70 mL/min/1.73 m^2. At week 6th after conversion, mean ± SD of eGFR was 81.27 ± 25.68 mL/min/1.73 m^2 (p = 0.10). No episode of rising serum creatinine more than 25 % was observed. TAC-OD was well tolerated and the adverse effect profile was consistent with TAC-BID. The most frequent adverse event of these two formulations was tremor. Incidences of new onset diabetes after transplantation (NODAT), hypertension and hyperlipidemia were not different between two formulations. Patients were not affected from biopsy-proven acute rejection (BPAR), lost graft or death during study period.
Conclusion: Conversion to TAC-OD at day 7th is associated with a significantly lower trough blood level, a similar systemic exposure and a higher correlation coefficient with systemic exposure when compared with TAC-BID. Our study suggested that there is no need to increase the dose of TAC-OD after early conversion from TAC-BID in kidney transplant recipients despite a lower trough level in TAC-OD group.

P.1510

Pharmacokinetics study of once daily immunosuppression in kidney transplantation

Natavudh Townamchai[1], Salin Watanatorn[1], Asada Leelahavanichkul[2], Boonchoo Sirichindakul[3], Yingyos Avihingsanon[1], Kearkiat Praditpornsilpa[1].
[1]Department of Medicine, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand; [2]Department of Microbiology, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand; [3]Department of Surgery, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand.

Background: Once-daily dose of immunosuppressive drug has better adherence. We study pharmacokinetic of once-daily regimen of sirolimus, minimized dose of prolonged-release tacrolimus, and prednisolone in kidney transplantation.
Methods: Low to moderate risk kidney transplant recipients with standard immunosuppression (tacrolimus with mycophoenolate and prednisolone) were enrolled for conversion to regimen of rapamycin®, low dose advagraf® and prednisolone were enrolled. Both rapamycin® and advagraf® were administered at the same time. Target sirolimus trough (C0) level was 5-8 ng/mL. The dose of advagraf® was 1 mg/day. The 24-hour-AUC of sirolimus and tacrolimus were measured by 10 time point blood levels (0, 1, 2, 3, 4, 6, 8, 10, 16, and 24 hours). Recipient with strong CYP450 inhibitor/inducer medication was excluded.
Results: Twenty recipients were enrolled. There were 4, 12, 3, and 1 recipients taking 1, 2, 3, and 4 mg/day of sirolimus. The dosages of rapamycin® and advagraf® were 0.36 ±0.03 and 0.0178 ±0.0008 mg/kg/day. The mean C0 of sirolimus and tacrolimus were 7.8 ±0.5 and 2.7 ±0.3 ng/mL. Both medication achieved peak concentration at 2 hours (C2). The 24-hour-AUC of rapamycin® and advagraf® were 257.1 ±81.0 and 45.9 ±22.9 ng.h/mL (figure 1).



Sirolimus blood levels from 2 hours till 24 hours provide good correlation with AUC (r > 0.9) while C8 had the best correlation (r = 0.977). None of our recipients developed rejection during 12 months follow up period.
Conclusions: The once-daily regimen of mTOR inhibitor with CNI minimization, rapamycin® together with advagraf®, provides good 24-hour-AUC with low interpatient variability. This regimen may improves adherence and suits for Asian which has high interpatient variability in tacrolimus dosing cause by variety of CYP3A5 expression.

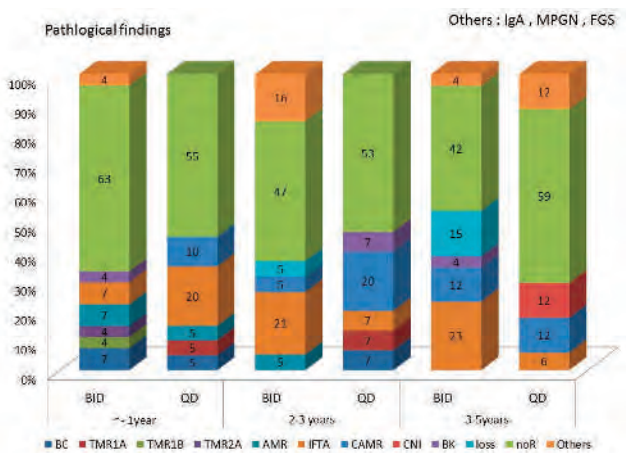
References:
[1] L. Guirado, et al. Efficacy and Safety of Conversion from Twice-daily to Once-daily Tacrolimus in a Large Cohort of Stable Kidney Transplant Recipients. *Am J Transplantation* 2011; 11: 1965-71

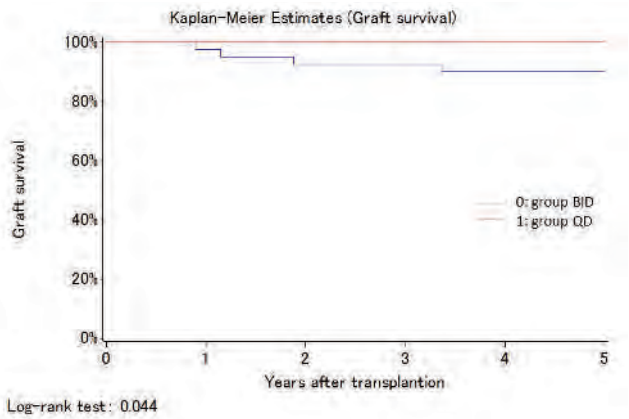
P.1511

Comparison of the pharmacokinetics and pathology for low-dose once-daily and twice-daily tacrolimus following living kidney transplantation: Five-year results from a prospective trial

Kohei Unagami[1], Masayoshi Okumi[2], Miyuki Furusawa[2], Toshihito Hirai[2], Kazuya Omoto[2], Tomokazu Shimizu[2], Masashi Inui[2], Hideki Ishida[2], Kazunari Tanabe[2], Kosaku Nitta[1].
[1]Nephrology, Tokyo Women's Medical University, Tokyo, Japan; [2]Urology, Tokyo Women's Medical University, Tokyo, Japan.

Background: Use of an appropriate immunosuppressant is necessary to ensure good graft survival following kidney transplantation (KTx). Currently, tacrolimus is the standard calcineurin inhibitor (CNI) used for KTx; however, high dose of tacrolimus leads to toxic kidney damage, whereas low dose leads to acute rejection and kidney damage[1]. Moreover, the individual differences in the bioavailability of tacrolimus are significant[2],[3]. Thus, caution needs to be exercised while adjusting the dosage of tacrolimus. Nowadays, a prolonged-release formulation of tacrolimus (Tacrolimus QD) has been recently developed and widely used. We previously reported the pharmacokinetics (PK) and protocol biopsy results within 1 year after KTx for Tacrolimus QD and twice-daily tacrolimus (Tacrolimus BID), and concluded that the clinical efficacy, safety, and PK profile were the same[4]. However, it is expected that Tacrolimus QD may be safer and more effective than Tacrolimus BID in the long-term after KTx[5],[6] because of the stable bioavailability. Moreover, to our knowledge, no study has compared the PK and pathology between tacrolimus QD and BID.
Methods: Between November 2009 and April 2011, a total of 77 KTx recipients were enrolled in a PK study, randomized to receive either low-dose Tacrolimus QD or Tacrolimus BID (group BID [N = 39] and group QD [N = 38]).
Results: Data were analyzed using the log-rank test for trends. During the 5-year follow up, characteristics of recipients and donors, tacrolimus trough levels, serum creatinine levels, and biopsy-proven rejection rates after KTx were not significantly different between groups.





No significant difference was observed in patient survival between groups (p=0.324).

Conclusions: Despite the lack of significant differences in patient survival, the use of tacrolimus QD was associated with better patient graft survival.

Katsunori Shimada, PhD. (STATZ Institute, Inc., Tokyo, Japan); Astellas Pharma Inc. (Tokyo, Japan)

References:

[1] Gaynor JJ. Lower tacrolimus trough levels are associated with subsequently higher acute rejection risk during the first 12 months after kidney transplantation. *Transpl Int*. 2016; 29: 216-226.

[2] Rodrigo E. Within-Patient Variability in Tacrolimus Blood Levels Predicts Kidney Graft Loss and Donor-Specific Antibody Development. *Transplantation*. 2015; [Epub ahead of print].

[3] Borra LC. High within-patient variability in the clearance of tacrolimus is a risk factor for poor long-term outcome after kidney transplantation. *Nephrol Dial Translant*. 2010; 25: 2757-2763.

[4] Tsuchiya T. Comparison of pharmacokinetics and pathology for low-dose tacrolimus once-daily and twice-daily in living kidney transplantation: prospective trial in once-daily versus twice-daily tacrolimus. *Transplantation*. 2013; 96: 198-204.

[5] Rostaing L. Novel Once-Daily Extended-Release Tacrolimus Versus Twice-Daily Tacrolimus in De Novo Kidney Transplant Recipients: Two-Year Results of Phase 3, Double-Blind, Randomized Trial. *Am J Kidney Dis*. 2015; [Epub ahead of print].

[6] Budde K. Novel once-daily extended-release tacrolimus (LCPT) versus twice-daily tacrolimus in de novo kidney transplants: one-year results of Phase III, double-blind, randomized trial. *Am J Transplant*. 2014; 14: 2796-2806.

P.1512

Conversion from twice-daily prograf to once-daily advagraf in multi-ethnic asian adult renal transplant recipients with or without concomitant use of diltiazem – impact of CYP3A5 genetic polymorphism on tacrolimus pharmacokinetics

Wai-Ping Yau[1], Anantharaman Vathsala[2,3].
[1]Department of Pharmacy, Faculty of Science, National University of Singapore, Singapore, Singapore; [2]National University Centre for Organ Transplantation, National University Hospital, Singapore, Singapore; [3]Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore.

Introduction: This study aimed to contribute information on tacrolimus (TAC) pharmacokinetics (PK) among multi-ethnic Asian adult renal transplant recipients (RTX) with or without concomitant use of diltiazem (DTZ) after conversion from twice-daily Prograf to once-daily Advagraf, as well as the impact of *CYP3A5*3* polymorphism on TAC PK.

Materials and Methods: A prospective, open-label, four-phase PK study was conducted at National University Centre for Organ Transplantation, Singapore. Participants included 24 stable Asian adult RTX (16 Chinese, 5 Malays, 3 Indians) maintained on the same immunosuppressive dosing regimen for ≥2 weeks prior to study. 12 RTX with DTZ participated in all four 24-h PK phases sequentially: (1) Phase P-D (Prograf with DTZ as per patient's regular regimen), (2) Phase P (Prograf after stopping DTZ for ≥1 week and titrated to achieve target TAC trough level (C_{24}) of 4–7 ng/mL prior to Phase P), (3) Phase A (Advagraf initially based on 1:1 dose conversion from Phase P and titrated to achieve target TAC C_{24} prior to Phase A), and (4) Phase A-D (Advagraf with DTZ restarted at the same dose as in Phase P-D for ≥1 week and titrated to achieve target TAC C_{24} prior to Phase A-D). The other 12 RTX without DTZ participated in only Phases P and A. TAC blood levels were measured at 19 time-points (0, 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, 12.5, 13, 13.5, 14, 15, 16, 18, 21 and 24 h after morning dose) for Phases P-D and P, and at 13 time-points (0, 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, 16, 20 and 24 h) for Phases A and A-D. All RTX were genotyped for *CYP3A5*3*.

Results and Discussion: TAC C_{24} was maintained within the target of 4–7 ng/mL after 1:1 dose conversion for 14 RTX (58.3%) while 10 RTX (41.7%) required dose titration (7 increased dose, 3 decreased dose) to achieve target TAC C_{24}. Overall, *CYP3A5* expressors (n=16; 87.5% Chinese, 12.5% Malays) received significantly higher TAC daily dose to achieve target TAC C_{24} and had significantly lower TAC exposure than *CYP3A5* non-expressors (n=8; 25% Chinese, 37.5% Malays, 37.5% Indians) for both formulations. When comparing Phase P with Phase A for all RTX with or without DTZ, TAC exposure was significantly lower after conversion for *CYP3A5* expressors (n=16; C_{24}: 6.5±1.2 vs 5.1±1.1 ng/mL, p<0.0005; dose-adjusted C_{24}: 78±34 vs 60±27 (ng/mL)/(mg/kg/day), p<0.0005; dose-adjusted AUC_{0-24}: 2758±1278 vs 2237±936 (ng·h/mL)/(mg/kg/day), p=0.004) but the decrease in TAC exposure was not significant among *CYP3A5* non-expressors (n=8). When comparing Phase P-D with Phase A-D for patients with DTZ, TAC exposure was lower, albeit not statistically significant, after conversion for *CYP3A5* expressors (n=10) and non-expressors (n=2).

Conclusion: TAC exposure generally decreased after conversion from Prograf to Advagraf in stable multi-ethnic Asian adult RTX with or without concomitant DTZ, particularly for *CYP3A5* expressors. An increase in dose with increased therapeutic drug monitoring after conversion may be warranted.

Angeline Goh; Chee Ling Goh; Sook Pui Khoo; Diana Chee Siang Teh; Tanusya Murali Murali

P.1513

Increased starting dose of Everolimus did not affect early renal function after renal transplantation

Mary Eng.
Surgery, University of Louisville, Louisville, KY, United States.

Background: The mTor class of immunosuppression has a reputation for prolonging delayed graft function in renal transplant recipients. Everolimus is a new mTor approved for use in the United States. The recommended initial starting dose is 0.75 mg twice daily, however, this dose yielded prolonged subtherapeutic levels. We, therefore, start Everolimus at 1.5mg twice daily to attempt to reach therapeutic levels faster. Delayed graft function (DGF) rates with higher starting doses has not been evaluated.
Purpose: We designed this study to see if the rate of decline of serum creatinine with the higher starting dose of Everolimus with tacrolimus was comparable to Mycophenolic acid (MPA) with Tacrolimus.
Study Design: This was a retrospective chart review conducted on renal transplants for the calender year 2015. Patient's calculated panel of reactive antibodies (cPRA), donor's kidney donor profile index (KDPI), induction and maintainence immunosuppression and serum creatinine level immediately after transplant and on post-operative days (POD) 1 to 3, and need for dialysis were recorded. Patient who received maintainence immunosuppression other than Everolimus, Tacrolimus, MPA or prednisone were excluded as were patient who received an extrarenal organ simultaneously. The groups were analyzed using student t-test and p-value < 0.05 was considered statiscally significant.
Results: A total of 64 patients were identified. There was no difference in patient demographics in regards to cPRA, KDPI and donor type. There was also no difference in the rate of DGF between the groups (19.5% in the Everolimus group vs. 33% in the MPA group, p-value 0.8). The decline in serum creatinine were similar (% decline compared to initial post-operative serum creatinine on POD #1,2,3, 20.5∓16.0 vs. 22.9∓17.0; 39.6∓23.0 vs. 36.6∓25.0; 51.9∓21.1 vs. 51.8∓26.1 respectively in the everolimus group vs. MPA group). Despite doubling the starting dose, no patients had supratherapeutic levels.
Conclusion: Initiating Everolimus at 1.5mg twice daily did not adversely affect early renal function after renal transplant.

P.1514

Postoperative rebound of anti-blood type antibodies and antibody-mediated rejection after ABO-incompatible living related kidney transplantation: Is prophylactic treatment necessary?

Hideki Ishida, Tomokazu Shimizu, Kazuya Omoto, Masayoshi Okumi, Kazunari Omoto.
Urology, Tokyo Women's Medical University, Tokyo, Japan.

The purpose of this study is to examine whether postoperative anti-blood type antibody rebound is attributed to kidney allograft rejection in ABO blood type-incompatible (ABO-I) living related kidney transplantation (KTx). A total of 191 ABO-I recipients who received ABO-I living related KTx between 2001 and 2013 were divided into two groups: Group 1 consisted of low rebound (≦1:32), N=170, and Group 2 of high rebound (≧1:64), N=21, according to the levels of the rebounded anti-blood type antibodies within one year after transplantation. No prophylactic treatment for rejection was administered for elevated anti-blood type antibodies, regardless of the levels of the rebounded antibodies. Within one year after transplantation, T cell-mediated rejection was observed in 13 of 170 recipients (13/170, 8 %) in Group 1, and in 2 of 21 recipients (2/21, 10 %) in Group 2 (Groups 1 vs. 2, P=0.432). Antibody-mediated rejection was observed in 15 of 170 recipients (15/170, 9 %) and 2 of 21 recipients (2/21, 10 %) in Groups 1 and 2, respectively (P=0.898). In this study, we found no correlation between the postoperative anti-blood type antibody rebound and the incidence of acute rejection. We concluded that no treatment is necessary for rebounded anti-blood type antibodies.

P.1515

Adherence to immunosuppression after conversion of twice-daily Tacrolimus to once-daily Tacrolimus in stable kidney transplant patients in Thailand: A prospective study

Sopon Jirasiritham, Surasak Kantachuvessiri, Atiporn Inksatid, Vasant Sumethkul, Siriwan Jirasiritham, Wiwat Thirapanich.
Department of Surgery, Faculty of Medicine Ramathibodi hospital Mahidol University, Bangkok, Thailand.

Background: Despite the clinical success of the kidney transplantation as the best treatment for end stage renal disease patients with acceptable rate of graft and patient survivals, still the long term outcome are disappointed. Non-adherence to immunosuppression has been shown in many studies to be one of the contributing factors for the late graft loss. In Thailand, twice-daily dose Tacrolimus is the most common used immunosuppression in kidney transplantation. The objective of this study is to evaluate the phamacoadherence to immunosuppression after conversion of the twice-daily dose Tacrolimus to the once-daily dose (prolonged released) Tacrolimus.
Methods: This study is an open-label, randomized parallel-group study of the clinical outcomes in the conversion of the twice-daily Tacrolimus to onec-daily Tacrolimus formulation among stable kidney transplant recipients in Thailand. 30 patients were recruited and randomly allocated into two groups. Group 1 immunosuppression were converted from the twice-daily Tacrolimus to once daily Tacrolimus. Group 2 continued with twice daily Tacrolimus. The assessment of adherece was done by questionnaire survey on treatment adherence which were conducted on Day 30, 90,180, and 365. The questionnaires were filled by the patients in each clinical visit. All patients were also individually interviewed by a trainded nurse during their visits.
Results: Of the 30 stable kidney transplant recipients, the patient characteristics in both groups are not different. All patients in both groups are assessed for their medical adherence. The rate of treatment adherence slightly dropped after baseline in the twice-daily group while those in the once daily group complied the treatment very well from D30. The difference of adherence rate between the two groups was 7.7% (95%CI:-22.2 to 6.8) m p-value=0.327. All 15 patients in the conversion group expressed their preferrence to take once daily Tacrolimus from D30 until the end of study (D365). The patients in conversion groups showd no change in the pharmacokinetic study, nor clinical adverse events, nor rejection or graft dysfunction throughout the study
Conclusion: In stable kidney transplant recipients in Thailand, the conversion of the twice-daily Tacrolimus to once-daily Tacrolimus improve the pharmacoadherencent to immunosuppression but not statistically significant. The long term impact need to be evaluated in the long term follow up. The patients also preferred the once-daily dose more than the twice daily-dose.

References:

[1] Chapman JR. Transplantation 2004:77;782
[2] Denhaerynck K, et al. Transpl Int. 2005;18(10):1121
[3] van Hooff JP, et al. ClinTransplant. December 16, 2010
[4] Kuypers D Transplantation 2013 ;95(2) : 333
[5] Pinsky BM Am J Transplant 2009 ; 9:2597
[6] Dharancy S Clin Transplant 2012 ; 293

P.1516

A change in insulin sensitivity and lipid profile in renal transplant recipients converted from cyclosporine or standard release tacrolimus to once-daily prolonged release tacrolimus

Soo Kyoung Kang[1], Sun Min Kim[1], Ye Na Kim[1], Ho Sik Shin[1], Yeon Soon Jung[1], Hark Rim[1], Bong Kwon Cheon[3], Hyun Yul Rhew[2].
[1]Internal Medicine, Kosin University College of Medicine, Busan, Korea; [2]Urology, Kosin University College of Medicine, Busan, Korea; [3]Pathology, Kosin University College of Medicine, Busan, Korea.

Background: New-onset diabetes after transplantation may be associated with the use of tacrolimus (Tac) causing impaired insulin release or reduced insulin sensitivity. And, dyslipidemia commonly occurred after transplantation. Such effects in insulin sensitivity and lipid profile have not been studied in renal transplant recipients receiving traditional twice-daily tacrolimus (TacBID) or cyclosporine and then compared to the new once-daily prolonged release formulation of tacrolimus (TacOD).
Methods: We performed an observational prospective study of 15 stable non-diabetic renal transplant recipients on change in insulin sensitivity and lipid profile in renal transplant recipients converted from cyclosporine or standard release tacrolimus to once-daily prolonged release tacrolimus. We evaluated the level of HbA1c, total cholesterol, HDL, LDL, TG, apolipoprotein A1, apolipoprotein B, serum creatinine, fasting plasma glucose, fasting insulin and HOMA-β at base line, two and four months. To analyze differences in parameter, we performed a t-test in both groups (cyclosporine to TacOD conversion group/TacBID to TacOD conversion group), and GLM-repeated measures ANOVA.
HOMA- β = (360 X Fasting insulin)/(Fasting glucoe-63)
HOMA-IR(insulin resistance) = (Fasting glucose X Fasting insulin)/405
Results: At baseline, parametes were not different in both groups (cyclosporine to TacOD conversion group/TacBID to TacOD conversion group). In GLM-repeated measures ANOVA, the result did not showed and any change in insulin sensitivity and lipid profile after conversion at baseline, two and four months.
Conclusion: Conversion from standard TacBID or cyclosporine to TacOD is safe. In spite of a reduced Tac exposure, there was no change in insulin sensitivity and lipid profile in renal transplant recipients.

Table 1. Evolution of clinical and laboratory data in cyclosprorine group and twice daily tacrolomus group at baseline

Variables	Cyclosporine (n=11)	Twice daily tacrolomus (n=4)	P value
Age (years)	51.8 ± 5.9	53.7 ± 8.9	0.632
Body weight (kg)	59.2 ± 10.0	66.5 ± 3.7	0.956
Abdominal circumference (cm)	79.7 ± 8.5	83.3 ± 3.0	0.275
SBP (mmHg)	120.1 ± 12.4	116.0 ± 11.5	0.548
DBP (mmHg)	74.2 ± 11.6	78.3 ± 10.0	0.581
Duration of transplantation	17.6 ± 3.7	7.2 ± 5.3	0.021
Serum creatinine (mg/dL)	1.1 ± 0.3	0.9 ± 0.2	0.145
estimated GFR (mL/min)	60.3 ± 18.5	96.2 ± 16.2	0.094
Total cholesterol (mg/dL)	163.8 ± 47.7	160.3 ± 45.2	0.564
LDL cholesterol (mg/dL)	73.3 ± 33.9	80.3 ± 8.6	0.651
HDL cholesterol (mg/dL)	52.2 ± 13.7	57.2 ± 10.2	0.578
Triglyceride (mg/dL)	140.1 ± 70.8	146.8 ± 56.0	0.874
Fasting glucose (mg/dL)	99.9 ± 9.2	99.3 ± 11.0	0.915
Apolipoprotein A1	169.0 ± 41.2	165.3 ± 33.1	0.573
Apolipoprotein B	82.1 ± 15.3	87.6 ± 14.4	0.593
HbA1c (%)	5.9 ± 0.5	5.8 ± 0.4	0.714
Fasting insulin	12.7 ± 13.0	8.2 ± 4.1	0.547
HOMA-IR	3.2 ± 3.2	1.8 ± 0.9	0.287
HOMA-β	148.3 ± 146.9	176.5 ± 185.7	0.815

Table 2. HOMA IR by before immunosuppressant

Variables	Baseline	2 months	4 months	P value*
cyclosporine (n=11)	4.2 ± 3.5	4.8 ± 5.0	2.4 ± 1.0	0.661
Tacrolimus (n=4)	1.8 ± 0.9	1.1 ± 0.9	1.1 ± 0.5	0.771
P value†	0.069	0.069	0.069	

*P-values by treatment period obtained from linear model using repeated measured ANOVA
†P values by content obtained from linear model using repeated measured ANOVA

P.1517

The prevalece of renal transplant recipients with low exposure to mycophenolic acid taking mofetil mycophenolate or enteric-coat sodium mycophenolate and possible influence factors

Elizete Keitel[1,2], Natiane Vizoli[1], Larissa Pacheco[1,2], Joao Carlos Goldani[1,2], Ronivan Dal Pra[2], Helen Zanetti[1,2], Gisele Meinerz[1,2], Bruna Cardoso[1,2], Rafael Linden[3], Valter Garcia[2].
[1]PPG Pathology, UFCSPA, Porto Alegre, Brazil; [2]Nephrology-Renal Transplant Unit, Santa Casa de Misericordia de Porto Alegre, Porto Alegre, Brazil; [3]Pharmacology, FEEVALE, Novo Hamburgo, Brazil.

The measurement of area under the curve (AUC) of mycophenolic acid (MPA) has not been used in the routine of out patient clinical transplant care. The aims of this study were to evaluate the prevalence of the MPA-AUC below 30 mcg*h ml-1 in a sample of renal transplant recipients taking mofetil mycophenolate (MMF) or enteric coat sodium mycophenolate (EC-MPS), also to analyse the influence of calcineurin inhibitor (CNI) and omeprazol interaction with MPA and renal function on AUC.

Methods: A transversal study of adults over 18 years old at least 6 months after transplant taking mycophenolate, CIN and corticosteroids with estimated glomerular filtration rate (MDRD) over 30ml/min. The MPA plasma concentration were measured by high performance liquid chromatography. Blood was collected 20 min pre-dose, 1h and 3h after morning dose of MMF and the AUC0-12 was calculated by an online tool (Imunossupressores Bayesian Dose Adjustment-ISBA). The patients taking MPS blood was collected 20 min pre-dose, 1, 2, 3 e 4 h after morning dose. The AUC0-12 was calculated by an algorithm described by Sommerer et al. (2010).

Results: One hundred recipients were included from Sep/2013 to Aug/2014. The mean age was 48.7±12.8 years, 46% were female, 62% received deceased donor kidney. The mean follow-up after transplant was 48.9 ± 46.4 month. Twelve 12 were taking MMF, full dose 50% of them (3 with cyclosporine and 3 with tacrolimus). The other 6 were under tacrolimus. Eighty-eight patients were taking MPS, only 33% full dose (7 with cyclosporine and 23 with tacrolimus). The other 58 (67%) took low dose MPS (52 with tacrolimus and 6 with cyclosporine).From the total 100 recipients evaluated 20% presented the AUC below 30mcg*h m, 63% between 30-60mcg*h ml-1 (target) and 17% over 60mcg*h ml-1. From the 64 patients with low dose of MPA, 26.2% presented ASC below 30mcg*h ml-1 and 9.2% over 60mcg*h ml-1.The MMF did not show a correlation between dose and exposition of MPA (r2:0.103 P:0.701), but MPS showed it (r2: 0.417 P<0.001).Considering the interaction of MPA exposure and CNI there was a negative correlation between cyclosporine levels AUC (r2=-0.925 P<0,001) but not with tacrolimus levels and AUC (r2=-0.59 P=0.592). From the 88 patients taking MPS, 62 were under omeprazole and 66.1% presented AUC into the targeting compared to 61.5% not taking it. Similar findings were observed in the group taking MMF (57.1% vs 40%, p=ns). There was no correlation between GFR and AUC.

Conclusion: The prevalence of patients with MPA-AUC below target was 20%. There was a correlation between dose and exposure to MPA-AUC in patients taking EC-MPS but not MMF. There was a negative correlation with cyclosporine level and MPA-AUC but not with tacrolimus. The omeprazole use and GFR did not interfere with MPA-AUC.

Novartis by parcial grant support

References:

[1] Langone A, Doria C, Greenstein S et al. Does reduction in mycophenolic acid dose compromise efficacy regardless of tacrolimus exposure level? An analysis of prospective data from the Mycophenolic Renal Transplant (MORE) Registry. *Clinical Transplantation.* 2012; 27:15-24.

[2] Van Hest R, Mathot RA, Pescovitz MD et al. : Explaining variability in mycophenolic acid exposure to optimize mycophenolate mofetil dosing: A population pharmacokinetic meta-analysis of mycophenolic acid in renal transplant recipients. *J Am Soc Nephrol.* 2006; 17: 871-880.

[3] https://pharmaco.chu-limoges.fr

[4] Sommerer C, Müller-Krebs S, Schaier M, Glander P, Budde K, Schwenger V, Mikus G, Zeier M. Pharmacokinetic and pharmacodynamic analysis of enteric-coated mycophenolate sodium: limited sampling strategies and clinical outcome in renal transplant patients. *Br J Clin Pharmacol.* 2010;69(4):346-57

P.1518

Effect of everolimus conversion in hepatitis C viremia in adult kidney recipients

Elizete Keitel[1,2], Larissa Pacheco[1,2], Ronivan Dal Pra[2], Bruna Cardoso[1,2], Mariana Rodrigues[2], Helen Zanetti[1,2], Jorge Neumann[2], Valter Garcia[2].
[1]PPG Pathology, UFCSPA, Porto Alegre, Brazil; [2]Nephrology-Transplant Unit, Santa Casa de Misericordia de Porto Alegre, Porto Alegre, Brazil.

Introduction: There is no specify immunosuppressive protocols for HCV positive renal transplants recipients. The aim of this study was to evaluate the conversion effect to everolimus in hepatitis C viremia in adult kidney recipients.
Methods: This is a pilot single-center, prospective, randomized, open label controlled trial with renal allograft recipients with positive serology for hepatitis C. Patients included were under calcineurin inhibitors, antiproliferative (mycophenolate/Azathioprin) and prednisone. They were randomized to convert to Everolimus or mainenance of calcineurin inhibitor. Prednisone and antiprolifertive were not changed. The exclusion cirteria were eGFR below 30ml/min and proteinuria/creatinine index >0,5.
Results: Thirty patients were randomized and 28 followed by 12 months (Conversion group=15 and control group=13). Real time PCR HCV levels expressed in log values were comparable in both groups and among patients in the same group. The statistical analysis showed no interaction effect between time and group (p-valueG*M= 0.852), overtime intra-groups (p-valueM=0.889) and between treatment and control group (p-valueG=0.286). The mean viral load at baseline, 3, 6, 9 and 12 months was 5.8 ±0,74, 5.7 ± 0,89, 5.8 ± 0.60, 5.7 ± 0.85, 5.8 ± 0.93 in the control group and 6.1 ± 0,83, 6.3 ± 0.95, 6.2 ± 0.87, 5.6 ± 1.8, 6.1 ± 0.62 in the treatment group. The group conversion showed a higher incidence of dyslipidemia (p=0.03) and proteinuria events (p=0.01). No difference was observed in the incidence of anemia (p=0,17), new onset of posttransplant diabetes mellitus (p= 1.00), urinary tract infection (p=0.60). The mean eGFR at the baseline, 1, 3, 6, 9 and 12 month after randomization was similar in both groups. During the follow-up there was no acute rejection episode, malignancies, graft loss or death.
Conclusion: Our pilot study did not show viral load decrease after conversion to everolimus with antiproliferative maintenance therapy.
Novartis by partial grant support
References:
[1] Wagner D, Kniepeiss D, Schaffellner S, Jakoby E, Mueller H, Fahrleitner-Pammer A, Stiegler P, Tscheliessnigg K, Iberer F. Sirolimus has a potential to influent viral recurrence in HCV positive liver transplant candidates. *International Immunopharmacology. 2010:10, 990–993*
[2] Lim WH, Eris J, Kanellis J, et al. A systematic review of conversion from calcineurin inhibitor to mammalian target of rapamycin inhibitors for maintenance immunosuppression in kidney transplant recipients. *Am J Transplant* 2014;14: 2106–2119
[3] Soliman A, Fathy Ahmed, Hhashab S, Shaheen N, Soliman M. Sirolimus Conversion may suppress viral replication in hepatitis c vírus-positive renal transplant candidates. *Exp Clin Transplant.* 2013;11(5):408-11.

P.1519

Association of tacrolimus trough levels with incidence of new onset of diabetes after transplantation and the graft function assessed by concentration and dose ratio

Sailaja Kesiraju[1,3], Uma Maheswara Rao Ch[2], Purna Paritala[2], Shreedhar Reddy[2], V.S. Reddy[2], Sahariah Sarbeshwar[2].
[1]Dept of Immunology, Bhagwan Mahavir Medical Centre, Hyderabad, India; [2]Transplantation Immunology& Research Centre, Transimmun, Hyderabad, India; [3]Department of Nephrology and Transplantation, Krishna Institute of Medical Sciences, Hyderabad, India.

Introduction: Tacrolimus(TAC) has established its usefulness in organ transplantation, however due to inter and intra-patient variability in absorption and clearance, due to its narrow therapeutic window, and drug-drug interactions, it is highly important to achieve optimal therapeutic efficacy while minimizing the risk of toxicity.
We hypothesized that a change in the metabolism of TAC would have a serious impact on allograft function, especially in the development of new onset of diabetes after transplantation (NODAT).Hence we evaluated the association between concentration by the dose (C/D ratio) on the development of NODAT, Calcineurin inhibitor(CNI) toxicity, acute rejection and graft function after kidney transplantation.
Methods: Graft function was assessed by S.creatinine levels and creatinine clearance. One hundred and seventy three live donor kidney transplant recipients with a mean age 37.4 (21 to 64 yrs) were included in the study. Immunosuppression comprised TAC, mycophnolatemofetil and prednisolone. TAC levels were measured with CMIE(ArchitectAbbottDiagnostics).TAC metabolism rate was determined by dividing trough concentration (C) with the daily TAC dose (D).
C/D ratio units: (ng/ml ÷ mg) ≡ 10-6 (ml-1) ≡ 10-3 (l-1)
Patients were categorized into three groups based on mean C/D ratio at the first month post transplantation. Group 1 (fast metabolizers, n=58): C/D <1.11 10-6/ml, Group2 (moderate metabolizers, n=76): C/D 1.82 (1.11-2.48) 10-6/ml, Group 3 (slow metabolizers, n=39):C/D ≥2.48 10-6/ml.
Results and Discussions: Incidence of NODAT (p=<0.05) was observed in slow metabolizers compared to the other two groups. Higher incidence of CNI toxicity (p=0.01) and chronic allograft dysfunction (p=<0.05) was noted in fast metabolizers. There was no significant difference in the occurrence of acute rejection between the groups. Renal function was better in moderate absorbers compared to other two groups with Crcl at 36 months 49±12.2,58±14.6 and 56±11.8 ml/mt/1.73m2,in fast, moderate and slow metabolizers respectively.
We noted that the trough levels of TAC have a strong correlation with the onset of NODAT, and secondly, the influence of metabolism of TAC has significant impact on the allograft function in renal transplant recipients. This measure could be important in deciding TAC dosage and also in achieving tailored immunosuppressive regimen. C/D ratio is a reliable yet easy and accurate measurement to define patients at risk of NODAT.
References:
[1] Thölking G, Fortmann C, Koch R, et al. The tacrolimus metabolism rate influences renal function after kidney transplantation. *PLoS One.* 2014 Oct 23;9(10):e111128. doi: 10.1371

P.1520

Conversion to once-daily tacrolimus extended-release formulation (TAC XL) in renal transplantation: A multicenter experience in Argentina

Rafael Maldonado[1], Gervasio Soler Pujol[2], Marisol Rodriguez[1], Jihan Sleiman[2], Santiago Carbel[1], Roberto Sabatiello[3], Hugo Petrone[4], Fabian Taylor[4], Nora Imperiali[5], Roxana Groppa[5], Silvina Grillo[6], Fabian Lopez[6], Monica Gomez[6], Carlos Diaz[2], Luis Gaite[7], Judith Gaite[7], Pablo Rafaelle[8], Fernando Mos[8], Ruben Schiavelli[3].
[1]Nephrology and Transplantation Service, Clínica Privada Velez Sarsfield, Cordoba, Argentina; [2]Nephrology and Transplant Section, CEMIC, Buenos Aires, Argentina; [3]Nephrology and Transplantation Service, Hospital Cosme Argerich, Buenos Aires, Argentina; [4]Renal Transplantation Service, CRAI Sur, La Plata, Argentina; [5]Nephrology Service, Hospital Italiano Buenos Aires, Buenos Aires, Argentina; [6]Renal Transplant Unit, Hospital JC Perrando, Resistencia - Chaco, Argentina; [7]Nephrology Service, Clinica de Nefrologia, Urologia y Enfermedades Cardivasculares, Santa Fe, Argentina; [8]Renal Unit, Hospital Universitario Fundación Favaloro, Buenos Aires, Argentina.

The extended-release formulation of Tacrolimus (TAC XL) was developed to provide a more convenient once daily dosing (QD) to improve patient´s adherence while showing pharmacokinetics, safety and efficacy profile comparable to the traditional twice daily formulation (BID TAC)[1],[3]. Although it is approved in renal transplantation to be used de novo many center chose to use it in conversion after the transplant either from a twice-daily formulation or from other non-TAC regimens[2],[4]. The aim of this study is to present a multicenter experience from 8 centers in Argentina with TAC XL used in different conversion modalities after renal transplantation.
Methods: Retrospective analysis of renal transplant recipients converted to TAC XL after transplantation and a follow up of at least 6 months. 215 patients were included from 8 centres converted between 02/2008 and 08/2015. Data recorded at baseline and at 6,12 and 24 months after conversion included creatinine, urine Pr/Cr, MDRD, CKD-EPI, haemoglobin lipids, uric acid, dose and trough TAC levels and adverse events.Depending on the time after transplantation we identified those who converted early (≤12 months) 28.4% or late (>12 months) 71.6% after the transplant procedure.Depending on the TAC dose ratio at conversion we identified 4 different groups, Group 1: same dose, Group 2: reduced dose, Group 3: increased dose, and Group 4: conversion from other regimes different from TAC (Ciclosporine or mTOR inhibitors). Statistical analysis: Variables were expressed as mean ± SD or frequencies as appropiate. Comparisons of data between base line, 12 and 24 months were performed using ANOVA or Kruskal-Wallis as appropriate. Survival estimates were plotted on Kaplan-Meier curves. p values less 0.05 were considered of statistical significance.
Results: Median time to conversion was 29,9 months (1,2 – 166). Renal function and proteinuria remain stable from baseline to 12, and 24 months: Creatinine (mg/dl) 1,48±0,6; 1,43±0,5 and 1,46±0,5 respectively, MDRD 55±21; 55±19,4; 54,2±19,7 respectively and urine Pr/Cr 0,16±0,4; 0,33±1,6; 0,31±1,18 respectively (p=NS). No differences were observed in CKD-EPI formula and the other blood parameters. TAC trough levels and dose remained stable and within the therapeutic range across the different conversion groups at 6, 12 and 24 months: TAC XL trough level (ng/dl) 7,8±6,6; 6.9± 2,5; and 6,6±2,1 respectively; and daily dose 5,1±3,5; 5,1±3,4; and 5,3±8,7 respectively (p=NS). There were no differences between groups concerning efficacy and safety variables and dose/trough level analysis. 11/211 (7%) patients experienced acute rejections post switch. Five year patients and events free survival were 95% and 80% respectively.
Summary: In multicentre experience with TAC XL used in conversion in renal transplants, was safe, and associated with stable renal function and proteinuria at two years. TAC XL shows a significant flexibility regarding different conversion strategies.
References:
[1] Guirado L et al. *Am J Transplant*. 2011 Sep;11(9):1965-71.
[2] Jeannot M et al.*Ann Transplant*. 2014 Jul 7;19:320-4.
[3] Spagnoletti G et al. *Transplant Proc*. 2014 Sep;46(7):2224-7.

P.1521

Efficacy and safety of enteric-coated mycophenolate sodium in Chinese living-donor kidney transplant recipients: A multicenter prospective cohort study

Chunbai Mo[1], Changxi Wang[2], Tongyi Men[3], Jinliang Xie[4], Aiming Zhang[5].
[1]Organ transplantation center, Tianjin First Center Hospital, Tianjin, People's Republic of China; [2]Organ transplantation center, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, People's Republic of China; [3]Organ transplantation center, Qian Fo-shan Hospital, Jinan, People's Republic of China; [4]Organ transplantation center, Xiang Ya Hospital Central South University, Changsha, People's Republic of China; [5]Organ transplantation center, Ji Nan Military General Hospital, Jinan, People's Republic of China.

Objective: To investigate the efficacy and safety of enteric-coated mycophenolate sodium (EC-MPS) in Chinese living-donor kidney transplant recipients.
Methods: Twenty-eight cases of living donor kidney transplant recipients from five centers were enrolled in this prospective cohort study. Maintenance immunosuppressive therapy was EC-MPS and calcineurin inhibitor (CNI) plus corticosteroids. The study was conducted according to the Declaration of Helsinki, and informed consent was obtained from all patients. The patients were followed up for 24 months. Biopsy-proven acute rejection (BPAR), patient / graft survival and adverse events were observed.
Results: All patients completed the follow-up. The patient and graft survival rates were both 100%, respectively. No biopsy-proven acute rejection was observed. The gastrointestinal adverse effects were assessed by using Gastrointestinal Symptom Rating Scale (GSRS) and the scores were 0 at 12, 15 and 21 months post-transplant, respectively. The scores were 0.1 ± 0.64 at 18 months and 0.0 ± 0.19 at 24 months. The medication adherence categories of 'excellent' and 'good' were 74.1% and 25.9% at 22-24 months, respectively. As to complete blood cell counts, one patient had significantly abnormal white blood cell counts and another had abnormal absolute neutrophil count at the visit of 24 months, respectively. No significantly abnormal variables of complete blood cell counts were found at other visits.
Conclusion: The triple therapy with EC-MPS and CNI plus corticosteroids for living-donor kidney transplant recipients has favorable clinical outcomes in a moderately long term. The combination regimen also demonstrated good gastrointestinal tolerance according to GSRS, which may contribute to good medication adherence.
Author Disclosure: The authors have declared no conflict of interest.

P.1522

Late onset of progressive speech impairment due to tacrolimus-associated leukoencephalopathy after kidney transplantation: A case report and literature review

Kazushige Sato[1], Yuhji Marui[2], Kiho Tanaka[2], Shinji Tomikawa[2].
[1]General Medicine, Tosendo Hospital, Wakuya, Japan; [2]Renal Transplantation Surgery, Toranomon Hospitral, Kawasaki, Japan.

Tacrolimus is one of the most commonly used immunosuppressants and its success in patient undergoing kidney and other solid-organ transplantation is well-established. However, tacrolimus-associated adverse drug effects are not unusual and include the development of neurological disorders, with clinical manifestations ranging from mild tremors to severe life-threatening stupor. Here we report an unusual case of a late-onset tacrolimus-associated complete loss of speech in a kidney-transplant patient. A 34-year-old man underwent kidney transplantation 9 months before he presented with dysarthria (slurred speech). His symptoms were initially treated as cerebrovascular disease but anarthria (complete loss of speech) developed on the third day after admission. A second round of cerebral magnetic resonance imaging (MRI) showed enhanced hyperintense lesions in the midbrain, seen on T2-weighted imaging. Tacrolimus was discontinued based on a suspicion of tacrolimus-associated leukoencephalopathy and the patient was instead administered cyclosporine. After speech therapy, his neurological manifestation resolved completely. He was discharged in good condition 48 days after admission. A follow-up MRI 14 months later showed the complete absence of the brain abnormalities. Our case shows that dysarthria, as an initial symptoms of tacrolimus-associated leukoencephalopathy, can develop even long after kidney transplantation. After organ transplantation, a loss of speech alone as the sole neurological manifestation, without generalized seizures or loss of consciousness, is very rare. In our review of published reports, there were only 13 cases in which impaired speech was the only TAC-associated complication after solid organ transplantation.

	Sex	Age (years)	Transplanted organ	Post-transplant days at symptom onset	TAC-related treatment	Outcome	Neurological manifestation(s) at symptom onset	Reference
1	M	43	Liver	12	Dose Reduction	Improved	Dysphasia, tremor	Reyes et al. 1990
2	F	38	Liver	9	Dose Reduction	Improved	Speech deficit, paraplegias	Reyes et al. 1990
3	F	46	Liver	7	Withdrawn	Improved	Adynetic mutism	Yamane et al. 1998
4	M	48	Liver	9	Withdrawn	Improved	Adynetic mutism	Bronster, 2000
5	F	5	Liver	3	Withdrawn	Unchanged	Akinetic mutism, hemiparesis	Yokoi, 2003
6	M	66	Liver	3	Withdrawn	Improved	Adynetic mutism	Sierra-Hidalgo et al. 2005
7	F	50	Liver	3	Withdrawn	Improved	Adynetic mutism	Vuerich et al. 2011
8	F	33	Kidney	21	Withdrawn	Improved	Akinetic mutism, seizures	Toledo et al. 2012
9	F	63	Liver	7	Withdrawn	Improved	Adynetic mutism	Bilbao et al. 2014
10	M	61	Liver	11	Withdrawn	Improved	Adynetic mutism	Bilbao et al. 2014
11	M	64	Liver	9	Withdrawn	Improved	Akinetic mutism, seizures	Bilbao et al. 2014
12	M	52	Liver	6	Withdrawn	Improved	Adynetic mutism	Bilbao et al. 2014
13	M	40	Liver	10	Withdrawn	Improved	Speech deficit, dysarthria	Jang et al. 2014

In 12 of these 13 cases, the patients had undergone liver transplantation; only one was a kidney-transplant patient.
In conclusions, unlike previously reported cases of TAC-associated leukoencephalopathy after solid-organ transplantation, this complication occurred in our patient 9 months after kidney transplantation. The only manifestation was progressive speech impairment. Dysarthria may thus be the presenting symptom of TAC-associated leukoencephalopathy, even long after transplantation. Careful attention to this clinical manifestation in transplant patient is crucial for a timely diagnosis, which will reduce the risk of permanent neurological disabilities.

P.1523

Impact of preoperative donor-specific anti-HLA antibody in adult living donor kidney transplantation

Youngmin Song[1], Kyo Won Lee[1], Jae Berm Park[1], Chan Woo Cho[1], Nuri Lee[1], Seounghyun Kim[1], Ji Soo Lee[1], Hyo Jun Park[1], Eun Suk Kang[2], Wooseong Huh[3], Sung Joo Kim[1].
[1]Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; [2]Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; [3]Division of Nephrology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea.

Recently, many centers tried to perform transplantation across HLA barrier with desensitization protocols including rituximab, plasmapheresis and intravenous immune globulin and reported promising short term outcome. The solid phase assay (SPA, Luminex) has recently become available for anti HLA antibody (HLAab) screening and has greater specificity and sensitivity than complement dependent cytotoxicity crossmatch (CDC-XM) in detecting HLAab. DSA detected on Luminex is being the subject of desensitization in many centers recently according to the result of previous studies shown that patients with low-level DSA are still at a higher risk of acute antibody mediated rejection (AMR). In the present study, we retrospectively investigated the impact of CDC positivity and preoperative DSA on graft survival and graft function in patients desensitized with protocol based on rituximab and rabbit antithymocyte globulin (rATG).
327 kidney transplantation recipients were enrolled. Among this 327, 9 patients (Group 1, 9/337, 2.8%) were complement dependent cytotoxicity crossmatch (CDC-XM) positive. 25 patients (Group 2, 25/337, 6.7%) were CDC-XM negative, donor specific antibody (DSA) positive on solid phase assay (SPA; Luminex assay). 55 patients (Group 3, 55/327, 16.8%) were CDC-XM negative, DSA negative and panel reactive antibody (PRA) > 50%. 241 patients (Group 4, 241/327, 73.7%) were CDC negative, DSA negative and PRA < 50%. CDC positive recipients got desensitization incluing rituximab (375mg/m2), IVIG (400mg/kg), plasmapheresis and rATG. DSA positive recipients got desensitization with rituximab and rATG.
Female and re-transplantation recipients were many in sensitized recipients (Group 1 and 2, $p < 0.001$)). Recipients with DSA (Group 2) experienced more AMR than other groups ($p = 0.005$). More de novo DSA were also developed in Group 2 ($p < 0.001$). 5-year graft survival rate were lower in Group 1 and 2 (0.5 and 0.675, respectively) than Group 3 and 4 (0.982, 0.967, repectively). Graft function estimated with serum creatinine level and estimated glomerulus filtration rate (eGFR) of Group 2 tended to decrease after 2 year. In Group 2, mean fluorescence intensity (MFI) of DSA tended to rebound more frequently than in Group 1 ($p = 0.0228$).
CDC negative and DSA positive status were at a higher risk of AMR. Decreased 5-year graft survival and graft function were also observed despite of desensitization with rituximab and rATG. More intense desensitization protocol would be necessary for these recipients.

P.1524

Optimal initial everolimus dose in renal transplant recipients administered tacrolimus

Hironori Takeuchi[1], Yuki Nakamura[2], Hitoshi Iwamoto[2], Osamu Konno[2], Yu Kihara[2], Takayoshi Yokoyama[2], Thoru Nagaishi[1], Sayuri Ohsato[1], Daich Nakayama[1], Miyuki Komazaki[1], Tatsunori Toraishi[3], Kiyoshi Okuyama[3], Chie Ikeda[2], Takashi Kawaguchi[1], Toshihiko Hirano[4], Sakae Unezaki[1], Shigeyuki Kawachi[2].
[1]Department of Practical Pharmacy, Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan; [2]Digestive Surgery and Transplantation Surgery, Hachioji Medical Center, Tokyo Medical University, Tokyo/ Hachioji, Japan; [3]Department of Pharmaceutics, Hachioji Medical Center, Tokyo Medical University, Tokyo/ Hachioji, Japan; [4]Department of Clinical Pharmacology, Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan.

Introduction: Everolimus (EVL) is administered in combination with cyclosporine (CYA) or tacrolimus (TAC) following renal transplantation. CYA inhibits EVL metabolism via competitive antagonism of CYP3A4, thereby elevating the blood EVL concentration. However, there are no pharmacokinetic interactions when administering combined EVL and TAC.
Objectives: The fixed initial EVL dose (1.5 mg/day) was confirmed in patients using CYA, and may be too low for patients receiving TAC. Consequently, we retrospectively investigated the suitable initial EVL dose in patients receiving combined TAC treatment.
Methods: Fifteen patients administered TAC (Graceptor®) and 16 patients administered CYA (Neoral®) who received renal transplantation were evaluated. EVL was administered at least 3 months after transplantation in patients treated with calcineurin inhibitors. The initial EVL dose was fixed (1.5 mg/day) in all patients. The EVL trough concentration (Ct) was measured after 1 week, allowing time to reach steady state. The Ct values were compared between patients receiving TAC and CYA, and the optimal initial EVL dose was calculated, to target the average EVL Ct of 5 ng/mL for patients receiving TAC using average values of actual Ct.
Results: The average EVL Ct was 3.25 ng/mL (95%CI:2.65-3.85 range: 1.24–5.50) in patients receiving TAC and 5.55 ng/mL (95%CI:3.59-7.51, range: 2.63–15.40) in patients receiving CYA (P = 0.0279). The average EVL Ct was significantly lower in patients receiving TAC (3.25 ng/mL, 95%CI:2.65-3.85, range: 1.24–5.50) compared with the patients receiving CYA (5.55 ng/mL, 95%CI:3.59- 7.51, range: 2.63–15.40)(P = 0.0279) Similarly, No patients receiving TAC had a higher EVL Ct than 8 ng/mL, as compared to 3 of 16 patients receiving CYA (19%). The patients that had the target EVL Ct (3-8ng/mL) and that a lower EVL Ct than 3 ng/mL were comparable between patients receiving TAC and CYA. The patients that had EVL Ct of more than 5mg/mL below target range were significantly fewer in patients receiving TAC (6.7%) compared with the patients receiving CYA (50%) (P = 0.0155). The calculated initial EVL dose to became the target Ct of 5 ng/mL in patients receiving TAC, was 2.3mg/day of half times than the patients receiving CYA
Conclusions: The EVL Ct significantly were lower in patients receiving TAC than in patients receiving CYA. Patients administered CYA had larger inter-individual EVL Ct variability. As The patients that had the target EVL Ct (3-8ng/mL) were comparable between both groups, we believe it may be better to start EVL administration using the same dose in patients receiving TAC and CYA, and then adjusting the dose after monitoring for safety However, the higher EVL Ct than target range existed in only patients receiving CYA and the number of patients with the higher EVL Ct than 5 ng/mL was significantly higher among those receiving TAC. Consequently, in patients receiving TAC who want to avoid a low initial EVL Ct, a initial higher dose may be necessary. In that case, we propose that an initial EVL dose was 2.25mg (0.75mg/tablet×3) of half times than the patients receiving CYA.

P.1525

Survival of HLA class two positive recipients compared to standard risk recipients after renal transplantation at the National Kidney and Transplant Institute from January 1, 2009 to December 31, 2013

Jonathan John Tamin, Arwin Ronan P.Ronsayro, Marc Anter E.Mejes, Adolfo C.Parayno, Benito V.C.Purugganan.
Organ Transplantation and Vascular Surgery, National Kidney and Transplant Institute, Quezon, Philippines.

Introduction: In the past, due to the high demand and limited supply for donor kidneys, only T- and B-cell cytotoxic cross-match-negative recipients were considered for donor renal transplantation. The objective of this study is to determine the outcome of HLA Class II positive cross matched recipient compared to Standard risk group within one year post-transplant at the National Kidney and Transplant Institute (NKTI).
Materials and Method: This is a retrospective chart review of patients who underwent renal transplantation from January 1, 2009 - December 31, 2013 at NKTI were reviewed. Patients who were HLA Class II positive and patients considered standard risk were included in the study. Patient's demographic profile, immunologic and non-immunologic complications occurring during renal transplant admission up to one year post-transplant was extracted. Patients with prior history of transplantation were not included in this study. Data will be presented in tables as frequencies and percentages.
Results and Discussion: This is a retrospective descriptive study of renal transplant cases done at NKTI from 2009 to 2013.

IMMUNOSUPPRESSION OUTCOMES

Immunosuppression Protocol	Subjects	Induction Medications	COMPLICATIONS Without	COMPLICATIONS With	Total
CYA + MPA	Standard Risk	Anti thymocyte globulin	26	8	34
		BASILIXIMAB	59	8	67
		ALEMTUZUMAB	21	13	34
		DACLIZUMAB	8	4	12
		NONE	131	42	173
		TOTAL	245	75	320
	HLA Class II positive	Anti thymocyte gobulin	2	1	3
		ALEMTUZUMAB	1	0	1
		NONE	3	1	4
		BASILIXIMAB	2	2	4
		TOTAL	8	4	12
Tacrolimus + MPA	Standard Risk	Anti thymocyte gobulin	76	32	108
		BASILIXIMAB	192	50	242
		ALEMTUZUMAB	21	11	32
		DACLIZUMAB	10	4	14
		NONE	40	8	48
		TOTAL	339	105	444
	HLA Class II positive	Anti thymocyte gobulin	14	4	28
		ALEMTUZUMAB	3	0	3
		BASILIXIMAB	2	2	4
		TOTAL	19	6	25

The revealed a **3.9%** mortality rate for standard risk group and **13%** mortality rate for HLA class II positive group.

CAUSES OF READMISSIONS

Causes of Readmission	Frequency (Standard risk)	Percent	Frequency (HLA Class II positive group)	Percent
Immunologic				
Acute cellular rejection	92	24.20%	3	14.28%
Acute humoral rejection	2	0.64%		
Chronic rejection	3	0.96%		
Non immunologic				
Genito Urinary	84	20.70%	6	28.57%
Respiratory	71	16.24%	4	19.07%
Gastro Intestinal	63	14.01%	2	9.52%
Infectious	39	7.96%	4	19.07%
Cardio Vascular	10	3.18%	2	9.52%
Endocrinology	7	2.23%		
Post op Complication	5	1.59%		
Neurologic	4	1.27%		
OB GYNE	4	1.27%		
Delayed Renal Function	3	0.96%		
Acute Humoral Rejection	2	0.64%		
Cancer	2	0.64%		
Ophthalmology	2	0.64%		
Orthopedic	2	0.64%		
Vascular	2	0.64%		
Acute gouty arthritis	1	0.32%		
Acute Kidney Injury sec to DEHYDRATION	1	0.32%		
ALLERGY	1	0.32%		
Acute Tubular Necrosis	1	0.32%		
Bell's palsy	1	0.32%		
Biopsy per study	1	0.32%		
Calcineurin inhibitor toxicity	1	0.32%		
Hematology	1	0.32%		
MULTI ORGAN FAILURE	1	0.32%		
t/c Acute Rejection	1	0.32%		
Total	314	100%	21	

Acute rejection was commonly encountered on both groups. A respiratory complication was the most common non-immunologic event and cause of mortality for both groups.

Conclusion: This study covers a transition period in the use of immunosuppression protocols due to the emergence of recommendations from the KDIGO Clinical Practice Guidelines. However, results of this study reveals that acute rejection is still the most common complication encountered for both groups. Mortality rate was 4 % for the standard risk group, and higher at 14 % for the HLA Class II positive group for up to 1 year post transplant. We hope to see lower incidence of immunologic and non-immunologic events in the coming years due to further compliance to recommendations, improvement in the medical services, and wider insurance coverage. We will recommend further studies in the near future to see this trend.

Keywords: Immunosuppression, Acute Rejection, Immunologic, Frequency

Department of Organ Transplantation and Vascular Surgery-NKTI;

Benito VC. Purugganan - Department Chairman; Dr. Arwin Ronan P. Ronsayro for initiating this study and for his invaluable contribution; Special thanks to Dr. Adolfo C. Parayno and Dr. Marc Anter E. Mejes for your patience and guidance in reviewing the paper; To Ms. Ana Karenina Rosana and Ms Pilar Ferrer for guiding us with the statistical aspect of the study; My heartfelt gratitude for their full support throughout the study

References:

[1] 1. Loredana PB, Raffaele C, Paola M, Gianluca L, Alice O, Federica R, Ercolino L, Maurizio T, Stefano R, Giuseppe PS, Antonio A. B cell positive cross-match not due to anti-HLA Class I antibodies and first kidney graft outcome. *Transplant Immunology* (Impact Factor: 1.46). 07/2008; 19(3-4):238-43

[2] 2. Newell KA, Asare A, Kirk AD, Gisler TD, Bourcier K, Suthanthiran M, Burlingham WJ, Marks WH, Sanz I, Lechler RI, Hernandez-Fuentes MP, Turka LA, Seyfert-Margolis VL. Identification of a B cell signature associated with renal transplant tolerance in humans. *J. Clin. Invest.* 2010; 120(6): 1836-47

[3] 3. Goh, A, Oei E, Vathsala A.Successful transplantation across positive B-cell cross-match in deceased donor renal transplants. *Transplantation proceedings* 2012;4(1): 193-9

[4] 4. Pollinger HS1, Stegall MD, Gloor JM, Moore SB, Degoey SR, Ploeger NA, Park WD, Pollinger HS, Stegall MD, Gloor JM, Moore SB, Degoey SR, Ploeger NA, Park WD. Kidney transplantation in patients with antibodies against donor HLA class II. *Am J Transplant* 2007 ;7(4):857-63

[5] 5. Lenhard V, Rettwitz W, Dreikorn K, Rohl L. Prognostic significance of B and T cell antibodies in kidney transplantation. *Transplant Association* 02/1980; 17:478-82.

[6] 6. Ozdemir FN1, Sezer S, Akcay A, Arat Z, Turan M, Gulmus S, Kulah E, Haberal M. Panel reactive antibody positivity and associated HLA antibodies in Turkish renal transplant candidates. *Transpl Immunol.* 2004 ;12(2):185-8.

[7] 7. Mishra MN1, Baliga KV. Significance of panel reactive antibodies in patients requiring kidney transplantation. *Saudi J Kidney Dis Transpl.* 2013 ;24(3):495-9.

[8] 8. Phayphet M, E. Alamartine E, Mariat C, Absi L, Berthoux F. Harmful Effect of Anti-Class II Antibodies in Kidney Transplant Patients who Experienced an Acute Rejection Episode. *Transplantations medizin.* 2006, 18. Jahrg., S. 78

P.1526

Successful early additional mTORi therapy with a low-dose calcineurin inhibitor in renal transplant patients: a single-center pilot study

Yuki Nakamura[1], Hitoshi Iwamoto[1], Takayoshi Yokoyama[1], Kouichiro Tomita[1], Yu Kihara[1], Osamu Konno[1], Takuya Ueno[2], Hironori Takeuchi[3], Shigeyuki Kawachi[1].
[1]Digestive and Transplantation Surgery, Tokyo Medical University Hachioji Medical Center, Tokyo, Japan; [2]Division of Acute and Critical Care Medicine, Tokyo Medical University Hachioji Medical Center, Tokyo, Japan; [3]Department of Practical Pharmacy, Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan.

Introduction: Calcineurin inhibitors (CNIs), standard immunosuppressive drugs used in renal transplantation, can have a negative effect on long-term prognosis. Nephrotoxic-free immunosuppressive therapy is an important goal in renal transplantation.
Objective: We examined the usefulness and safety of adding everolimus (EVL) at an early stage (within 6 months) with CNI reduction in renal transplant patients.
Methods: This was a prospective study of 50 kidney recipients. Initial immunosuppression used a four-drug combination therapy. Rituximab was added in cases of ABO incompatibility. Three months after transplantation, patients who experienced acute rejection were divided into urine protein-positive, EVL administration (n = 24), and EVL non-administration (n = 26) groups in the presence or absence of hyperlipidemia (group Convention). In the EVL group, EVL (1.5 mg/day) was added 120-180 days after transplantation, steroids were withdrawn, and we initiated a 50% reduction in CNI and mycophenolate mofetil.
Results: We compared results between the EVL and Convention groups. The EVL group consisted of 17 men and 7 women with an average age of 49.5 years. The Convention group consisted of 18 men and 8 women with an average age of 49.7 years. Two patients in the EVL group were excluded due to stomatitis. Example the onset of rejection is administered after the start ATMR in EVL group 1 (4.1%), example AAMR1 (4.1%), example CAMR1 (4.1%), ATMR is 5/26 (19.2%) in group Conv; CAMR1 patients (one month after transplantation, 3.8%) renal function EVL group serum creatinine (Cr): 1.34 mg/dL; estimated glomerular filtration rate (eGFR), 46.3 mL/min/1.73 m2; 3-month Cr, 1.38 mg/dL; eGFR, 47.8 mL/min/1.73 m2; 6-month Cr, 1.35 mg/dL; eGFR, 48.1 mL/min/1.73 m2; 1-year Cr, 1.32 mg/dL; eGFR, 50.0 mL/min/1.73 m2; 2-year Cr, 1.44 mg/dL; and eGFR, 27.9 mL/min/1.73 m2. In the Convention group, 1-month Cr was 1.23 mg/dL and eGFR was 52 mL/min/1.73 m2; 3-month Cr, 1.26 mg/dL; eGFR 50.1 mL/min/1.73 m2; 6-month Cr, 1.29 mg/dL, eGFR 49.6 mL/min/1.73 m2; 1-year s-Cr 1.31 mg/dL, it was possible to maintain an eGFR of 49.2 mL/min/1.73 m2. There was no statistically significant difference respectively. According to the multiple logistic analysis, factors that affect the IFTA were CNI, PRA positive, donor age and donor preoperative eGFR. In addition, factors that affect the subsequent 6 months ATMR was a malignant tumor and six months earlier ATMR.
Discussion: Rejection was suppressed with EVL early after conversion. Interstitial fibrosis and tubular atrophy and moderate (IFTA) progression slowed. Additionally, it preserved renal function in the medium- to long-term.
Conclusion: The rejection-suppressing effect in early-conversion EVL, expected to IF / TA effect to suppress the progress is be popular.

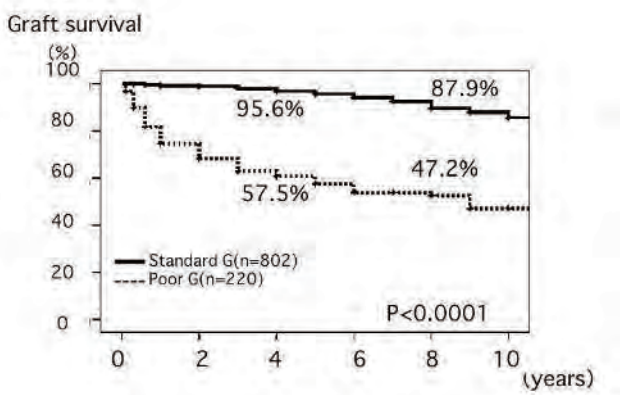
P.1527

The examination of the breaking point of expanded criteria deceased donor in Japan

Yuki Nakagawa[1], Masayuki Tasaki[1], Kazuhide Saito[1], Kota Takahashi[2], Atushi Aikawa[3], Masami Kikuchi[3], Yoshihiko Tomita[1].
[1]Division of Urology, Department of Regenerative and Transplant Medicine, Niigata University, Niigata, Japan; [2]Otukadai Clinic, Otukadai Clinic, Tokyo, Japan; [3]Japan Organ Transplant Network, Japan Organ Transplant Network, Tokyo, Japan.

Background: The lack of donors is a serious problem around the world. Definition of Expanded Criteria Donor (ECD) has been accepted and is being applied for kidney transplantation in Europe and North America[1]. However, definition of ECD for deceased donor kidney transplantation has not been developed yet in Japan.
Patients and Methods: We analyzed data of 1051 recipients who received deceased donor kidney transplantation in East Japan. Based on kidney function one year after transplantation, we divided the recipients into the standard groups (Cr < 2.0 mg/dl, n=802) and the poor groups (Cr ≧ 2.0 mg/dl, n = 220). We examined the graft survival and analyzed relevant risk factors.
Results: The graft survival of Japanese renal graft is good results (5 year graft survival 94.4% 10 year graft survival. 82.2%) .There are not the differences between brain-dead donor and cardiac-dead donors.
The renal graft survival rates were significantly worse in the poor groups than in the standard group ten years after transplantation (87.9% vs. 47.2 % P < 0.0001).

figure1



Significant donor risk factors for ECD graft failure were age, donation after circulatory death, a history of high blood pressure, presence of cerebrovascular disease, average urine flow and blood pressure just before kidney procurement, TIT and WIT.

Table 1 The risk factors(donor factor)

	Standard Group (n=802)	Poor Group (n=220)	Poor Group (n=220)
Donor age(years)	46.1 ± 15.5	55.7 ± 11.7	< 0.001
Presence of cerebrovascular disease	382 / 800 (47.7%) (loss n=2)	156 / 219 (71.9%) (loss n=1)	< 0.0001
Donation after circulatory death (brain death／heart death)	189 / 613 (30.8%)	23 / 197 (11.6%)	< 0.0001
History of high blood pressure	134 / 802 (16.7%)	69 / 220 (31.3%)	< 0.0001
Donor Cr (On admission)	0.86 ± 0.30	0.82 ± 0.29	0.394
Donor Cr (Just before renal extraction)	2.16 ± 2.20	2.34 ± 2.17	0.256
Average urine flow≦ 50 ml／h (just before kidney procurement)	269 / 682 (39.4%) (loss n=120)	269 / 682 (39.4%) (loss n=120)	< 0.0001
Average blood pressure ≦ 60 mmhg (just before kidney procurement)	324 / 736 (44.0%) (loss n=66)	324 / 736 (44.0%) (loss n=66)	0.038

The significant recipient risk factors were gender (male) and the length of time on dialysis prior to renal transplantation.

Conclusion: There is a need for developing definition of ECD for deceased donor kidney transplantation in Japan. To develop ECD we should thoroughly analyze factors influencing renal graft function.

References:

[1] Hoshino J. An analysis of long-term survival and renal function in simultaneous pancreas and kidney transplantation from the OPTN/UNOS registry. Clin Transpl. 2011:17-28.

P.1528

5 year follow-up results of the HERAKLES study: superior renal function after early conversion to an everolimus-based calcineurin inhibitor free regimen.

Claudia Sommerer[1], Klemens Budde[1], Wolfgang Arns[1], Petra Reinke[1], Markus Guba[1], Peter Weithofer[1], Bruno Vogt[3], Johannes Jacobi[1], Rolf Stahl[1], Thomas Rath[1], Ingeborg A Hauser[1], Martina Porstner[2], Christoph May[2], Frank Lehner[1], Oliver Witzke[1].
[1]Herakles, Study Group, Germany; [2]Novartis, Pharma, Germany; [3]Herakles, Study Group, Switzerland.

Background: To follow up on renal function (GFR) 5 years after kidney transplantation (KTx) in patients (pts) on immunosuppressive regimens with different calcineurin inhibitor (CNI) exposures.

Methods: 802 patients (pts) were included in this 1 year, prospective, open-label, randomized, controlled multi-center study with observational follow-up (FU) to Mo 60 post Tx. After induction therapy all pts received cyclosporine A (CsA), enteric-coated mycophenolate sodium (EC-MPS) and steroids. 3 Mo post Tx, 499 pts were randomized 1:1:1 to either a) continue standard CsA (100-180ng/ml) + EC-MPS (n=166) (STD) or convert b) to a calcineurin inhibitor (CNI)-free regimen with everolimus (EVR) (5-10ng/ml) + EC-MPS (n=171) or c) to a CNI-reduced regimen with EVR (3-8ng/ml) + reduced CsA (50-75ng/ml; n=162). All pts continued on steroids according to centers practice. In total 77% of pts completed the FU period: 75% of STD, 84% of CNI-reduced and 84% of CNI-free group pts.

Results: GFR (Nankivell, ITT) was similar at randomization 3 Mo post Tx and had significantly improved at Mo12 by +5.6mL/min (95% CI:[+2.9;+8.3]; p<0.001) and remained significantly improved by +6.7mL/min in favor of the CNI-free regimen after 5 years (ANCOVA, LOCF, p<0.001; Tab1). Similarly, when missing values were not replaced, LS mean of GFR-Nankivell at Mo 60 was highest in the CNI free group (68.92 mL/min) and was significantly greater than in the CNI reduced group (60.0 mL/min; p =0.0001) and the STD group (62.69 mL/min; p=0.0076). There was no significant difference between the CNI reduced and STD groups (p=0.2490). Benefit on renal function was highest for non-switcher pts: CNI-free Mo60 GFR-Nankivell was 78.33 mL/min vs. 64.33 mL/min for CNI-reduced and 61.68 mL/min for STD pts (unadjusted mean eGFR, LOCF; p<0.0001; Tab1). Mean CsA C0 levels at Mo60 were 80 ng/mL in CNI-reduced group and 109 ng/mL in STD group (ITT).

eGFR (Nankivell) [mL/min/1.73m2] ITT FU pop. to Mo60	CNI-free	CNI-reduced	Standard CNI	Difference: CNI-free vs standard CNI (95% CI) P value*	Difference: CNI-reduced vs standard CNI (95% CI) P value*	Difference: CNI-free vs CNI-reduced (95% CI) P value*
Nankivell formula (ITT population)						
Unadjusted eGFR, mL/min/1.73m², mean(±SD)	68.94 (21.21)	59.27 (19.27)	59.20 (19.82)	-	-	-
Adjusted (ANCOVA) eGFR, mL/min/1.73m², LS mean (95%CI)*	68.93 (65.76-70.17)	56.74 (55.58-61.80)	60.24 (57.11-63.38)	8.73 (3.22-10.24) 0.0002	1.50 (-5.01-3.51) 0.4008	8.23 (4.73-11.73) <0.0001
Nankivell formula (non-switcher)						
Unadjusted eGFR, mL/min/1.73m², mean(±SD)	78.33 (19.82)	64.33 (19.78)	61.68 (18.56)	-	-	-
Adjusted (ANCOVA) eGFR, mL/min/1.73m², LS mean (95%CI)*	74.69 (69.21-79.77)	64.87 (58.51-72.71)	60.32 (58.04-64.59)	14.37 (9.14-19.59) <0.0001	4.25 (-2.29-10.67) 0.1966	10.08 (3.04-17.12) 0.0053

(*) ANCOVA model with treatment, center, donor type as factors and GFR value at V4/M3/BL2 as covariate
Replacement of missing values: LOCF = last observation (=Randomization) carried forward

Conclusions: CNI-free as well as reduced CNI in combination with EVR represent both efficacious and safe regimen. CNI-reduced group had higher CsA levels than anticipated per protocol. The fact that CNI reduction was not fully accomplished might have prevented GFR differences compared to STD in this randomized treatment group. However, CNI-free regimen was associated with significant higher eGFR maintained for 5 years post Tx. The results of this large trial confirm previous reports of improved GFR after CsA withdrawal with EVR in combination with EC-MPS.

P.1529

Efficacy and safety of three different treatment regimen in de novo renal transplant patients: 5 year follow-up results of the HERAKLES trial

Klemens Budde[1], Claudia Sommerer[1], Wolfgang Arns[1], Volker Kliem[1], Bruno Vogt[3], Markus Guba[1], Johannes Jacobi[1], Ingeborg A Hauser[1], Rolf Stahl[1], Petra Reinke[1], Thomas Rath[1], Christoph May[2], Martina Porstner[2], Oliver Witzke[1], Frank Lehner[1].
[1]Herakles, Study Group, Germany; [2]Novartis, Pharma, Germany; [3]Herakles, Study Group, Switzerland.

Background: To compare safety and efficacy of 3 different immunosuppressive regimens at month (Mo) 60 after kidney transplantation (KTx).
Methods: 802 patients (pts) were included in this 1 year, prospective, open-label, randomized, controlled multi-center study with observational follow-up (FU) to Mo 60 post KTx. After induction therapy all pts received cyclosporine A (CsA), enteric-coated mycophenolate sodium (EC-MPS) and steroids. 3 Mo post KTx, 499 pts were randomized 1:1:1 to either a) continue standard CsA (100-180ng/mL) + EC-MPS (n=166) (STD) or convert b) to a calcineurin inhibitor (CNI)-free regimen with everolimus (EVR) (5-10ng/mL) + EC-MPS (n=171) or c) to a CNI-reduced regimen with EVR (3-8ng/mL) + reduced CsA (50-75ng/mL; n=162). All pts continued on steroids according to centers practice. In total 77% of pts completed the FU period: 75% of STD, 84% of CNI-reduced and 84% of CNI-free group pts.
Results: During FU period to Mo60 efficacy events from ITT were reported as follows: BPAR was reported in 13/165 (8%) STD, 13/171 (8%) CNI-free and in 12/161 (7%) CNI-reduced pts (p value =ns). 7 deaths (4%) occurred in STD, 4 (2%) in CNI-free and 9 (6%) in the CNI-reduced group. 7(4%) graft losses were observed in the STD, 7 (4%) in the CNI-free and 3 (2%) in the CNI-reduced group. Composite failure (BPAR, death, graft loss, loss to FU) occurred in 38 (23%) STD, 35 (20%) CNI-free and in 36 (22%) CNI-reduced treated pts. eGFR (Nankivell; ITT, adjusted LS-Mean, LOCF) was significantly improved by +6.7mL/min in favor of the CNI-free regimen at Mo60 (p<0.001). Safety profile did not differ between groups.

Overview on efficacy events during follow-up (ITT population)

Frequencies, n (%)	Standard (N=165)	CNI free (N=171)	CNI reduced (N=161)
Events starting after Month 12			
Biopsy-proven acute rejection (BPAR)	13 (7.88)	13 (7.60)	12 (7.45)
Graft loss	7 (4.24)	7 (4.09)	3 (1.86)
Death	7 (4.24)	4 (2.34)	9 (5.59)
Lost to follow-up	17 (10.30)	15 (8.77)	13 (8.07)
Toxicity	10 (6.06)	8 (4.68)	4 (2.48)
Therapy failure (composite endpoint)*	38 (23.03)	35 (20.47)	36 (22.36)
p-value (Fisher's exact Test): no significant differences found			
*Treatment failure as composite endpoint is defined as occurrence of at least one of the events: biopsy proven acute rejection, graft loss, death, lost to follow-up, discontinuation due to toxicity.			

Overview on adverse and other safety relevant events during follow-up (Safety population)

Event	Standard N=165 n (%)	CNI-free N=171 n (%)	CNI-reduced N=161 n (%)
Death, n (%)	7 (4.2)	4 (2.3)	9 (5.6)
Hospitalization, n (%)	99 (60.0)	102 (59.6)	107 (66.5)
Discontinuation due to AE [3], n (%)	10 (6.1)	8 (4.7)	4 (2.5)
Infection, n (%)	83 (50.3)	85 (49.7)	81 (50.3)
Severe Infection, n (%)	19 (11.5)	21 (12.3)	19 (11.8)
Infection leading to hospitalization, n (%)	50 (30.3)	50 (29.2)	54 (33.5)
Of these the most common:			
Urinary tract infection, (%)	10.9%	9.4%	10.6%
Pneumonia, (%)	6.1%	9.9%	4.3%
Urosepsis, (%)	6.1%	4.7%	5.0%
CMV, (%)	3.0%	2.9%	3.1%
Gastroenteritis	3.0%	1.8%	4.3%
[3] documented as primary reason for prematurely discontinuation of follow-up period (reported at any follow-up visit); Footnote: The CRF and the clinical data base do only include serious adverse events for the follow-up period. Nevertheless, AEs were reported to Novartis drug safety but are not included in this analysis.			

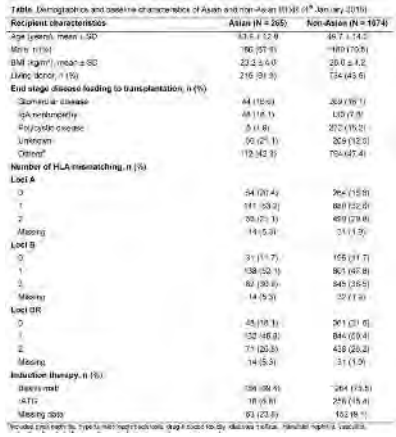
Conclusion: Mo60 results from HERAKLES show that immunosuppressive regimens using EVR with reduced-dose or without CNI-exposure reflect an efficacious and safe therapeutic approach offering the opportunity for an individualized immunosuppression to minimize CNI-exposure.

P.1530

Baseline characteristics of Asian and non-Asian renal transplant recipients: Findings from the TRANSFORM trial

Po-Chang Lee[1,3], Steven J.Chadban[2,3].
[1]Department of Surgery, National Cheng Kung University Hospital, Tainan, Taiwan; [2]Royal Prince Alfred Hospital, Camperdown, Australia; [3]On behalf of the TRANSFORM Investigators, Novartis, Basel, Switzerland.

Introduction: Geographic distribution and race may have some influence on graft function and acute rejection after renal transplant (RTx). Asian RTx recipients (RTxR) have typically been under-represented in immunosuppression trials. The TRANSFORM study combines a novel clinically relevant endpoint of anti-rejection efficacy and preservation of renal function, to determine the benefits of an everolimus (EVR)-based regimen vs standard CNI (sCNI)-based triple therapy in *de novo* RTxR. Here, we present the baseline data of Asian and non-Asian recipients.
Methods: TRANSFORM (NCT01950819) is an ongoing 24-month (M), randomized, open-label, multicenter trial to evaluate the effect of EVR+reduced calcineurin inhibitor (rCNI) vs mycophenolic acid (MPA)+sCNI, along with induction and steroids in *de novo* RTxR. The primary endpoint of the study is to evaluate the binary composite of treated biopsy-proven acute rejection (tBPAR) or estimated glomerular filtration <50 mL/min/1.73 m^2 (using 4-variable modification of diet in renal disease formula) at M12, after RTx. The key secondary endpoint is to assess composite efficacy failure (tBPAR, graft loss or death) at M12 and M24. The baseline characteristics of the RTxR based on geographic distribution are evaluated here.
Results: TRANSFORM completed recruitment of the planned 2038 RTxR from 218 sites across 43 countries on 26th January 2016. As per database, a total of 1978 RTxR had been randomized by 4th January 2016; 13.7% of the recipients were Asians. RTxR were predominantly male and mean age was comparable between the groups. A majority of Asian recipients (91.9%) received living donor grafts vs non-Asians (43.5%). RTx due to glomerular disease (including immunoglobulin A nephropathy) was higher in Asians than non-Asians (34.7% vs 23.8%, respectively). HLA mismatch was comparable between the groups. Irrespective of region, most of the RTxR received basiliximab as induction therapy (Table).
Conclusion: TRANSFORM is the largest prospective trial in RTx to date, recruiting more than 2000 RTxR which includes a large proportion of Asian recipients from various countries throughout Asia. This will enable post-hoc subgroup analyses to provide much needed data on the outcomes of Asian RTxR treated with both sCNI, MPA and steroids and, in comparison, EVR, rCNI and steroids. Given the size of the Asian Tx population and its potential for growth, such data is urgently required.



P.1550

Intracranial hemorrhage after liver transplantation; A case

Seong-Hwan Chang.
Surgery, Konkuk University School of Medicine, Seoul, Korea.

Neurologic complications after liver transplant (LT) occur frequently. Usually they are not fatal but intracranial hemorrhage (ICH) seldom happens and it is one of fatal complications after liver transplantation. Some investigators insisted that the cause of hemorrhage would be coagulopathy, aspergillosis, or both. Others insisted that massive bleeding leading to brain infarction and coagulopathy would be the cause of posttransplantation intracranial bleeding. Between June 2006 and December 2015, 52 adult patents received 54 liver transplants at Konkuk University Medical Center, Seoul, Korea. We experience one case of ICH and the patient died. She was admitted for melena and abdominal distension. She denied any history of hypertension, and MELD score was 23 just prior to cadaveric donor LT. During LT, estimated blood loss was 5000 cc, which was more than twice than mean estimated blood loss, so that massive transfusion was done. After LT, there was no severe coagulopathy, and her consciousness was very clear and had a conversation with her family over the phone. As liver graft function was good, we administered a low molecular weight heparin, alprostadil, and so on. On postoperative day 2, she complained a sudden headache, and then lost consciousness in 30 minutes later. A rapid consultation to a neurologist was made and brain CT was taken. The CT revealed that there is a massive acute ICH with hemorrhage-fluid level in the cerebellum, mainly right cerebellum and superior vermis, and acute intraventricular hemorrhage in the fourth and third, and acute ICH with low-density in right occipital lobe, and acute subdural hemorrhage along the right cerebral convexity, right posterior falx and the right tentorium, and 3 mm sized unruptured saccular aneurysm of right distal ICA bifurcation. The neurosurgeon's opinion for surgery was futile, we managed her conservatively and she died on postoperative day 9. There was a massive bleeding during LT but she recovered well after LT. And as we could not find any other causes for ICH, we concluded that the intracranial hemorrhage occurred by chance. ICH after LT should be investigated more to find clear causes and to find risk groups for ICH after LT.

References:

[1] Stein DP, Lederman RJ, Vogt DP, Carey WD, Broughan TA. Neurological complications following liver transplantation.*Ann Neurol*.1992;31:644-649.
[2] Boon AP, Adams DH, Buckels J, McMaster P. Cerebral aspergillosis in liver transplantation.*J Clin Pathol*. 1990;43:114-118.
[3] Wijdicks EF, de Groen PC, Wiesner RH, Krom RA. Intracerebral hemorrhage in liver transplant recipients.*Mayo Clin Proc*. 1995;70:443-446.
[4] Wang W, Yang Z, Lo C, Liu C, Fan S. Intracerebral hemorrhage after liver transplantation.*Liver Transpl*. 200;6:345-348.

P.1551

Serum osteoprotegerin is an independent marker of peripheral arterial disease in kidney transplantation patients

Bang-Gee Hsu[1,3], Ming-Che Lee[2,3].
[1]Division of Nephrology, Buddhist Tzu Chi General Hospital, Hualien, Taiwan; [2]Department of Surgery, Buddhist Tzu Chi General Hospital, Hualien, Taiwan; [3]School of Medicine, Tzu Chi University, Hualien, Taiwan.

Introduction: Osteoprotegerin (OPG) levels may be a potential biomarker of complications and severity of cardiovascular disease. Peripheral arterial disease (PAD) is associated with an increased risk of death in kidney transplantation (KT) patients. Our aim was to evaluate the relationship between serum OPG levels and PAD among KT patients.
Materials and Methods: Fasting blood samples were obtained from 74 KT patients. Serum OPG levels were measured using a commercial enzyme-linked immunosorbent assay kit. Left or right ankle-brachial index (ABI) values that were < 0.9 were included in the PAD group. The predictive ability of OPG levels for PAD of KT patients was assessed using receiver operating characteristic (ROC) curve and multivariate logistic regression analyses.
Results: Thirteen patients (17.6%) were enrolled in PAD group. KT patients with diabetes (P = 0.025), smoking (P = 0.010), and increased serum OPG levels (P = 0.001) were noted in the PAD group. Multivariate logistic regression analysis showed that OPG (Odds ratio: 1.297, 95% confidence interval: 1.102-1.527, P=0.002), and diabetes (Odds ratio: 4.846, 95% confidence interval: 1.041-22.550, P=0.044) were the independent predictors of PAD in KT patients. The area under the ROC curve (AUC) indicates the diagnostic power of OPG levels at predicting PAD of KT patients was 0.799 (95% confidence interval: 0.690-0.884, P < 0.001), with a sensitivity, specificity, positive predictive value (PV), and negative PV of were 61.5%, 86.9%, 50.0%, 91.4%, respectively.
Discussion: There is evidence of the role of OPG in the pathogenesis of atherosclerosis, calcification, and cardiovascular disease. OPG may be expressed, be regulated, and function in vascular physiology and pathology in unique ways to promote endothelial cell survival, angiogenesis, monocyte, or endothelial cell recruitment, and smooth muscle cell osteogenesis, and calcification. In our study, serum OPG level is an independent marker of PAD in KT patients using ROC curve and multivariate logistic regression analyses.
Conclusion: Serum OPG level was positively associated with PAD among KT patients.

P.1552

Positive correlation of serum adipocyte fatty acid binding protein levels with metabolic syndrome in kidney transplantation patients

Bang-Gee Hsu[1,3], Ming-Che Lee[2,3].
[1]Division of Nephrology, Buddhist Tzu Chi General Hospital, Hualien, Taiwan; [2]Department of Surgery, Buddhist Tzu Chi General Hospital, Hualien, Taiwan; [3]School of Medicine, Tzu Chi University, Hualien, Taiwan.

Introduction: Adipocyte fatty acid binding protein (A-FABP) is significantly expressed in white and brown adipose tissue, monocytes and macrophages and is a central regulator of systemic insulin sensitivity. Metabolic syndrome (MetS) is a risk factor for post-transplant diabetes mellitus, chronic graft dysfunction, graft loss, and patient death in kidney transplantation (KT) patients. This study was undertaken to evaluate the relationship between MetS and fasting serum A-FABP concentration in KT patients.
Materials and Methods: Fasting blood samples were obtained from 70 KT patients. Serum A-FABP levels were measured using a commercial enzyme-linked immunosorbent assay kit. MetS and its components were defined using the diagnostic criteria of the International Diabetes Federation.
Results: Twenty-two patients (31.4%) had MetS. Hypertension (P=0.011), diabetes (P=0.002), body weight (P=0.004), body mass index (P=0.001), waist circumference (P<0.001), body fat mass (P<0.001), systolic blood pressure (P=0.017), total cholesterol (P=0.028), triglyceride (P=0.001), blood urea nitrogen (P=0.003), insulin (P<0.001), HOMA-IR (P<0.001), and A-FABP level (P<0.001) were higher, while high density lipoprotein cholesterol (HDL-C) was lower in KT patients with MetS. Serum A-FABP levels correlated with a number of diagnostic MetS criteria in KT patients (P<0.001). Hypertension ($\beta = 0.326$, P=0.004), waist circumference ($\beta = 0.229$, P=0.039) and logarithmically transformed triglyceride (log-TG, $\beta = 0.331$, P=0.004) was associated with A-FABP levels in a multivariable forward stepwise linear regression analysis among KT patients. The area under the ROC curve (AUC) indicates the diagnostic power of A-FABP levels at predicting MetS of KT patients was 0.843 (95% confidence interval: 0.749-0.938, $P < 0.001$), with a sensitivity, specificity, positive predictive value (PV), and negative PV of were 90.9%, 72.9%, 60.6%, 94.6%, respectively.
Discussion: The A-FABP is expressed in adipocytes and macrophages, and integrates inflammatory and metabolic responses. Studies in A-FABP-deficient mice have shown that this lipid chaperone plays a significant role in several aspects of MetS. In our study, serum A-FABP level is also positively associated with MetS in KT patients.
Conclusion: The results of our study showed that the fasting A-FABP level was positively associated with MetS in KT patients. Serum A-FABP levels correlated with a number of MetS criteria. Hypertension, waist circumference, and log-TG was an independent predictor of the serum A-FABP level among KT patients.

P.1553

Patient and graft outcomes among kidney transplant recipients who underwent pre-transplant coronary angiography

Jason Paul Medina, Romina A.Danguilan, Concesa C.Casasola.
Adult Nephrology, National Kidney and Transplant Institute, Quezon City, Philippines.

Introduction: Kidney transplantation is the treatment of choice for Stage V Chronic Kidney Disease by offering improved quality of life and overall survival. Cardiovascular complications continue to be the leading cause of death, even in the transplant population. The limited supply of organs makes pre-operative cardiac risk assessment crucial in optimizing patient selection and outcomes. This study investigates the profiles, graft and patient outcomes of kidney transplant recipients who underwent coronary angiography as part of their pre-transplant cardiac evaluation.
Methodology: This is a retrospective cohort that includes all adult recipients from either living or diseased donors who underwent coronary angiography as part of their pre-transplant workup, transplanted from November 2011 to May 2015 in the National Kidney and Transplant Institute.
Results: Among 830 kidney transplants performed, there were 22 Filipinos who underwent pre-kidney transplant coronary angiography. Among the 22 who underwent coronary angiography, 10 underwent revascularization (9 stenting and 1 bypass) while 12 did not require intervention. Among the 10 who required revascularization, 3 had three-vessel disease, 4 had 2-vessel, and 3 had one-vessel involvement, all which had either left main or left anterior descending coronary vessel stenosis. Nine had diabetes mellitus. In this group, one patient developed restenosis requiring repeat stenting within 3 months post-coronary intervention and within the first week post-transplant and recovered, while one patient died 9 months post-transplant due to infection. All 10 patients were alive at 6 months post-transplant.
Among the 12 who did not require intervention, 3 had mild coronary artery disease, 2 had moderate disease, and 7 had normal coronaries. Only one patient had diabetes, 6 had chronic glomerulonephritis, 2 had hypertensive kidney disease, one had NSAID nephropathy, one polycystic kidney disease and one obstructive nephropathy. In this group, one patient died due to infection within 7 months post-coronary angiogram and 1 month from transplant. 11 out of 12 patients were alive at 6 months post-transplant.
Both of the patients who died had a functioning graft. All of the patients had functioning grafts at 6 months post-transplant whether they had coronary stenting or not.
Conclusion: Patients who had severe coronary artery disease who had coronary stenting had the same outcome compared to patients with normal coronary arteries or mild to moderate CAD that did not require stenting.
References:
[1] Hakeem et. Al Screening and risk Stratification of CAD in ESRD JACC Cardiovasc Imaging 2014 Jul 7 (7):715-28
[2] Jong P, Sternberg L.. Assessing coronary artery disease in women: how useful is coronary angiography?. Medscape Womens Health. 1998 May;3(3):1.
[3] Delos Santos RB, Gmurczyk A, Obhrai JS, Watnick SG. Cardiac Evaluation prior to Kidney Transplantation. Seminars in dialysis. 2010;23(3):324-329
[4] Kasiske BL, Cangro CB, Hariharan S, Hricik DE, Kerman RH, Roth D, Rush DN, Vazquez MA, Weir MR. American Society of T. The evaluation of renal transplantation candidates: Clinical practice guidelines. Am J Transplant. 2001;1:3–95.
[5] Matas, A.J. et al. OPTN/SRTR 2013 Annual Data Report: Kidney. American Journal of Transplantation. Special Issue: OPTN/SRTR 2013 Annual Data Report. Volume 15, Issue S2, pages 1–34, January 2015
[6] Kriesche, H.M. Kidney Transplantation Halts Cardiovascular

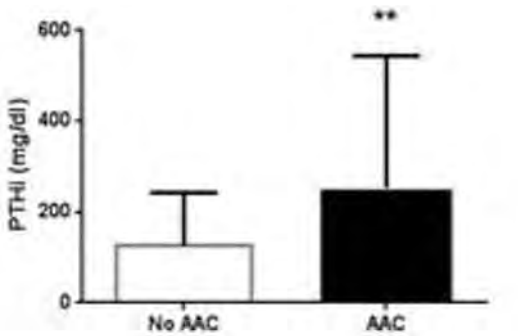
Disease Progression in Patients with End-Stage Renal Disease. American Journal of Transplantation 2004; 4: 1662–1668
[7] Kammerer et.al. Use of Drug-eluting balloon coronary intervention prior to living donor kidney transplantation: BMC Cardiovasc Disorder. 2014:14:112. Dobutamine stress echocardiography in the diagnosis of asymptomatic Ischemic Heart Disease in patients with CKD – review of literature and single center experience.
[8] U.S. renal data system. USRDS 2006 annual data report: Atlas of end-stage Renal disease in the united states, national institutes of Health, national institute of diabetes and digestive and kidney diseases. Bethesda, MD: 2009.
[9] Gradaus F, Ivens K, Peters AJ, Heering P, Schoebel FC, Grabensee B, Strauer BE. Angiographic progression of coronary artery disease in patients with end-stage renal disease. Nephrol Dial Transplant. 2001;16:1198–1202
[10] Sharma R, Pellerin D, Gaze DC, Gregson H, Streather CP, Collinson PO, Brecker SJ. Dobutamine stress echocardiography and the resting but not exercise electrocardiograph predict severe coronary artery disease in renal transplant candidates. Nephrol Dial Transplant. 2005

P.1554

Mineral metabolism and vascular calcification before and at one year of the kidney transplantation

Luis León[1], Elena Minué[1], Ana Uriona[1], Olga Guardia[1], Nela Ancieta[1], Alfredo Percischini[1], Daniel Flores[1], Eduardo Chuluyan[2], María C.Rial[1], Domingo Casadei[1].
[1]Instituto de Nefrología de Buenos Aires, Nephrology, Buenos Aires, Argentina; [2]Facultad de Medicina, CEFYBO-UBA, Buenos Aires, Argentina.

Patients with chronic kidney diseases have disorders in mineral metabolism. Kidney transplantation improves some of them. The aim of this study was to survey the state of mineral metabolism and vascular calcification score in patients before and after one year of kidney transplant. Furthermore, we assessed the impact of the immunosuppressive treatment on the mineral metabolism and the vascular calcifications.
In this observational, prospective study, 108 kidney transplant patients were included. Simple vascular calcifications (SVC) were evaluated on plain radiographic films of pelvis and hands, while the calcifications of abdominal aortic wall (AAC) were evaluated on lateral lumbar films. The maintenance immunosuppressive treatment was rapamycin+MMF+steroids (n = 40) and tacrolimus+MMF+steroids (n = 68).
We found that, before transplantation 16.7 % and 39.2 % of patients had SVC and AAC, respectively. At one year, after kidney transplantation, SVC and AAC were observed in 18.3 % and 32.5 % of patients. Different maintenance immunosuppressive treatments did not modify the values of the vascular calcifications. However, patients with AAC treated with tacrolimus+MMF+steroids showed higher values of PTH compared to those patients without AAC.



This was not observed with patients treated with rapamycin+MMF+steroids. Instead, high values of calcium were observed in AAC patients treated with rapamycin+MMF+steroids.
Next we determined the presence of rejections and infections at one year of the transplant. Among the patients with AAC there were a lower number of rejections regardless the immunosuppressive treatment, but also more infections.

		Rejections	Infections
Tacrolimus + MMF + Steroid	NO AAC	14	33
	AAC	0	58
Rapamycin + MMF + Steroid	NO AAC	33	57
	AAC	15	50

Overall these results suggest that patients with AAC, exhibit different characteristics from those patients without AAC, and perhaps might be treated with a different immunosuppressive regimen.

P.1555

Ureteral reconstruction for ureteral complications after kidney transplantation

Daisuke Ishii[1], Masahiro Hagiwara[1], Mizuho Kawamura[1], Masae Ikeda[1], Fumino Noguchi[1], Takahiro Hirayama[1], Kenichi Tabata[1], Haruki Wake[1], Yasuo Takeuchi[2], Kazunari Yoshida[3], Masatsugu Iwamura[1].
[1]Urology, Kitasato University, Sagamihara, Japan; [2]Nephrology, Kitasato University, Sagamihara, Japan; [3]Organ Transplantation and Regenerative Medicine, Kitasato University, Sagamihara, Japan.

Objective: To describe our experience with surgical management of transplant ureteral strictures.
Patients and Methods: In this study identified patients who underwent urinary reconstruction for transplant ureteral strictures between January 2000 and December 2015 after kidney transplantation in our institution. We underwent 168 kidney transplantations. Clinical characteristics including age at transplantation, renal function, immunosuppressive drug regimen, periods of double-J stent using, and reconstruction technique were analyzed. Successful reconstructions were defined as stable allograft function with unobstructed outflow not requiring repeat dilation or stent placement.
Results: Five of 168 patients (3.0%) were needed open urinary reconstruction after kidney transplantation. Median age at the time of reconstruction was 37 years old and the mean time from transplantation was 28 months. Main cause of ureteral stricture was urinary leakage (60%) and low blood flow to the ureter (40%). Uretero-ureterostomy was selected for two cases and pyelo-pyelostomy, uretero-neocystostomy and uretero-calicostomy was selected 1 case each for reconstruction. The patients were followed for a median of 28 months and there is no patient recurrence urinary re-stricture.
Conclusions: After renal transplantation with ureteral strictures are severe and difficult to manage only stenting or endoscopic technique. Open surgical ureteral reconstruction has variable choice with using native urinary tract. In ureteric reconstruction, we need to choose correct technique for each case at an appropriate time.

P.1556

Prevalence of Transfusion-transmitted virus infection and its association with renal post-transplant complications

Hassan Nikoueinejad[1], Eghlim Nemati[1], Alireza Soleimani[2], Behzad Einollahi[1].
[1]Department of Nephrology and Urology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran (Islamic Republic of); [2]Department of Internal Medicine, Kashan University of Medical Sciences, Kashan, Iran (Islamic Republic of).

Introduction: Renal transplant patients show a higher risk of Transfusion-transmitted virus (TTV), a single-stranded DNA virus, infection. The aim of this study was to detect TTV infection in such patients and evaluate its correlation with post renal transplant complications.
Methods: Our cross sectional study was performed on 120 ESRD patients transplanted during 2011 to 2013 in 2 referral Iranian hospitals since 1-2 years after the transplant. TTV infection in the peripheral blood samples were measured by semi-nested polymerase chain reaction.
Results: 34.2% of the patients showed a TTV infection. There was a significant correlation between the TTV infection with diabetes, acute transplant rejection and urinary tract infection ($P \leq 0.004$). However, we didn't find any direct correlation between the presence of TTV with hypertension, hyperlipidemia, respiratory infection and CMV infection.
Conclusions: Our study showed that the TTV infection may be an important predictor of some post renal transplant complications.
References:
[1] Szládek G, Juhász A, Asztalos L, Szöke K, Murvai M, SzarkaK, et al. Persisting TT virus (TTV) genogroup 1 variants in renal transplant recipients. Arch Virol. 2003; 148(5):841-51
[2] Gallian P, Berland Y, Olmer M, Raccah D, de Micco P, Biagini P, et al. TT Virus Infection in French Hemodialysis Patients: Study of Prevalence and Risk Factors.J ClinMicrobiol .1999; 37(8):2538-42

P.1557

Recalcitrant warts in solid organ transplantation

Hugo Petrone[1], AnaA Ochoa [1], Silvia Di Pietrantonio[1], Mariana Arias[2], Alejandra Abeldaño[2], Ricardo Skare[1].
[1]Servicio de Trasplante renal ., CRAI Sur. CUCAIBA, La Plata, Argentina; [2]Unidad de Dermatología, Hospital Cosme Argerich, Buenos Aires, Argentina.

Background: Warts develop in up to 95% of patients with solid organ transplants. These lesions are often multiple, recalcitrant and preneoplasic.
Objectives: To describe clinical characteristics of recalcitrant warts in solid organ transplantation.To analyse therapeutic approaches and factors determining the treatment of choice.To highlight the importance of dermatologic control due to the higher incidence of skin cancer in patients with solid organ transplants.
Patients and Methods: We describe three patients with recalcitrant warts.Patient 1: a 37 year-old woman with chronic renal failure due to lupus nephropathy who received a cadaveric graft in 2008. She received triple therapy with steroids, mycophenolate and tacrolimus. A kidney biopsy performed in 2010 revealed changes related to chronic anticalcineurinic toxicity, which prompted switching to rapamycin.Patient 2: a 42 year-old woman with chronic renal failure of unknown etiology, who received a cadaveric graft (expanded criteria) in 2006. She received induction with thymoglobulin and maintenance with steroids, mycophenolate and tacrolimus. She developed BK nephropathy in 2007, which was treated with leflunomide and tacrolimus.Patient 3: a 40 year-old man with lung transplantation due to cystic fibrosis who was treated with mycophenolate, steroids, azathioprine and tacrolimus.All three cases were performed skin biopsies of the affected lesions.Patient 1 showed remission of skin lesions under rapamycin.Patient 2 received topic cidofovir due to partial response at last control.Patient 3 was treated with acythretin 25 mg PO in a daily basis due to a diagnosis of acquired epidermodysplasia verruciformis, but outcome could not be properly evaluated.
Conclusions: We report on three patients with multiple warts, refractory to conventional therapy. Treatment of choice in each case was challenging, due to different skin lesions, serotype and host clinical conditions. Dermatologic control is essential due to the possibility of developing skin cancer.

P.1558

Endovascular treatment of arteriovenous fistulas secondary to renal graft biopsy. Presentation of 3 cases

Hugo Petrone, Silvia Di Pietrantonio, Marcelo F.Taylor, Ricardo Skare, María Bayon.
Servicio de Trasplante Renal, CRAI Sur-HIGA Gral. San Martín de La Plata, La Plata, Argentina.

Three cases of renal transplant patients with arteriovenous fistula (AVF) after biopsy were diagnosed and treated by endovascular procedure are presented.
Patient 1: biopsy implant 10 days after Tx was performed by ultrasound because delayed function. AVF of 4 cm in diameter is found. Glomerular filtration rate 55.4 ml / min. Coil occlusion by catheterization procedure is performed.
Patient 2: 2nd transplant.two renal biopsies were performed clinically indicated. In ultrasound AVF is confirmed by hemodynamic study is found. Plug placement is done by endovascular occlusion of it. Glomerular filtration rate 30 ml / min.
Patient 3: surveillance biopsy is performed at 3 months. FG 55.2 ml / min. Persistent hematuria after it. Hemodynamic Study: AVF. It is embolized with coils. Cessation of hematuria. Current FG 39 ml / min. In all three cases, diagnosis and endovascular treatment of arteriovenous fistula after renal biopsy without surgery was performed.

P.1559

Post-transplantation lymphoproliferative disorder (PTLD) intestinal after renal transplantation: a case report and review of the literature

Hugo Petrone[1], Horacio Belhart[1], Ricardo Skare[1], Fabián Taylor[1], Andrea Martinoia[1], Franco Moreschio[2], Sebastián Giantorno[3].
[1]Servicio de Trasplante Renal, CRAI Sur-HIGA Gral. San Martín de La Plata, La Plata, Argentina; [2]Servicio de Cirugía, HIGA Gral. San Martín de La Plata, La Plata, Argentina; [3]Servicio de Hematología, HIGA Gral. San Martín de La Plata, La Plata, Argentina.

Introduction: The Post-Transplant Lymphoproliferative Disorders (PTLD) are lymphoid cell proliferation occurring as a result of immunosuppression, in the context of transplantation. They are usually related to the presence of Epstein-Barr virus (EBV). It is limited the number of kidney transplant recipients who developed PTLD of small bowel and there are few publications with histologically proven.
Case Report: Male, 29 y-o, diagnosed with ESRD secondary to type I MPGN in hemodialysis since April 1997 to April 2001.The patient was transplanted with deceased donor. Serology of the donor and receptor were positive for EBV. Immunosuppressive therapy: Induction with OKT3, Steroids,Mycophenolate and Cyclosporine. 14 years after transplantation he developed abdominal pain, anemia, weight loss, fever and palpable abdominal mass. CT of the abdomen shows jejunal tumor. Enteroscopy was made and demostrated tumor mass located in proximal jejunum. Biopsies were taken and They showed polymorphic PTLD, so immunosuppression were changed to Sirolimus-Steroids. Three weeks later he underwent surgery for intestinal perforation. resection portion is made with thin lateral end anastomosis. In a third intervention, regional nodes near to jejunal área were resected whose pathology report confirmed the diagnosis of monomorphic PTLD (diffuse large B-cell lymphoma).Because poor response to decrease immunosuppression, he begins chemotherapy (CHOP-R) with improvement of general condition and disappearance of abdominal nodal masses six months after started treatment.
Conclusion: It present a rare case of PTLD (diffuse large B-cell lymphoma) of small bowel, in renal transplant patients positive EBV.

P.1560

Surgical complications of ureteric anastomosis following renal transplantation - a study in techniques

Abheesh Prasad, D. Roy, Simon Fletcher, T. Fernando, L.C. Tan, S.H. Kashi, C Imray, F.T. Lam, Nithya Krishnan.
Renal Transplant Unit, University Hospitals Coventry and Warwickshire NHS Trust, Coventry, United Kingdom.

Introduction: Ureteric complications following renal transplantations are an issue. We aim to look at our experiences over the last 5 years and the incidence of complications with differing surgical techniques and type of kidney implanted.
Aim: To study the rate of ureteric complications in renal transplant patients done at our centre.
Methods: A retrospective analysis of all adult single kidney transplant patients performed at our centre, from 1st January 2011 to 31st December 2015.
Results: A total of 266 consecutive renal transplants were studied. There were 164 men and 102 women. 141 were recipients from live donors, 94 from DBDs and 31were from DCDs. 2 (0.75%) patients had ureteric strictures. No ureteric leakages or ischaemia were identified. Neither of the patients needed re-implantation of the ureter. Both were males. One was a DCD and the other a DBD, recipient. The ureteric anastomosis in one was performed as a continuous closure and the other had an interrupted anastomosis. Intra operatively both were stented with a double 'J' stent. The patients were noticed to have a rise in creatinine, 2 months and 3 months post-transplant, respectively. An ultrasound scan showed moderate hydronephrosis, this was confirmed with a CT KUB. The patients had nephrostomies inserted and was followed by antegrade ureteric stenting under radiological guidance. The stents were removed in 4 months and 12 months respectively.
Conclusion: A very low incidence of ureteric complications is noted. Compared to published studies (5%)[1]-[3], the ureteric complication rate at our centre is 0.75%. No obvious correlation is noted to the method of anastomosis. No obvious correlation is noted to the source of the kidneys, other than that they were both from deceased donors. Preserving blood supply to the ureter, avoiding close dissection of hilar fat, keeping a short ureter and avoiding kinking of the ureter by inserting a stent are commonly practiced at our centre.
References:
[1] Urological complications in 980 consecutive patients with renal transplantation. Davari HR, Yarmohammadi H, Malekhosseini SA. Int J Urol. 2006 Oct;13(10):1271-5.

P.1561

Cancer incidence after kidney transplantation in Saudi Arabia

Sayed Raza[1], Ibrahim Alahmadi[1], Abbas Ghazaanfar[2], Dieter Broring[1], Tariq Ali[1], Hassan Aleid[1].
[1]Department of Kidney and Pancreas Transplantation, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia; [2]Department of Transplant Surgery, St Georges Healthcare NHS Trust, london, United Kingdom.

In the current era of renal transplantation the patient and graft survival are continuously improving. However there are some factors that are counterbalancing the effect of such success. One such factor is post renal transplant malignancies (PRTxM). The longer the patient survival is with functioning allograft, the higher is the risk of developing malignancy. In this present study we describes our experience of PRTxM among 2346 renal transplants performed in our centre.
Material and Methods: We performed retrospective analysis of prospectively collected data of 2346 renal transplant performed at King Faisal Specialist Hospital and Research Centre KSA between 1981 and 2013 with a median follow-up period of 153 months. We studied incidence and outcome of PRTxM among these patients. Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS 21). Non-variables were analyzed using the T-Test, and variables were analyzed by means of the Fisher exact test and Chi square test. We also analyzed for relative risk ratio among different factors. Survival analysis was conducted using Kaplan-Meier survival graphs and log-rank testing was used where differences in survival were significant. In general, a P value of <0.05 was considered statistically significant for the purpose of our study.
Results: Overall there was 4.5% (n=106) incidence of post renal transplant malignancies recorded in our patients with equal distribution among paediatric and adult recipients [RR 1.0106; 95% CI 0.5927 to 1.7231; P = 0.96921. The median time from transplant to malignancy was 53 months. Non-cutaneous carcinomas (n=42) were the commonest malignancy followed by PTLD (n=33) [Table I]. The incidence of PTLD was higher in paediatric group as compared to adults [RR 3.4667; 95% CI 1.2050 to 2.4504; P <0.0001]. Likewise the incidence of non-cutaneous carcinoma was higher in adult as compared with paediatric population [RR 2.343; 95% CI 1.2204 to 2.042; P <0.0001]. The over all mortality was 7.45% during the median follow-up period of 153 months. Patients with malignancy had higher mortality rate of 29.24% as compared to 6.64% in non-malignancy group [P= <0.0001] [Figure I] Death with functioning graft (DWFG) was significantly more in malignant group [P=<0.0001].
Conculusion: The overall rate of malignancy remain low(4.5%) when compared to the international rat, this is inspite of high rate of follow up. Viral driven cancer were more common when compared to national rates, however non cotaneous carcinoma were comparable to local standarized rate. Mortality was significantly higher with cancer development and PTLD was common among perdiatric patients particularly EBV negative .

P.1562

Predictive model for new onset diabetes in renal transplant recipients

Hassan Aleid[1], Tariq Ali [1], Ammar Abdulbaki[1], Ahmad Alhuraiji[3], Abdullah Alqararaawi[3], Mai Altalhi [1], Mohamed Shoukri[2], Abdelmoneim Eldali [2], Dieter Broring[1], Raef Hussain[3].
[1]Department of Kidney and Pancreas Transplantation, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia; [2]Department of Biostats & Epidemiology, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia; [3]Medicine, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia.

Introduction: Previous studies have identified various risk factors for the development of New Onset Diabetes after Transplantation (NODAT) but data on predictive models are limited.
Aims: In this study, we aimed to identify risk factors for the development of NODAT and to develop predictive model to identify at risk of developing NODAT.
Design and Methods: This is a single centre retrospective study of all adults who received a kidney transplant between January 2003 to December 2009. NODAT was defined according to the criteria outlined in the 2003 international consensus guidelines. Multivariate logistic regression analyses were used to develop predictive model.
Results: A total of 500 patients were included in this study, 136 patients (27%) developed diabetes (NODAT group). Mortality was 6% in the NODAT group 0.5% in the non-diabetic group had died (p < 0.001). In the multivariate analysis, Older age (OR 1.06), family history of diabetes (OR 1.09), Hepatitis C infection (OR 1.92) and impaired glucose intolerance (OR 1.79) were found to be significant risk factors for the development of NODAT. Based on multivariate analysis, we have developed following predictive model:
Risk = (1 + e-h)-1
h (linear predictor)= -5.1987 + 0.0529(age) + 0.1058(Family history) + 0.7524 (impaired glucose tolerance) + 0.5892(HCV infection)
Conclusion: In this study we have developed a 4 variable model to predict diabetes in renal transplant recipients. This study has shown that around quarter of patients develop diabetes after kidney transplantation and mortality in these patients is high.
References:
[1] (1) Suthanthiran M, Strom TB. Renal Transplantation. N Engl J Med 1994;331:365-376
[2] (2) Port FK, Wolfe RA, Mauger EA, Berling DP, Jiang K. Comparison of survival probabilities for dialysis patients vs cadaveric renal transplant recipients. JAMA 1993;270:1339-1343.
[3] (3) Schnuelle P, Lorenz D, Trede M, Van Der Woude FJ. Impact of renal cadaveric transplantation on survival in end-stage renal failure: evidence for reduced mortality risk compared with hemodialysis during long-term follow-up. Journal of the American Society of Nephrology 1998;9:2135-2141.
[4] (4) Sam R, Leehey DJ. Improved graft survival after renal transplantation in the United States, 1988 to 1996. N Engl J Med 2000;342:1837-1838.
[5] (5) Silkensen JR. Long-term complications in renal transplantation. Journal of the American Society of Nephrology 2000;11:582-588.

P.1563

Urological cancers after renal transplantation

Wael Alshomali, Mahmoud Samhan.
Organ Transplant Centre, Ministry of Health, Kuwait, Kuwait.

Introduction: A higher incidence of urological cancers after renal transplantation has been reported in the literature with variable results**.**
Aim of Study: To explore the prevalance and outcome of urological cancers after renal transplantation in our centre.
Patients and Methods: Between 19798-december 2015, anumber of 2545 kidney recipients have been followed up in our centre, the medical records of these recipients were retrospectively reviwed**.**
Results: 19 instances og urological cancers were detected in 18 recipients with overall incidence of 0.74%. of these, 17 were males, and one patient was a child at time of renal transplantation. 13 grafts were from live donors. 12 renal transplantation procedure were performed in kuwait, and the another remaning 6 were performed abroad. Cancers were detected 3-176 months after transplantation (nean 66.8 month). The involved organs were: native kidney in 11, bladder in 6, prostate in 1, and alograft in 1. The histopathology diagnosis was: RCC in 12, TCC in 5, adenocarcinoma in 1, and rabdomyosarcoma in 1. Management included: surgery in 16 cases, radiotherapy in 2 cases, and one recipient died befor starting treatment. 3 patients died with functioning graft at one week to 62 months after cancer diagnosis, another graft was lost 2 months after cancer diagnosis.
Conclusion: The incidence of urological cancers after renal transplantation is similar to what reporte in literature, recipient and graft survival rates in the present study is satisfactory and comparable to what reported in larger centres.

P.1564

Chemoprevention of cutaneous squamous cell carcinoma in renal transplant recipients: A case-controlled analysis

Rakesh Anand[1], Rachel Hung[2], Sharon Frame[1], Kate Bramham[1], Mary Wain[3], AntoniaJ. Cronin[1,4].
[1]Department of Nephrology and Transplantation, Guy's & St Thomas' NHS Foundation Trust, London, United Kingdom; [2]Department of Nephrology and Transplantation, Royal Free London NHS Foundation Trust, London, United Kingdom; [3]St John's Institute of Dermatology, Guy's & St Thomas' NHS Foundation Trust, London, United Kingdom; [4]Kings College London, London, United Kingdom.

Organ transplant recipients are up to 200 times more likely to develop cutaneous squamous cell carcinoma (SCC) than age-matched general populations. Systemic retinoids, such as acitretin, may prevent the development of SCC. However, their use has been associated with adverse side effects and should be used with caution in patients with renal impairment (RI). We aimed to determine the safety and effectiveness of acitretin as a suitable chemopreventative agent against the development of SCC in long-term renal transplant recipients (LRTR).
We collected retrospective data from a large cohort (n=469) of LRTR (>7 years), of which n=108 had been diagnosed with non-melanoma skin cancer. We identified patients (n=12) on treatment with acitretin for the prevention of SCC. We matched these to an equal number of controls by age, total years from transplant and Fitzpatrick skin type. We compared GFR, liver function (LFTs) and lipid profile at 1 year pre, and 1, 3 and 5 years post commencing acitretin. We also compared the total number of SCCs pre and post acitretin. Wilcoxon signed-rank test was used to compare blood chemistry values before and after acitretin treatment within cases, and a Mann-Whitney test was used to compare differences between cases and controls.
Serum total cholesterol concentrations and LDL were significantly lower (p=0.007 and p=0.012 respectively) in patients prescribed acitretin at 5 years post treatment compared with baseline measurements. There were no other statistically significant differences in lipid profile, GFR or LFTs at baseline parameters and at 1, 3 and 5 years after starting treatment within cases or comparing cases and controls. During the 5 years after starting acitretin treatment the median number of new SCCs per patient was 2 (range 0-4), significantly lower than the median number prior to treatment of 6 (range 3-10) p=0.005.
Acitretin treatment did not adversely affect lipid profiles, liver or renal function when compared with baseline or matched untreated control data, and was associated with a statistically significant reduction in total number of new SCCs during 5-year follow-up. Acitretin should be considered as a safe and effective chemoprevention agent in carefully selected LRTR with multiple SCCs who are under regular dermatology surveillance. Further research to assess whether its use may circumvent the need to reduce or withdraw immunosuppression treatment in LRTR with SCCs is warranted.

P.1565

Complete molecular response of de novo chronic myelogenic leukemia with dasatinib therapy in renal transplant recipient

Heungman Jun[1], Sungyoon Lim[2], Yong Park[2], Cheol Woong Jung[1].
[1]Surgery, Korea University Anam Hospital, Seoul, Korea; [2]Internal Medicine, Korea University Anam Hospital, Seoul, Korea.

Introduction: Chronic myeloid leukemia (CML) is a rare malignant complication in solid organ recipients. The majority of malignancies diagnosed following immunosuppression are non-Hodgkin's lymphoma, squamous cell carcinoma and Kaposi sarcoma. Also, in CML treatment, dasatinib, administered once daily, as compared with imatinib, administered once daily, induced significantly higher and faster rates of complete cytogenetic response and major molecular response. We report complete molecular response of de novo chronic myelogenic leukemia with dasatinib therapy in renal transplant recipient.
Case Report: A 48-year-old female received two renal transplants. In 1991, she received living donor renal transplant from mother, and in 2009, received deceased donor renal transplant. In preparing for her 2nd transplant, due to high panel reactive antibody (PRA) level, rituximab, intravenous immunoglobulin G (IV IgG), plasmapheresis were conducted as a preventive measure. She received renal graft from a 38-year-old female with half mismatch HLA type. The recipient was induced with basiliximab and received mycophenolate mofetil and tacrolimus for maintenance. In addition, donor-specific antibodies (DSA) levels in pre and post-transplant revealed continuous increase. Therefore, plasmapheresis, IV IgG, bortezomib were repeatedly used post operatively. In 2014, 5 years after her 2nd transplant, she complained of myalgia, and showed leukocytosis, thrombocytosis in laboratory.
A bone marrow examination in 2014 was consistent with CML in chronic phase, and cytogenetic studies confirmed the presence of t(9;22)(q34;q11.2). Treatment was initiated with dasatinib of 100 mg daily. In follow-up with polymerase chain reaction test, she showed major molecular response in 6 months, and complete molecular response in 8 months. Currently, for over a year, she shows no specific symptoms or recurrence.
Conclusion: We report complete molecular response of de novo chronic myelogenic leukemia with dasatinib therapy in renal transplant recipient. In CML after transplant, dasatinib can be a simple and effective treatment with minimal side effects.
References:
[1] Pelloso LA, Campos MG, Nascimento M, Silva MR, Pestana JO, Chauffaille Mde L. Chronic myeloid leukemia following kidney transplantation. Leuk Res 2005;29:353-5.
[2] Eishei Oskuei A, Makhdoomi K, Abkhiz S, Vossoghian S, Farrokhpour M. Successful treatment of Chronic Myelogenic Leukemia (CML) with imatinib after renal transplantation. Arch Iran Med 2014;17:388-90.
[3] Eishei Oskuei A, Makhdoomi K, Abkhiz S, Vossoghian S, Farrokhpour M. Successful treatment of Chronic Myelogenic Leukemia (CML) with imatinib after renal transplantation. Arch Iran Med 2014;17:388-90.

P.1566

High incidence of urothelial carcinoma in Thai renal transplant recipients, 24 years experience

Touch Ativitavas [1], Wisit Liwlompaisan [2], Pongpan Thanak[2], Viroon Mavichak [2], Sopon Jirasiritham [2], North Techawattanawanna [2], Rungrote Khunprakant[2], Kaenchai Pipatpannawong [2], Nattakan Naitook[2].
[1]Oncology, Ramathibodi hospital, Bangkok, Thailand; [2]Nephrology, Praram 9 hospital, Bangkok, Thailand.

From 1992 to 2016, 617 Renal Transplantations were performed at Praram 9 Hospital. 361 cases were Living Related, 31 cases were Spouse Related and 225 cases were Deceased Renal Transplantations. There were 42 cases of malignancy, 16 cases (38%) were Urothelial Carcinoma (Transitional Cell Carcinoma, TCC), 7 cases were Hepatoma, 3 cases were Colon Carcinoma. There were 3 cases of Post Transplantation Lymphoproliferative Disease (PTLD) and 3 cases of Renal Cell Carcinoma. Other malignancies were as followed : Breast = 2, Lungs = 2, Skin = 2, Thyroid = 1, Prostate = 1, Parotid = 1, Gastrointestinal Stromal Tumor (GIST) = 1.
The overall incidence of malignancies in our studies was 6.8% (as compared to USA = 7.5%, Australia and New Zealand = 9.4%, Spain = 6.2%, Taiwan = 6.8%, Japan = 8.2%, Hong Kong = 6.1%, Korea = 8.3%). Of those 42 patients, 21 were dead, (mortality = 50%).There were 7 death in TCC patients, (mortality = 44%). Interesting finding that of those 16 cases with TCC, 11 cases were female and 5 cases were male.
Our findings suggested high percentage of TCC in renal transplant recipients with malignancies in Thai population (16 out of 42 = 38%) and female were more predominant in TCC than male (Female = 69%, Male = 31%)
There were report evidence of higher risk of cancer of kidney and bladder among female patients who were on dialysis treatment in Caucasian populations (USA, Europe Australia and New Zealand). We suggested a more aggressive surveillance for malignancy in Kidney and Urinary system in pre and post transplantation in Thailand and also in other Asian countries, especially in female recipients.
References:
[1] 1. Bertram L. Kasiske, Cancer after Kidney Transplantation in the United States, American Journal of transplantation 2004;4:905-913
[2] 2. Germaine Wong, ANZDATA registry 2012 report
[3] 3. Pendon-Ruiz de Mier V, Incidence and Long-Term Prognosis of Cancer After Kidney Transplantation. Transplant Proc. 2015 Nov;(9):2618-21
[4] 4. Wei-Hsean Li, Malignancies after renal transplantation in Taiwan: a nationwide population base study, Nephrol dial transplant (2012) 0:833-839
[5] 5. Masahiko Okamoto, Incidence, Site and Risk Factor of Post-Transplant Malignancies Analysis of 771 Renal Transplant Recipients for 40 Years in Japanese Single Center J Transplant Technol Res S1:006
[6] 6. Jeong-Kye Hwang, Malignancy after kidney transplantation: a 40-year single-center experience in Korea, European Society for Organ Transplantation 24(2011) 716-721
[7] 7. C.Y.Cheung, Malignancies after kidney transplantation: Hong Kong Renal Registry, American Journal of transplantation 2012; 3039-3046
[8] 8. Stewart JH, Buccianti G, Agodoa L, et al. Cancer of the kidney and urinary tract in patients on dialysis for end-stage renal disease: analysis of data from the United States, Europe, and Australia and New Zealand. J Am Soc Nephrol 2003;14:197

P.1567

Patient tailored risk score for the novo tumors in kidney transplantation. Is it possible to improve the outcome?

Anna Rossetto[1], Patrizia Tulissi[3], Francesco De Marchi[4], Maria Gropuzzo[3], Clotilde Vallone[3], Gian Luigi Adani[2], Umberto Baccarani[2], Dario Lorenzin[2], Domenico Montanaro[3], Andrea Risaliti[2]. [1]Oncological Surgery, IRCCS CRO Aviano, Aviano, Italy; [2]General Surgery and Transplantation University Hospital of Udine, Udine, Italy; [3]Nephrology, Dialysis, and Renal Transplantation, AOU S Maria della Misericordia, Udine, Italy; [4]General Surgery and kidney transplantation, Hospital San Bortolo of Vicenza, Vicenza, Italy.

Tumors, after cardiovascular diseases are the second cause leading to death in kidney transplant patients.
Besides the most frequent tumors (Kaposi sarcoma, non melanoma skin cancer and lympho prolipherative disease, many of those strictly related to viral infections and viral reactivation), also solid tumors have increased incidence in transplant population than in general population; many studies have shown that the transplanted organ is related to different rate of risk for different kind of solid tumors.
Urogenital cancer and gastrointestinal cancer were the most occurring solid cancer in the kidney transplant population at the centre of Udine. Those tumors were responsible of more of 50% of de novo tumors with 22% of mortality for urogenital cancer and 41% mortality for gastrointestinal cancer. Even though those tumors are perhaps not difficult to be diagnosed during scheduled follow up according to the guide lines, what was surprising was the aggressiveness leading in few times to "late" diagnosis with advanced disease and exitus for some patients. So maybe, for some curable tumors, an increased evaluation and a tailored risk assessment could help early diagnosis.
In our population we found two statistically demonstrated factors related to increased occurrence of de novo tumors: age and the disease (if glomerulonephritis) which lead to renal failure.
Alchool intake and smoke seem to be relevant as risk factors to develop some kind of tumors in our data collection, but we did not find correlation with familiarity.
Conserning the pretransplant study, since at the moment 16 months is the median time for kidney transplant, many patients can stay in the waiting list even for many years, without clear indication to repeat blood test, imaging and endoscopic studies.
We would suggest that a possible improvement in early diagnosis and prevention could be the result of a personalized program starting with the pretransplant medical history and going through the perioperative period and the late follow up. So we could design for each patient a unique risk assessment, including pretransplant medical history, habits (smoke and alcohol intake) and exposure (asbestos, steroids, imunosuppressive drugs), perioperative events (such as donor age and medical history, acute rejection, steroids and other drugs, viral infection or reactivation) and post transplant follow up.
In conclusion we suggest to define differences in risk and consequently modulate the pre-peri and post transplant period for each patient in order to ameliorate the outcome.

P.1568

Immortal time bias correction: Comparing the cancer risk between patients who had undergone kidney transplantation and those waiting for the procedure

Shih-Feng Weng[1], Yi-Chen Chen[2], Chih-Chiang Chien[3]. [1]Department of Healthcare Administration and Medical Informatics, Kaohsiung Medical University, Kaohsiung, Taiwan; [2]Departments of Medical Research, Chi-Mei Medical Center, Tainan, Taiwan; [3]Departments of Nephrology, Chi-Mei Medical Center, Tainan, Taiwan.

Introduction: Both end-stage renal disease (ESRD) patients and renal transplantation (RT) recipients are well known to have a higher cancer risk compared to the general population. Although a few analyses of relative risk for cancer associated with transplantation vs. comparable ESRD patients without transplantation have been done, the immortal time bias correction and competing death causes were not included in those analyses. The aim of this study is to correct immortal time bias and then exam the relative risk for cancer associated ESRD patients with RT vs. matched ESRD patients without RT after adjusting death as competing risk event.
Materials and Methods: This study will use claims data from Taiwan National Health Insurance Research Database (NHIRD). The cohort included new diagnosed ESRD patients between 1999 and 2011. The cases of renal transplantation (n=2,987) were matched to four ESRD patients without RT (n=11,948) on gender, year of birth, date of cohort entry, and duration of dialysis before RT by using the propensity score to avoid immortal time bias. The subjects were followed up until death or the end of 2011. The sub-distribution hazard ratio (sdHR) for getting cancer was estimated by Cox proportional hazard models in considering death as a competing risk event.
Results and Discussion: Our results showed that ESRD patients with RT in Taiwan had a significantly 1.52 times higher cancer risk than those without RT after accounting for death as the competing risk, and 1.40 times higher cancer risk if the competing risk was not taken into account. Patients whose age greater than 50 years old had 1.71 times risk to develop cancer (sdHR=1.71) in ESRD patients with RT than their counterparts without RT. The cumulative malignancy hazard ratio was significantly higher in female ESRD patients with RT than that in those female ESRD counterparts without RT (sdHR=1.66). The risk increased in the following 1 to 3 (sdHR=1.47; 95%CI= 1.14-1.90), 3 to 5(sdHR=1.49; 95%CI= 1.11-1.99) and >5 (sdHR=1.65; 95%CI= 1.27-2.13) follow-up years, but there was non-significant risk in the first year after transplantation. In addition, Hematological (sdHR=2.91, 95% CI=1.21-7.01) and kidney (sdHR=2.84,CI= 2.13-3.80) cancer had the top 2 adjusted sdHR between the ESRD patients with RT and their ESRD counterparts without RT, followed by the digestive, liver, and bladder cancer.
Conclusion: The cancer risk in ESRD patients with RT was higher than that in their ESRD counterparts without RT, and the risk with adjusted death as a competing risk event was higher than those without. It indicated that the results of the standard survival analysis tend to underestimate the event rate, especially when the rate of competing mortality is high in the ESRD patients without RT.
MOST 103-2314-B-384-001

P.1569

First case of spindle cell lipoma in an end-stage renal allograft

Zitong Zhao, Sathiyamoorthy Selvarajan, Li Yan Khor.
Pathology, Singapore General Hospital, Singapore, Singapore.

Case Summary: A 60-year-old Chinese man with IgA nephropathy and end-stage renal disease received living donor kidney transplant from his sister 20 years ago. He suffered from graft failure 10 years later and was restarted on hemodialysis. He was found a 2.9cm renal graft mass by ultrasound 4 years ago, and decided on follow-up. The mass grew to 6.2cm 3 years later. CT scan showed an enhancing renal graft mass suspicious for renal cell carcinoma. The patient underwent renal graft radical nephrectomy.

Grossly, the kidney was atrophic with a yellowish solid lesion in the renal sinus fat, measuring 6.5 x 5 x 2.5cm. Microscopically, the lesion was composed of variably-sized adipocytes and cellular areas of bland spindle cells. There were also scattered inflammatory cells and prominent slender blood vessels, with focal myxoid change. Lipoblasts were not identified.

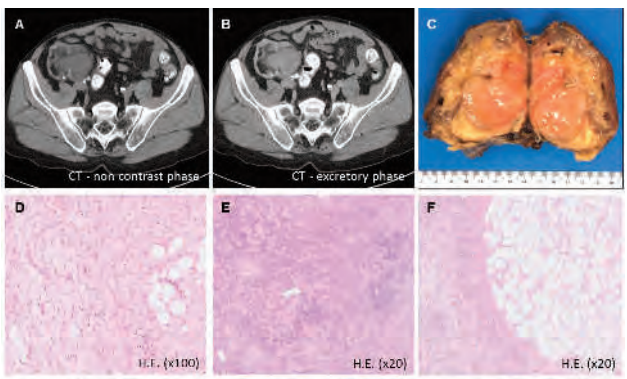


Figure 2. (A & B) CT scan of renal graft lesion. (C) Gross picture of renal graft. (D) Cellular areas of lesion (magnification x100). (E) Renal graft parenchyma (x20). (F) Lesion with adjacent renal parenchyma (x20).

The spindle cells were positive for CD34, and negative for Melan-A, HMB45, S100 and SMA. Pax-2 stain was non-specific. MDM2 expression by FISH and immunohistochemistry was negative. The Ki-67 proliferation index was less than 1%.

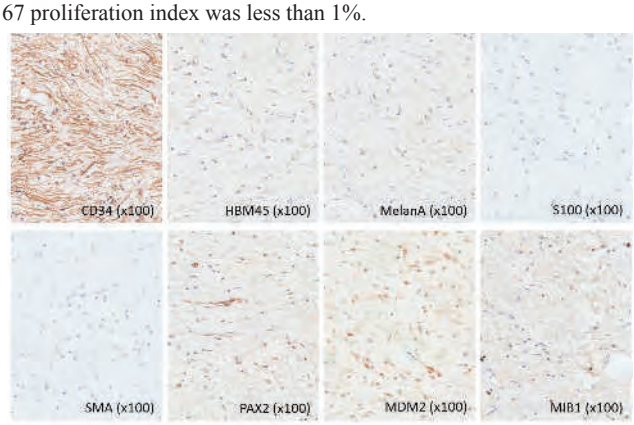


Figure 2. Immunohistochemistry.

The renal parenchyma was thinned and showed glomerulosclerosis, interstitial inflammation and thyroidisation of the tubules.

Discussion: Lipomas are the most common mesenchymal tumors, but primary renal lipomas are quite rare. Only 26 cases are reported in the literature, most commonly in the renal cortex and afftecting middle-aged women[1]-[9]. Spindle cell lipoma (SCL) is an uncommon histologic variant of lipoma and usually occurs as a solitary, subcutaneous, and well-circumscribed lesion in the posterior neck, shoulders and back of older men. To our knowledge, no renal SCL has been reported. It is composed of mixed mature lipocytes and uniform bland spindle cells in a mucinous and fibrous background.

Angiomyolipoma (AML) and well-differentiated liposarcoma (WDL) are the top two differential diagnoses. AML is the most common benign mesenchymal neoplasm of the kidney and is composed of variable proportions of mature adipose tissue, blood vessels and smooth muscle. Positivity for HMB45 and Melan-A distinguishes this entity from others. WDL is featured by lipoblasts and prominent plexiform capillaries. MDM2 amplification is a sensitive and specific marker for the diagnosis, since the morphological distinction from SCL is quite subtle. The origin of renal lipoma is unknown, possibly from lipomatous differentiation of primitive mesenchymal cells or embryonal adipose rests in the kidney[10]. Immunosuppressive therapy is well-known to increase post-transplant tumour. Although the patient in this case had stopped immunosuppressants for 10 years, his initial exposure may have had contributed to neoplastic transformation.

Conclusion: We report the first case of SCL in an end-stage renal allograft. It is a benign tumour and immunohistochemistry is helpful in differentiation from other spindle cell lesions.

References:

[1] Cakiroglu B, Tas T, Esen T, et al. Myxolipoma of the renal capsule: A case report. Int J Surg Case Rep. 2015;6:48-50.
[2] Cheng B, Cai Q, Wu Y, et al. Primary renal sinus tumor: Three case reports with a review of the literature. Oncol Lett. 2015;9(2):829-832.
[3] Lmezguidi K, Hajji F, Sinaa M, et al. Upper urinary tract lipoma: A case report. Can Urol Assoc J. 2015;9(9-10):E673-E675.
[4] Vukmirovic F, Vukmirovic M, Vukmirovic Irena, et al. Renal lipoma: a case report. Cent Eur J Med. 2013;8(3):328-330.
[5] Liu X, Wu X, He D, et al. The first case of renal lipoma in a child. J Pediatr Surg. 2011;46(6):1281-1283.
[6] Chiang IC, Jang MY, Tsai KB, et al. Huge renal lipoma with prominent hypervascular non-adipose elements. Br J Radiol. 2006;79(946):E148-E151.
[7] Ke HL, Hsiao HL, Guh JY, et al. Primary intrarenal lipoma: a case report. Kaohsiung J Med Sci. 2005;21(8):383-386.
[8] Safak M, Baltaci S, Akyar S, et al. Intrarenal lipoma: report of a case. Urol Int. 1989;44(2):113-5.
[9] Dineen MK, Venable DD, Misra RP. Pure intrarenal lipoma: report of a case and review of the literature. J Urol. 1984;132(1):104-107.
[10] Tamboli P, Ro JY, Amin MB, et al. Benign tumours and tumour-like lesions of the adult kidney. Adv Anat Pathol. 2000;7(1):47-66.

P.1570

Posterior reversible encephalopathy syndrome after solid organ transplantation

Aydin Ulas[1], Aycan Ozdemirkan[1], Ufuk Can[2], Pinar Zeyneloglu[1], Arash Pirat[1].
[1]Department of Anesthesiology and Intensive Care, Baskent University Medical School, Ankara, Turkey; [2]Department of Neurology, Baskent University Medical School, Ankara, Turkey.

Introduction: Posterior reversible encephalopathy syndrome (PRES) is a neurotoxic state that is known to occur following solid organ transplantation (SOT). In this study we evaluate the incidence, timing, and clinical characteristics of PRES.
Materials and Methods: We retrospectively analyzed the data of 12 solid organ transplant recipients who were diagnosed with PRES after SOT between 2010 and 2015. The analyzed data included the demographics, timing of PRES, blood pressure measurements preoperatively and following PRES development, and mortality.
Results: PRES developed in 12 (2.3%) out of 522 solid organ transplant recipients during the study period. Six of these patients had liver, 4 had heart, and 2 had renal transplant. PRES developed at a median time of 21 days after SOT. The median age was 10.1 years. The incidence of headache, visual disturbances, convulsions, and altered mental status were 3 (25%), 1 (8%), 9 (75%), and 6 (50%), respectively. The median percent change of 24 hour systolic, diastolic, and mean blood pressure measurements from preoperative period to after development of PRES were 42.0 %, 28.5%, and 35.7 %, respectively. Eleven (92%) of patients were admitted to intensive care unit (ICU) following development of PRES. The median duration of ICU stay was 4 days. Two patients died during ICU stay. The median time between PRES and mortality was 32 days.
Conclusion: In our cohort of solid organ recipients who developed PRES in the postoperative period, neurological symptoms and higher blood pressures compared to preoperative measurements were noted at presentation. Since PRES is often underdiagnosed, recognition of symptoms and observation of higher blood pressures should raise suspicion of PRES.
References:
[1] C Roth, A Ferbert. The posterior reversible encephalopathy syndrome: what's certain, what's new? *Practical Neurology* 2011; 11: 136–144
[2] Servillo G. Posterior reversible encephalopathy syndrome in intensive care medicine. *Intensive Care Med*. 2007; 33: 230-236.

P.1571

Acute respiratory distress syndrome in solid organ transplant recipients

Pinar Zeyneloglu, Aycan Ozdemirkan, Ozgur Komurcu, Aydin Ulas, Funda Atar, Ender Gedik, Arash Pirat.
Department of Anesthesiology and Intensive Care, Baskent University Medical School, Ankara, Turkey.

In solid organ transplant recipients (SOTRs), multiple risk factors predispose to acute respiratory distress syndrome (ARDS), such as pulmonary and extrapulmonary infections, prolonged surgical times, blood transfusions, gastric aspiration and ischemia-reperfusion injury of the transplanted organ. The aim of this study was to determine the epidemiology and outcomes of ARDS after kidney, liver and heart transplantation according to Berlin definition of ARDS.
We retrospectively analyzed the data of 804 SOTRs who received their grafts including kidney, liver and heart at a single transplant center over a 10-year period from 2005 to 2015. Patients diagnosed with ARDS were determined. The Berlin definition of ARDS was used for ARDS diagnosis. These criteria are the onset of hypoxemia within 1 week of a known clinical insult or new/worsening respiratory symptoms, bilateral opacities on chest radiograph, respiratory failure not fully explained by cardiac failure or fluid overload. Further the level of hypoxemia is used to determine the severity of the hypoxemia as mild (P/F:200-300), moderate (P/F:100-200), and severe (P/F<100). Patient demographic, clinical and laboratory data were collected.
During the study period, 540 patients underwent renal transplantation, 173 patients underwent liver transplantation, and 91 patients underwent heart transplantation. Among the 804 SOTRs, 65 (8.1%) were admitted to ICU for acute respiratory failure and 46 (5.7%) developed ARDS. The number of patients with mild, moderate, and severe ARDS were 13 (28.3%), 18 (39.1%), and 15 (32.6%), respectively. Mean patient age was 46.6 years and average duration between transplantation and ARDS onset was 120 days. The leading cause of ARDS was pneumonia (88.9%) due to bacterial pathogens in 84.2% of the cases. Hospital mortality was 54.3% overall. Mortality rates in mild, moderate, and severe ARDS patients were 4, 44, and 52%, respectively ($p<0.05$).
Among patients receiving kidney, liver and heart transplants, ARDS had an incidence rate of 5.7% with a 54.3% of hospital mortality in our cohort. The mortality rates for mild ARDS was lower and for moderate and severe ARDS were higher than expected according to Berlin definition. Further studies with larger number of patients are required to determine the ARDS mortality rates of this specific group of patients using Berlin definition.
References:
[1] Antonelli M. Noninvasive ventilation for treatment of acute respiratory failure in patients undergoing solid organ transplantation: a randomized trial. *JAMA*. 2000; 283: 235-241.
[2] Zeyneloglu P. Respiratory complications after solid-organ transplantation. *Exp Clin Transplan.*.2015; 13: 115-125.

P.1572

Venoarterial extracorporeal membrane oxygenation support as a bridge to heart transplantation: report of three cases

Ender Gedik[1], Aydın Ulaş[1], Özgür Ersoy[2], Funda Atar[1], Aynur Camkıran Fırat[1], Pınar Zeyneloğlu[1], Atilla Sezgin[2], Arash Pirat[1].
[1]Anesthesiology and Critical Care, Baskent University, Ankara, Turkey; [2]Cardiovascular Surgery, Baskent University, Ankara, Turkey.

Introduction: Heart transplantation (HT) is the only definitive treatment of end-stage heart failure. Venoarterial extracorporeal membrane oxygenation (VA-ECMO) is one of the several mechanical circulatory support devices used for patients with refractory cardiac failure (RCF). This technique may be used as a bridge to HT. The advantage of ECMO over other percutaneous devices results from the ease of insertion, ability to support right, left, or biventricular failure at high blood flows[1]. We present our experience in 3 cases that VA-ECMO was performed as a bridge to HT in the ICU.
Materials and Methods: We retrospectively screened the data of 31 HT recipients performed between 2014-2016 at our center. Among them patients who were admitted to ICU before HT, did not respond to optimal medical therapy, and required VA-ECMO for circulatory support were included.
Results and Discussion: *Case1:* A 51-years old male with ischemic biventicular dilated cardiomyopathy was admitted to ICU for RCF. His RCF did not improve with IV therapies and VA-ECMO support was initiated. Right femoral vein and left femoral artery with a leg perfusion cannula were performed percutaneously. HT was performed after 6 days on VA-ECMO support. He was discharged from the ICU and hospital on postoperative days12 and 19, respectively. He is on post-HT day159 and surviving right now.
Case2: A 12-years old girl with biventricular dilated cardiomyopathy of unknown etiology was admitted to ICU because of failure of medical therapy and worsening heart failure. She suffered from a cardiac arrest 10 days after her ICU admission. VA-ECMO was initiated during cardiopulmonary resuscitation (E-CPR). Left femoral vein and right femoral artery with leg perfusion cannulae were inserted percutaneously. She gained full neurological recovery and remained on HT list. Peripheral VA-ECMO was continued for 15 days. However she had lower leg ischemia on the day24. Peripheral VA-ECMO support was converted to central VA-ECMO and continued for 7 days. HT was done on the day 22 without ECMO support. Her ECMO related complications were lower limb ischemia with no sequels, thrombosis of arterial cannula, bleeding during central VA-ECMO. She was discharged from ICU on the day66 and she is on her post-HT day72 currently.
Case3: A 50-years old male with ischemic biventicular dilated cardiomyopathy was admitted to ICU for worsening heart failure symptoms, despite medical therapy. Although he initially improved with optimal IV therapy, he developed cardiogenic shock on day of ICU admission and VA-ECMO was commenced. Left femoral vein and right femoral artery with leg perfusion cannulae were used for vascular access. Percutaneous ventricular septostomy was performed for left ventricle venting. Peripheral VA support was continued for 23 days. He underwent HT on day28 of ICU admission. He is on his post-HT day29 currently.
Conclusion: For patients on HT list who are worsening despite optimal medical therapy VA-ECMO support is a safe and viable last resort.
References:
[1] Eur Heart J. 2014;35:156–67

P.1573

Left ventricular assist device as the bridge to heart transplantation: five-case series

Aynur Camkiran Firat[1], Nukhet Akovali[1], Ender Gedik[1], Pinar Zeyneloglu[1], Murat Ozkan[2], Atilla Sezgin[2], Arash Pirat[1].
[1]Anesthesiology, Baskent University Hospital, Ankara, Turkey; [2]Cardivascular Surgery, Baskent University Hospital, Ankara, Turkey.

Introduction: Left ventricular assist device (LVAD) is an effective therapeutic option for end-stage heart failure patients either as the bridge to heart transplantation or as the destination therapy. Although the basics of anesthetic management in LVAD as the bridge to heart transplantation is well-known, the practical approach differs with the experience and available technology. The purpose of this case series was to assess our early anesthetic experience and to arise future directions in management of LVAD as the bridge to heart transplantation.
Materials and Methods: Five patients in whom LVAD was implanted as the bridge to heart transplantation between June 2013-December 2015 were retrospectively evaluated from anesthetic and surgical charts. Beside standard invasive monitoring, pulse contour cardiac output measurement device and pulmonary artery monitoring were performed in all patients. Anesthetic technique included induction with midazolam and fentanyl followed by maintenance with desflurane, air/O2 and fentanyl infusion. During the procedure, the re-opening and dissection phase is the most challenging. The procedures were performed through median sternotomy and hypothermic femoro-femoral cardiopulmonary bypass was used in all patients.
Results: Of the 5 patients considered, 4 patients (80%) were male. The mean age at the time of transplantation was 32.1±16.6 years and mean body weight was 76.6±9.7 kg. Preoperatively, 5 patients had internal cardiac defibrillator device and 1 patient had device-related thrombi. One patient was transferred to our hospital in anticipation of mechanical circulatory support before LVAD implantation due to low cardiac output preoperatively. She was treated with dopamine, adrenaline, milrinone and extracorporeal membrane oxygenation. Other patients required LVAD for advanced cardiac failure.
There were not any major events other than transient hemodynamic changes in patients. Ultrafiltration was performed in 4 patients with an average amount of 1260±920 mL per day. Total ischemic time was 204.6±43.1 minutes. The mean duration of postoperative mechanical ventilation was 63.8±62.1 hours. There were no early mortalities. One patient who had device-related thrombi died on postoperative first day after transplantation because of hematologic problem.
Discussion: In this report we describe five heart transplant recipients who required LVAD implantation for hemodynamic support as the bridge to transplantation. Cardiac transplantation as treatment for severe heart failure has been successful but organ donor supply is limited. This situation has led to the successful improvement of LVAD.
Conclusion: LVAD is a good alternative cardiac replacement option, but LVAD causes thromboembolic events. Our findings demonstrate that patients undergoing LVAD as the bridge to heart transplantation operations constitute high-risk for thromboembolic event. Construction of standard institutional protocols may provide better evaluation and safer perioperative follow-up.
References:
[1] Koji Takeda. J Heart Lung Transplant 2015; 34:89-99.
[2] J J Brugts. Neth Heart J 2014;22:176-181.
[3] Robert A. Sorabella. Ann Thorac Surg2015; 99(2):582-88.

P.1574

Extracorporeal membrane oxygenation after liver transplantation in a patient with hepatopulmonary syndrome and refractory hypoxemia

Ozgur Komurcu[1], Arash Pirat[1], Pinar Zeyneloglu[1], Aydin Ulas[1], Gokhan Moray[2], Mehmet Haberal[2].
[1]Department of Anesthesiology and Intensive Care, Baskent University Medical School, Ankara, Turkey; [2]Department of General Surgery, Baskent University Medical School, Ankara, Turkey.

Hepatopulmonary syndrome (HPS) is a life-threatening complication of end-stage liver disease. Although HPS may be the only reason of hypoxemia, it may also contribute to hypoxemia in the presence of other causes. We present the use of extracorporeal membrane oxygenation (ECMO) after liver transplantation in a patient with HPS.
A 14 years old, 70 kg, male, diagnosed with HPS, underwent living donor liver re-transplantation for late graft loss 8 years after his first transplantation. His postoperative course was complicated by refractory hypoxemia. Different mechanical ventilation settings were used to improve oxygenation. He was extubated 47 hours postoperatively and was transferred to ward on postoperative day (POD) 4. During the postoperative period mild hypoxemia was the only problem. On POD 12 it was decided to discharge the patient and his central venous catheter was removed. Severe hypoxemia occurred immediately after catheter removal and cardiac arrest ensued. After 15 minutes of cardiopulmonary resuscitation spontaneous circulation was established. During the post-CPR period his oxygen saturation was ranging between 65% and 75%, despite FiO2 of 100%. Despite use of several mechanical ventilation modes, recruitment maneuvers, prone positioning, and vasopressors, an acceptable level of oxygen saturation could not be achieved. It was decided to use veno-venous ECMO (VV-ECMO). A 23 F-50 cm drainage cannula was inserted through the right femoral vein into the right atrium. A 17 F-15 cm infusion cannula was inserted through the right internal jugular vein into the superior vena cava. Oxygen saturation increased to 95% after initiation of VV-ECMO. A percutaneous dilational tracheotomy was performed on day 5 after cardiac arrest. He was weaned from VV-ECMO and mechanical ventilation on post-cardiac arrest days 7 and 10, respectively. However, he never gained consciousness. He suffered from two episodes of infections and several episodes of gastrointestinal bleeding due to arteriobiliary fistula. He died 30 days after his cardiac arrest.
In conclusion VV-ECMO may improve oxygen saturation after LT in patients with refractory hypoxemia that is due to HPS. However, VV-ECMO has its pitfalls, such as risk of bleeding and infection, and should be used when all other measures have failed to improve oxygenation.

References:

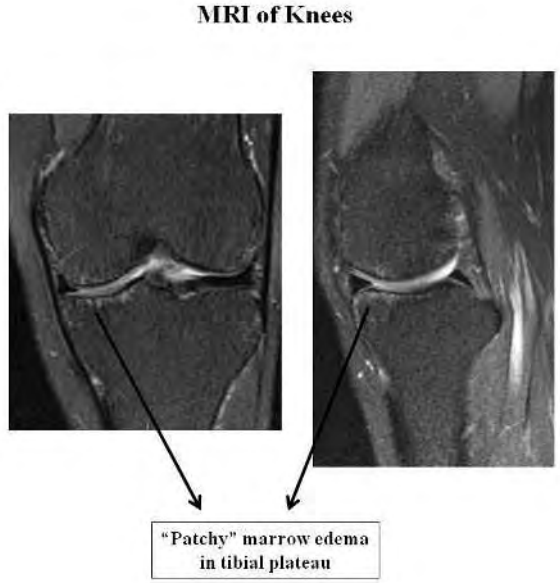
[1] Sharma NS. Extracorporeal membrane oxygenation after liver transplantation in a patient with hepatopulmonary syndrome and an atrial septal defect. *The International Journal of Artificial Organs*.2015; 3: 170-172.
[2] Park YH. Effect of pulmonary support using extracorporeal membrane oxygenation for adult liver transplant recipients with respiratory failure. *Transplantation Proceeding*. 2012; 3: 757-761.

P.1575

Calcineurin-inhibitor induced pain syndrome (CIPS): A review of 4 cases in renal retransplantation

Fabio Acosta[1], Juan Oddino[1], Maria Laura Benitez[1], Silvina Lema[1], Silvia Maiorano[1], Juan Loureiro[1], Mariela Stur[2].
[1]Renal Transplant, Hospital Provincial del Centenario, Rosario, Argentina; [2]Diagnostic Imaging, Hospital Provincial del Centenario, Rosario, Argentina.

Introduction: Kidney transplantation is the treatment of choice for End Stage Renal Disease. However, the use of immunosuppressive drugs may be associated with adverse effects that have an impact on the patient's quality of life. The calcineurin inhibitors such as cyclosporine and tacrolimus play a central role in the success of transplanted organs. However they can cause toxicity, including painful musculoskeletal disorders[6].
Bone disease, often occurring in solid organ and bone marrow transplants, is of particular concern in kidney patients due to a higher prevalence of pre-transplant bone disease (renal osteosdistrofia) In 2001 Grotz et al. coined the term Calcineurin-Inhibitor Induced Pain Syndrome (CIPS) to describe a condition characterized by symmetrical, acute and disabling bone pain localized exclusively in both lower limbs (feet, ankles and knees) with characteristic patterns in bone scans and MRI[1].
We report 4 cases of CIPS in patients with kidney retransplantation, with tacrolimus as the main inmunosupressor agent and a diagnosis by MRI.
Materials and Methods: Four patients (3 female and 1 male) who underwent kidney transplantation between March 2014 and September 2015 presented severe bone pain in both legs. The patients were 55, 52, 35 and 30 years old respectively, all retransplantations (2nd in 3 cases and 3rd in 1 case) from cadaveric donors.
Images of the MRIs of the lower limbs were performed.



Medical treatment with calcium channel blockers[2] was given and if toxic levels appeared an adjustment in the Tacrolimus dose was made.

Results and Discussion: In all patients, the CIPS began on average at 2 months after transplantation and about 2 weeks after detection of toxic levels of tacrolimus in blood. The pain lasted more than two months until it gradually disappeared. The pain was located symmetrically in both lower limbs spreading into feet, ankles and knees. Patients described the pain as sharp, throbbing and disabling which worsened when standing. It ceased when lying with legs elevated[4].

All patients presented good graft function and persistent hyperparathyroidism with iPTH average of 319.7 pg / ml. when the symptoms started. An MRI confirmed the diagnosis, revealing a pattern of bone marrow edema in tibial plateau and basically astragals[5],[7].

All cases were reversible with the indicated treatment and none required Tacrolimus discontinuation.

The CIPS diagnosis was based on clinical suspicion and exclusion of other clinical conditions[3].

Conclusion: This is the first study in the literature which registers 4 cases of Calcineurin-inhibitor induced pain syndrome exclusively related to the use of Tacrolimus as primary immunosuppressant agent with the singularity that all were cadaveric kidney retransplantations.

All cases were reversible and the diagnosis was reached by the form and location of pain, high index of clinical suspicion and characteristic pattern in MRIs.

References:

[1] Grotz, W.H., et al., Calcineurin-inhibitor induced pain syndrome (CIPS): a severe disabling complication after organ transplantation. Transpl Int, 2001. 14(1): p. 16-23.

[2] Gauthier, V.J. and L.M. Barbosa, Bone Pain in Transplant Recipients Responsive to Calcium Channel Blockers. Annals of Internal Medicine, 1994. 121(11): p. 863-865.

[3] Collini, A., et al., Calcineurin-inhibitor induced pain syndrome after organ transplantation. Kidney Int, 2006. 70(7): p. 1367-70.

[4] Elder, G.J., From marrow oedema to osteonecrosis: Common paths in the development of post-transplant bone pain (Review Article). Nephrology, 2006. 11(6): p. 560-567.

[5] Gurin, L., R. Gohh, and P. Evangelista, Pain syndrome with stress fractures in transplanted patients treated with calcineurin inhibitors. Clinical Kidney Journal, 2012. 5(1): p. 13-16.

[6] Grotz, W., K. Breitenfeldt, and M. Cybulla, Immunosuppression and skeletal disorders. Transplant Proc, 2001. 33(1-2): p. 992-3.

[7] Hetzel, G.R., et al., Post-transplant distal-limb bone-marrow oedema: MR imaging and therapeutic considerations. Nephrol Dial Transplant, 2000. 15(11): p. 1859-64.

P.1576

Adherence to bone-mineral metabolism guidelines among kidney transplant candidates

Fahad Alotaibe, Khalid B.Akkari, Meteb M.Al Bugami, Aisha Al Dowihi, Malak Bamashmoos.
Multi-Organ Transplant Center, King Fahad Specialist Hospital - Dammam, Dammam, Saudi Arabia.

Introduction and Aims: Chronic kidney disease-mineral and bone disorders (CKD-MBD) is linked to cardiovascular disease (CVD). Since CVD is the most common cause of death among kidney transplant (KT) recipients, it would be prudent to optimize CKD-MBD among KT candidates. This study aimed to measure the extent to which KT candidates complied with the National Saudi Bone Biochemistry Guidelines.

Methods: All potential KT recipients evaluated at the Kidney and Pancreas Transplant Department at King Fahad Specialist Hospital-Dammam, between January 2009 and December 2011 were reviewed. Data were collected from electronic database. Blood samples were obtained during patients' initial visit to the pre-transplant evaluation clinic. For patients on hemodialysis, pre-dialysis samples were obtained.

Results: A total of 678 candidates were evaluated. The mean age was 43 years ± 13.7. 396 (58%) subjects were males, and 33 % were diabetic. 82 % and 8 % of patients were on conventional hemodialysis and peritoneal dialysis, respectively. 10% were seen for pre-emptive KT. 44% achieved the guideline standard for phosphorus (Mean 1.73±0.56 mmol/l); 42% for corrected calcium (2.3±0.24mmol/l) and 19% for PTH (range 6.9 to 4396 pg/ml, median 438.2 pg/ml). 260 patients (38 %) had a PTH level >500 pg/ml, while 28 % had PTH below 150 pg/ml. Only 2.3% of patients met all the 3 standards for corrected calcium, phosphorus and PTH.

Conclusions: Substantial proportion of KT candidates referred for pre-transplant evaluation failed to meet the national Saudi guideline targets of CKD-MBD. This should prompt us to place greater and more rigorous emphasis on adherence measures to the guidelines in order to improve the the cardiovascular risk of transplant recipients.

P.1577

Histopathological compilcations in the new onset diabetes mellitus after kidney transplantation

Bernadett Borda[1], Edit Szederkényi[1], Zoltan Hódi[1], Éva Kemény[2], Csilla Keresztes[3], Gyorgy Lazar[1].
[1]Department of Surgery, University of Szeged, Szeged, Hungary; [2]Institute of Pathology, University of Szeged, Szeged, Hungary; [3]Department for Medical Communication and Translation, University of Szeged, Szeged, Hungary.

Introduction and Aims: New-onset diabetes after kidney transplantation (NODAT) is one of the most common complications following kidney transplantation. The diagnosis of NODAT is often late or missed; therefore, it impairs the implanted renal allograft. Diabetes and acute rejection are major contributors to morbidity and mortality. Immunosuppressive medications decrease acute rejection, but increase the frequency of new-onset diabetes after transplantation. The aim of our study was to compare the incidence of new-onset diabetes mellitus one year after kidney transplantation in patients taking cyclosporine-A and tacrolimus and the frequency of T-cell mediated rejection (TCMR) in new onset diabetes.
Methods: Patients were randomized to receive cyclosporine A- or tacrolimus-based immunosuppression. Patients who had no zero-hour biopsy did not agree to the biopsy in accordance with the protocol bei g performed, died during the study, had diabetes mellitus diagnosed before the transplantation, were younger than 18 years, or were living-donor recipients were excluded from the study; therefore, a total of 197 patients were enrolled in the study. The ultrasound-guided protocol biopsy was performed (with prior consent) after the 1-year fasting laboratory testing.
Results: Before the oral glucose tolerance tests, NODAT developed in 8% of patients receiving cyclosporine A-based immunosuppression and in 17% of patients taking tacrolimus (P = 0.03) but after the OGTT, 12% vs. 28% (P = 0.002). Albumin levels were similar, but uric acid level (P = 0.002) and the age of the recipients (P = 0.003) were significantly different between the diabetic and the normal groups. Evaluation of tissue samples revealed that TCMR (within IIA, IIB and III) (P = 0.01) and interstitial fibrosis/ tubular atrophy (IF/TA) (grade II and III) (P = 0.02) and similarly the borderline changes (P = 0.003) were significantly different between the normal and NODAT groups.
Conclusions: Regular control of carbohydrate metabolism is essential especially in high risk patients. Harmonized care may be essential in preserving the function of the allograft, as well as in preventing macrovascular complications, and in decreasing mortality in the long-term.

P.1578

Computed tomography measures of nutrition deficits in patients with liver and intestine failure

Jason Davis, Richard Mangus, Weston Bush, Jared Salisbury, A. J.Tector.
Department of Surgery, Transplant Division, Indiana University School of Medicine, Indianapolis, IN, United States.

Introduction: Patients with end-organ failure may develop severe nutrition deficits that impact perioperative morbidity and mortality at the time of transplant. Previous research demonstrates that measures of nutrition status using clinical exam or laboratory values fail to fully assess clinical deficits in muscle mass and fat tissue. This study employs computed tomography (CT) imaging to assess (1) muscle mass and (2) subcutaneous fat (SF) and (3) visceral fat (VF) stores in patients with long standing failure of the liver or intestine.
Methods: A 1:1 case-control study design is used to compare subject patients with healthy controls. Study patients were selected from a database of transplant patients, known to have long-standing end-organ failure. Controls were selected from the trauma database, using patients with an injury severity score less than 10, who received an abdominal CT scan upon presentation to the emergency department. Cases and controls were matched for age +/- 5 years, gender, and BMI +/- 2.
Results: For intestine failure (IF), there were 30 subjects and 30 controls. Compared to healthy controls, the IF patients had a higher serum protein (6.7 vs 6.0, p=0.03), but a lower serum albumin (3.1 vs 3.4, p=0.11). The IF patients had deficits in muscle mass (-22%) and SF (-7%), but no difference in VF. Subgroup analysis by gender demonstrated a greater loss of muscle mass in IF males compared to females, (-36% vs -16%, p=0.26); a greater loss of VF for males (-14% vs +6%, p=0.45) and similar deficits in SF (-9% vs -6% p=0.86). Three age groups were analyzed: < 40, 40-60, and >60 years. These three groups had an incrementally greater deficit of muscle mass (-42%, -25%, +12%) with lower age (p=0.03) and did not differ significantly for fat deficits.
For liver failure (ESLD), there were 90 subjects and 90 controls. The ESLD patients had a lower albumin level (p<0.001), similar total protein level (p=0.72), a -19% muscle mass deficit (p<0.001), a similar distribution of SF (-1%, p=0.35), and a -13% VF deficit (p<0.001). Subgroup analysis of ESLD patients found a lower albumin level only among high MELD patients (<0.001, compared to low MELD). Females had a significantly lower muscle mass and perinephric fat volume, but a higher SF volume when compared to ESLD males. There was a loss of muscle mass and VF with increasing age, while SF was similar among age groups. Finally, ESLD patients with a high BMI had a lesser deficit of muscle mass and SF and VF when compared to low BMI ESLD patients.
Conclusions: These results support previous research demonstrating substantial nutritional deficits in IF and ESLD patients that are not adequately measured by laboratory testing or clinical physical exam. Standard CT scans can be employed to assess the distribution and quantity of nutritional deficits in a more precise manner. These measures will allow for targeted intervention and an earlier time point to prevent nutrition deficits.

P.1579

Use of cinacalcet in the treatment of post-renal transplantation hyperparathyroidism. Experience from an Argentinean site

Jorge Camacho, Gustavo Laham, Jihan Sleiman, Gervasio Soler Pujol, Carlos Diaz.
Nephrology and Transplant Section, Cemic, Buenos Aires, Argentina.

Introduction: Renal transplantation is associated with a decrease in serum levels of parathyroid hormone (PTH). The persistence of high levels of PTH associated with hypercalcemia is indicative of persistent hyperparathyroidism[1].
Persistent hyperparathyroidism is a risk factor for vascular calcification, bone loss and graft survival[2]. Cinacalcet acts on sensitive calcium receptors (Ca) by increasing its activation by ionic Ca, lowering levels of plasma PTH, Ca and phosphorus (P)[3].
The aim of this study is to evaluate the effectiveness and safety of cinacalcet in renal transplant patients with persistent hyperparathyroidism and hypercalcemia.
Materials and Methods: A retrospective, observational study was conducted in 14 renal transplanted patients who received cinacalcet for at least 3 months for the treatment of hyperparathyroidism. Ca level, P level and PTH level were measure before and after treatment at 1, 3, 6, 12 months
Results: Pre-Cinacalcet PTHi was 159 ± 70 pg/mL; 151 ± 110 pg/mL after one month; 150 ± 96 pg/mL after 3 months, 142 ± 64 pg/mL after 6 months, and 139 ± 75 pg/mL after one year. PTHi decrease was not significant. Serum Ca lowered significantly from 11.3 ± 0.8 to 10.0 ± 0.8 mg/dL after one-month therapy ($p<0.001$) keeping steady values after 3 (10.2 ± 1.0), 6 (10.3 ± 0.5) and 12 (10 ± 0.4) months. P was 2.7 ± 0.79 mg/dL at therapy onset, keeping steady values after 3, 6, and 12 months. Load mean Cinacalcet dose was of 30 mg, being non-significantly increased at the third month to 32 ± 12 mg/d, 40 ± 22 mg/d at the 6th month, and 41.6 ± 18 mg/d after one year.
Conclusion: In this small study cohort of patients with persistent hyperparathyroidism and hypercalcemia, cinacalcet was effective in lowering Ca levels ($p<0.001$), while not exerting the same effect on PTHi.
References:
[1] Torregrosa V. Alteraciones del metabolismo oseo mineral tras el trasplante renal. *Nefrologia Sup Ext*. 2013; 4(1:10-8)
[2] Torres A. Parathyroid function in long-term renal transplatation: importance of pre-transplant PTH concentration. *Nephrol Dial Transplant*. 1998;13:94-7
[3] Serra AL. Effective control of persistent high hyperparathyroidism with cinalcalcet in renal allograft recipients. *Nephrol Dial Transplant.2007; 22(2):577-83*

P.1580

A prospective study of renal transplant recipients: the inter-relationship between insulin secretion and sensitivity underpins dysglycemia post following renal transplantation

KarenM. Dwyer[1,2,3], David Langsford[1], Varuni Obeyesekere[4], Jessie Teng[1], Sara Vogrin[2], Richard MacIsaac[2,4], Glenn Ward[4], Frank Alford[4].
[1]Nephrology, St.Vincent's Hospital Melbourne, Fitzroy, Australia; [2]Medicine, University of Melbourne, Melbourne, Australia; [3]School of Medicine, Deakin University, Geelong, Australia; [4]Endocrinology, St.Vincent's Hospital Melbourne, Fitzroy, Australia.

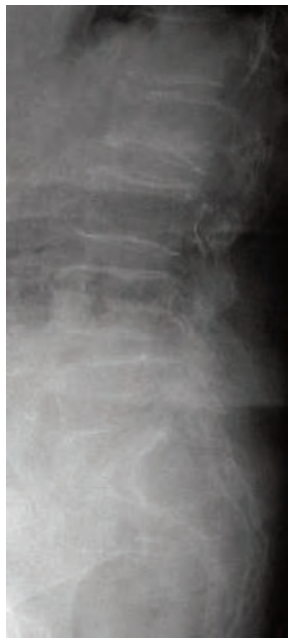
Background: Dysglycemia (encompassing impaired glucose tolerance and diabetes) arising after renal transplantation is common and confers a significant cardiovascular mortality risk. The aim of this study was to prospectively and comprehensively assess glucose handling in renal transplant recipients from before to 12 months after transplantation in order to determine the underpinning pathophysiology.
Methods: Complete 12 month data was obtained on 14 renal transplant recipients. Intravenous and oral glucose tolerance testing was conducted prior to and at 3 and 12 months following transplantation. An additional intravenous test was performed on day 7. Insulin secretion, resistance and calculation of the disposition index (DI), a measure of beta cell responsiveness in the context of prevailing insulin resistance were determined.
Results: At 12 months 50% of renal transplant recipients had dysglycemia. This cohort were older with features of the metabolic syndrome. Dysglycemia was associated with a dramatic fall in DI and this loss in beta cell function was evident as early as 3 months post transplantation. Differences in the beta cell response to oral glucose challenge were evident pre-transplant in those in which DI fell at 3 months.
Conclusion: Dysglycemia following renal transplantation occurs in older patients with features of the metabolic syndrome. The predominant mechanism is loss of insulin secretion. Subclinical differences in glucose handling are evident pre-transplant in those destined to develop dysglycemia potentially heralding a susceptible beta cell which under the stressors associated with transplantation fails.

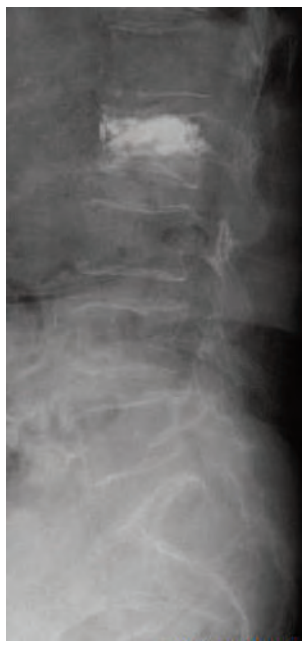
P.1581

Balloon kyphoplasty is a safe and effective option for the treatment of vertebral compression fractures in solid organ transplant recipients

Erkin Sönmez[1], Serhat Cömert[1], Aydıncan Akdur[2], Emre Karakaya[2], Salih Gülşen[1], Cem Yılmaz[1], Nur Altınörs[1], Mehmet Haberal[2].
[1]Department of Neurosurgery, Başkent University School of Medicine, Ankara, Turkey; [2]Department of General Surgery, Başkent University School of Medicine, Ankara, Turkey.

Introduction: Osteoporotic vertebral compression fractures (VCFs) affect many individual worldwide. Solid organ transplant recipients are also at great risk for osteoporotic VCFs because of both underlying pretransplant bone diseases and posttransplant immunosuppressive treatments. Balloon kyphoplasty (BK) is a minimally invasive procedure that is used in the treatment of painful osteoporotic vertebral fractures. It involves injection of viscous polymethylmethacrylate (PMMA) into the vertebral body. Injected PMMA stabilizes the fracture and alleviates the pain immediately. In this study, we report the results of balloon kyphoplasty procedure for the treatment of VCFs seen in solid organ transplant recipients.
Material and Methods: We retrospectively reviewed 512 liver transplantation and 2248 kidney transplantation procedures that were performed in our center between the years 1985 and 2015. Seven transplant recipients (4 liver and 3 kidney) with a total of 10 acute, symptomatic VCFs who were unresponsive to the conservative treatment for 3 weeks underwent BK. All fractures were treated with BK within 3 months of fracture onset. Clinical outcome were graded using the visual analog scale (VAS). Radiographic evaluation included measurement of the segmental kyphosis by Cobb method.
Results: There were 4 females and 3 males in the study. The patients' age ranged from 56 to 63 with an average of 58.8. The affected vertebral levels varied from T12 to L4. The mean follow-up time after BK was 3.4 years, with a range of 1 to 7 years. Mean time interval from transplantation to BK was found to be 8.6 years ranging from 4 months to 27 years. Statistically significant difference was found in terms of VAS scores especially in the first 24 hours after surgery and this decrease lasted for 3 years ($p<0.05$). Sagittal alignment improved (>5 degree) in 2 of 7 patients (28%).
Discussion: Transplant recipients are at great risk in terms of VCF development especially within a year after transplantation. High dose immunosuppressive treatment and long term of immobilization are the mostly suspected mechanisms. Although conservative treatment has been the first choice for VCF treatment, long time and high costs may be needed to achieve cure. BK shows perfect and fast pain relieving effect in organ transplant recipients with an acceptable complication rate.
Conclusion: Experience with our small patient population showed that BK has been very effective and safe in order to obtain rapid pain relief and earlier mobilization with less complications.





References:
[1] Hsieh MK, Chen LH, Chen WJ. Current Concepts of Percutaneous Balloon Kyphoplasty for the Treatment of Osteoporotic Vertebral Compression Fractures: Evidence-based Review. *Biomed J* 2013;36:154-161.

P.1582

Diabetic nephropathy in patients with new onset diabetes after kidney transplantation

Hyeon Joo Jeong[1,3], Hyang Sook Chung[1], Seung Hwan Song[2,3], Yu Seun Kim[2,3], Beom Jin Lim[1,3], Soon Il Kim[2,3], Kyu Ha Huh[2,3], Myoung Soo Kim[2,3].
[1]Pathology, Yonsei University College of Medicine, Seoul, Korea; [2]Transplantation Surgery, Yonsei University College of Medicine, Seoul, Korea; [3]The Research Institute for Transplantation, Yonsei University, Seoul, Korea.

Introduction: Diabetic nephropathy (DN) may develop *de novo* in some patients with new onset diabetes after kidney transplantation (NODAT). However, there have been few data on DN among NODAT patients.
Method: We retrospectively reviewed 194 cases of NODAT from 2005 to 2012 period and explored the predisposing factors and graft morphology of DN.
Results: DN was diagnosed in 18 cases. Of clinical factors, the proportion of patients with high body mass index (>25 kg/m^2) and the prevalence of hypertension, female donors, living donors and hepatitis C viral infection tended to be higher in DN patients than in non-DN patients. However, smoking history was more prevalent in non-DN patients. The prevalence of coronary heart disease and hepatitis B viral infection, the degrees of HLA mismatches, and donor ages were not different between the two groups. Renal allograft histology was advanced in DN patients: the percentage of global (38.8% versus 17.8%) and segmental (4.9% versus 3.0%) glomerulosclerosis, the degrees of tubular atrophy (1.4 versus 0.8), interstitial fibrosis (1.3 versus 0.6), arteriolar hyalinosis (1.8 versus 0.8) and arteriosclerosis (0.9 versus 0.4) were higher in DN patients than those of non-DN patients. Glomerular morphology of DN was persistent and showed progression in repeated biopsies.
Conclusion: Some clinical factors are more prevalent in DN patients, which may influence on the development of DN. Since DN accompanies higher degrees of graft injuries, early diagnosis of DN may be useful for active therapeutic intervention.

P.1583

Early onset hyperuricemia is a prognostic marker for kidney allograft dysfunction

In Mok Jung[1], Miyeun Han[2], Seung Hee Yang[2], Su-Kil Park[3], Duck Jong Han[3], Yon Su Kim[3], Young Hoon Kim[3], Jung Pyo Lee[1].
[1]Internal Medicine, Surgery, SMG-SNU Borame Medical Center, Seoul, Korea; [2]Internal Medicine, Seoul National University Hospital, Seoul, Korea; [3]Internal Medicine, Surgery, Asan Medical Center, Seoul, Korea.

Aim: It is not certain whether hyperuricemia is a true risk factor of kidney allograft failure. We investigated the association of hyperuricemia and graft outcome.
Methods: We performed a retrospective, multi-center cohort study including 2440 kidney transplant recipients. Patients were classified into the normouricemic or hyperuricemic group according to their level of serum uric acid (SUA) at post-transplant 3 month. Hyperuricemia was defined as a SUA level of≥7.0 mg/dL in male and≥6.0 mg/dL in female or when hypouricemic medications were prescribed. The effect of hyperuricemia on graft outcome, cardiovascular events, and recipient mortality were analyzed.
Results: 661 (27.1%) recipients were classified as the hyperuricemic group, among which 115 were prescribed urate lowering agents. There were 208 cases of graft failure, 44 cases of post-transplant cardiovascular events, and 84 cases of patient death during a median follow-up period of 71.0 months. Hyperuricemia was significantly associated with graft failure in multiple Cox regression analysis (Hazard ratio (HR) 1.383, 95% confidence interval [CI] 1.010 – 1.894, P=0.043). Major adverse cardiac events (HR 0.965, 95% CI 0.412-2.262, P=0.935) and recipient survival (HR 0.891, 95% CI 0.504-1.574, P=0.691) were not associated with hyperuricemia.
Conclusion: Hyperuricemia at post-transplant 3 month is associated with graft outcome. The patients showing early onset hyperuricemia after kidney transplant should be carefully monitored.

P.1584

Post transplant diabetes mellitus (PTDM) in Kuwait

Tarek Mahmoud, Torki Al-Otaibi, Osama Gheith, Jude Yagan, Yahia Makiya, Ahmad Fathi, Medhat Abdel-Halim, Rashad Hasan, Mohamed Balah, Khaled A. T.Atea, Nair Prasad, M.R.N. Nampoory.
Nephrology, Hamed Al-Essa Organ Transplant Center, Kuwait, Kuwait.

Introduction: Diabetes mellitus (DM) is considered the second leading cause for chronic kidney disease (CKD) in Kuwait (24%). Kidney transplantation is the treatment of choice for patients with CKD. PTDM is a known entity that can affect both graft and patient survival.
Patients and Methods: We conducted a survey on kidney transplant recipients (KTR) who are following up in our Center. A questionnaire was attached to all active patients' records in OPD and filled up by the attending physician. The data were collected in Excel sheet and analyzed by SPSS statistics software.
Results: we reviewed 1392 KTR over 6 months. There were 48 (3.45%), 301 (21.6%) and 356 (25.6%) patients labeled as type I, type II and PTDM respectively and remaining 684 (49.1%) patients were non-diabetics (ND). The mean BMI was 27.8, 31, 29.8 and 28.3 for type I, type II, PTDM and ND respectively. Risk factors for DM in PTDM compared to ND group were significantly higher for physical inactivity (40.7 Vs 33.3%), first degree relative (63.4 Vs 46%), hypertension (88.7 Vs 73.8%), dyslipidemia (64.9 Vs 42.8%), peri-operative hyperglycemia (38.5 Vs 9.9%) and cardiovascular disease (12 Vs 4.2%) respectively. Type of immunosuppression was not significantly different between the two groups.
Conclusion: PTDM has a major contribution to the diabetic pool in our center. Risk factors for PTDM are similar to those in general population.

P.1585

Obesity and overweight as a potential risk factors of graft function loss and death in renal transplant recipients

Przemyslaw Miarka, Dominik Cieniawski, Malgorzata Walus-Miarka, Ewa Ignacak, Alina Bentkowska-Prokop, Katarzyna Krzanowska, Barbara Idzior-Walus.
Department of Nephrology and Metabolic Diseases, Jagiellonian University Medical College, Krakow, Poland.

Introduction: Obesity and overweight are known risk factors for cardiovascular complication. In patients with renal failure (including kidney transplant-KTx) the risk of cardiac complications is very high because of long-term renal replacement therapy. In recent years it has been a steady increase in number of people with elevated BMI.
The aim of the study was to evaluate the influence of BMI on graft function KTx patients.
Material and Methods: The study was performed in years 2010-2014 and include 82 KTx patients (30 female and 52 male, aged 23 to 70 years) divided in 3 groups: 19 with diabetes before TX, 28 with post-transplantation diabetes and 35 with glomerulopathy. The occurrence of the composite endpoint (return to dialysis and/or death) was evaluated during a 4-year observation period. The measurements of height, weight and biochemical parameters were conducted at the start and the end of the study.
There was no significant difference in age and the time of KTx. In patients with pre-transplantation diabetes and post-transplantation diabetes we observed significantly higher percentage of BMI>30, higher glucose and creatinine concentration ($p<0.05$) in comparison to patients with glomerulopathy. In both group of patients with diabetes, obesity occurred in 24 patients (29.3%) and overweight in 26 (31.7%). Too high BMI was related to the total of 61% of patients. Both, the presence of overweight ($p=0.02$; $X2=5.39$) and obesity ($p=0.008$; $X2=6.93$) were associated with significantly increased risk of the composite endpoint. BMI in the group of patients who died or returned to hemodialysis (16 persons) were significantly higher than in patients who have completed 4 years of follow-up ($p=0.011$; 30.2 vs 26.7 kg/m2).
Conclusions: Overweight and obesity are a common problem in patients after KTx. Patients with abnormally high BMI are saddled with an increased risk of serious complications - death and loss of transplanted kidney function.

P.1586

Change of bone densitometry after kidney transplantation

Han Ro[1], Yeon Ho Park[2], Hyun Hee Lee[1], Ae Jin Kim[1], Ji Yong Jung[1], Jae Hyun Chang[1], Wookyung Chung[1].
[1]Department of Internal Medicine, Gachon University Gil Medical Center, Incheon, Korea; [2]Department of Surgery, Gachon University Gil Medical Center, Incheon, Korea.

Background: Numerous studies have shown that osteoporosis is common in kidney transplant recipients. However, change of bone densitometry after kidney transplantation (KT) is not fully understood.
Methods: Twenty-two kidney transplant recipients (14 males, 8 females) with bone densitometry at pre-transplant and 24 month after KT were reviewed.
Results: Recipients' median age (43.5 years, range 21.3 ~61.7) and dialysis duration before KT (2.6 years, range 0.04~12.3) were recorded. T score after KT did not show significant change (p=0.710 in lumbar spine, p=0.138 in femur neck). T score of lumbar spine or femur neck at 24 month after KT was positively associated with T score at pretransplant (p=0.001 in lumbar spine, p=0.001 in femur neck). However, change of T score during 24 months (delta T score) of lumbar spine was negatively associated with T score at pretransplant (p=0.001 in lumbar spine). Recipients with osteopenia (T score of femur neck ≤ -1.0) increased at 24 month after KT (p=0.048).
Conclusion: Careful follow-up of bone densitometry for KT recipients was needed.

P.1588

Comparison of metabolic syndrome rates in living-donor and deceased-donor kidney recipients – a three-year follow-up

Jolanta Gozdowska[1], Aleksandra Chabior[1], Ewelina Jędrych[1], Dominika Dęborska-Materkowska[1], Marta Serwańska-Świętek[2], Artur Kwiatkowski[2], Magdalena Durlik[1].
[1]Department of Transplantation Medicine, Nephrology and Internal Diseases, Medical University of Warsaw, Warsaw, Poland; [2]Department of General and Transplantation Surgery, Medical University of Warsaw, Warsaw, Poland.

Background: Metabolic syndrome (MS) is characterized by coexistent pro-atherogenic disorders and insulin resistance. MS also increases cardiovascular risk.
Materials and Methods: A total of 112 living-donor (n=54) and deceased-donor (n=58) kidney transplant recipients were evaluated for metabolic syndrome (MS) in months 6, 12, and 36. The National Cholesterol Education Program – Adult Treatment Panel III (NCEP-ATP III) criteria were used. Both groups were compared in terms of MS rates. Moreover, correlations between MS and other parameters (age, gender, dialysis type and duration, donor type, immunosuppressant drugs, acute rejection episodes, smoking, levels of triglycerides, uric acid, creatinine, MDRD eGFR, and proteinuria) were evaluated. The chi-square, McNemar's test, Student's t test, Welch's t test, Mann-Whitney U test, Fisher's test, and Shapiro-Wilk test were used in the statistical analysis.
Results: MS rates following living-donor (LD) and deceased-donor (DD) kidney transplantation (KTx) in months 6, 12, and 36 were 0.148 vs 0.276; 0.173 vs 0.316; 0.235 vs 0.182, respectively. MS rates in LD KTx recipients were lower than those in DD KTx recipients in months 6 and 12, especially in males (0.14 vs 0.379; p=0.0251), but they increased systematically in subsequent months of follow-up. MS was more commonly diagnosed in older recipients (p=0.019), with lower MDRD eGFR values (p=0.009), who received more anti-hypertensive drugs (p=0.046). The dialysis type, donor type and the number of transplantations had no effect. The logistic regression model indicated that the factors contributing to MS were elevated uric acid levels and proteinuria.
Conclusions:
1. MS rates in LD KTx recipients in month 6 and 12 following transplantation are lower than those in DD KTx recipients.
2. MS rates in LD KTx recipients tended to progressively increase during follow-up.
3. MS was more common in older patients with poorer kidney function, higher uric acid levels and proteinuria.
References:
[1] Prasad GV, Huang M, Silver SA, Al-Lawati AI, Rapi L, Nash MM, Zaltzman JS. Metabolic syndrome definitions and components in predicting major adverse cardiovascular events after kidney transplantation. Transpl Int. 2015; 28: 79-88.
[2] Nagaraja P, Sharif A, Ravindran V, Baboolal K. Long-term progression of abnormal glucose tolerance and its relationship with the metabolic syndrome after kidney transplantation. Transplantation. 2014; 97: 576-581

P.1589

Epidemiology and risk factors of cholangitis after liver transplantation – single center experience

Maciej Gaciong[1], Aleksandra Majek[1], Jolanta Gozodwska[1], Olga Tronina[1], Marek Pacholczyk[2], Magdalena Durlik[1].
[1]Department of Transplantation Medicine, Nephrology and Internal Deseases, Medical University of Warsaw, Warsaw, Poland;
[2]Department of General and Transplantation Surgery, Medical University of Warsaw, Warsaw, Poland.

Introduction: Bacterial cholangitis is common complication after liver transplantation (LTx) causing significant morbidity and mortality.
Aim of the Study: We analyzed demographic, clinical and microbiological characteristics of patients (pts) with bacterial cholangitis after cadaveric LTx performed in single transplant center.
Materials and Methods: After retrospective analysis of medical records we identified 50 pts(30/20, M/F, age during transplantation 45.7±16,6) with bacterial cholangitis among recipients of cadaveric liver transplanted between 2011 and 2015. Diagnosis of cholangitis was based on clinical symptoms, laboratory findings (elevation of markers of acute inflammatory reaction and cholestasis) and positive results of blood/bile cultures. Analysis included recipients' demographic and clinical data, results of laboratory tests (CRP, bilirubin, AST, ALT, GGTP, ALP), bacterial etiology with antibiotic resistance. Choice of therapy, duration of treatment and clinical outcomes were also evaluated.
Results: Causes of liver failure included cirrhosis due to HCV or/and HBV infection (n=21) and autoimmune hepatitis: PSC, AIH, PBC (n=17). Among 33 pts with bile samples obtained during endoscopic retrograde cholangiopancreatography, 32 had positive cultures. Most common isolates were: Enterococcus faecium (18%), Enterococcus faecalis (18%), Escherichia coli (13%), Klebsiella pneumoniae (13%), Pseudomonas aeruginosa (11%). Multiple drug resistant (MDR) species were found in 22% pts and in 27% of bacterial isolates. Among them were: vancomycin resistant Enterococcus (33%), extended-spectrum beta-lactamases-producing K. pneumoniae (33%), high-level aminoglycoside resistant Enterococcus (17%) and methicillin resistant coagulase negative Staphylococcus (17%). Antibiotic regimen included carbapenems (34% pts), vancomycin (22% pts) and colistin (4% pts). Multiple regression analysis showed that patients with higher CRP level on admission required longer antibiotics treatment and had higher blood tacrolimus level (p=0.047). Within this group more often was obtained growth of multidrug resistant bacteria culture from bile (p=0.014). Duration of hospitalization was 15±8days and was longer in patients with higher ALP plasma activity (p=0.047). Recurrent bacterial cholangitis developed in 64% pts.
Conclusion: Cholangitis after LTx is a recurring infection frequently caused by MDR bacteria. MDR is associated with more severe clinical course and requirement for combined antibiotic therapy.
References:
[1] Aberg F, Mäkisalo H, Höckerstedt K, Isoniemi H. Infectious complications more than 1 year after liver transplantation: a 3-decade nationwide experience. Am J Transplant. 2011; 11 :287-295

P.1590

Post-transplantation lymphoproliferative disease in a patient after two liver transplant because of cirrhosis of the liver's own post-inflammatory etiology HCV and HBV

Marta Soboniak[1], Jolanta Gozodwska[1], Olga Tronina[1], Marek Pacholczyk[2], Magdalena Durlik[1].
[1]Department of Transplantation Medicine, Nephrology and Internal Deseases, Medical University of Warsaw, Warsaw, Poland;
[2]Department of General and Transplantation Surgery, Medical University of Warsaw, Warsaw, Poland.

Background: Post-transplantation lymphoproliferative disease (PTLD) is a complication of parenchymal organ transplant that involves to immunosuppressive treatment and often coexisting infection of EBV. PTLD is group of disorders having in base uncontrolled growth of B lymphocytes mainly. PTLD have different clinical course (mononucleosis-like symptoms, the fulminant course with lymphadenopathy or graft dysfunction). The aim of therapy is to cure PTLD while preserving the function of the transplanted organ.
Case Report: 70-year-old patient with liver cirrhosis etiology HCV and HBV, had performed the procedure of the liver transplantation from a deceased donor with creation an arterial bypass graft between an aorta and hepatic artery. Because of septic complications related to the infection of biliary tract caused by stenosis in place of anastomosis and biliary necrosis caused by late thrombosis of the hepatic artery, the patient was qualified for retransplantation. After few months, there was a recurrence of HCV infection of a graft. The patient was treated pegylated interferon alpha 2B and ribavirin obtain a sustained viral response. Patient had recurrent infections of biliary tract in the course of stenosis of common hepatic duct. There was several procedures of prosthetic biliary tract. In the liver biopsies are described a borderline cellular rejection, because of this increased dose immunosuppressive therapy. Maintenance immunosuppression was low doses of prednisone, CsA and mycophenolate mofetil. Afterwards in the ultrasound revealed an abnormal mass in hilum of the liver which in further studies was enlarged (51x45x40mm). Diagnostic laparotomy was performed with biopsy of the mass. In the histopathology was shown B cell lymphoma with high proliferative activity of the image nearest with DLBCL (diffuse large B-cell lymphoma, not otherwise specified). The patient was qualified for immunochemotherapy accordance a treatment of PTLD - 4 administration of rituximab and 4 courses of CHOP at reduced doses of cytostatic, because of the aggravating history. As a result of treatment achieved complete regression of changes confirmed in the CT. The current state of the patient's health is good. The liver function is normal.
Conclusions: The occurrence of PTLD is serious complication, burdened with high mortality. It is recommended to reduction dose of immunosuppression therapy with rituximab and CHOP. Sequential treatment with risk stratification reduces the number of severe and fatal complications.
References:
[1] Kamdar KY, Rooney CM, Heslop HE. Posttransplant lymphoproliferative disease following liver transplantation. Curr Opin Organ Transplant. 2011;16: 274-280

P.1591

Adherence to treatment among renal post-transplant patients

Jolanta Gozdowska[1], Mateusz Zatorski[2], Agnieszka Bojanowska[2], Patrycja Torchalla[1], Łukasz Białek[1], Rafał Kieszek[3], Aleksandra Tomaszek[3], Artur Kwiatkowski[3], Magdalena Durlik[1].
[1]Department of Transplantation Medicine, Nephrology and Internal Diseases, Medical University of Warsaw, Warsaw, Poland; [2]University of Social Sciences and Humanities, Faculty in Poznan, Poznan, Poland; [3]Department of General and Transplantation Surgery, Medical University of Warsaw, Warsaw, Poland.

Background: One of the decisive factors in maintaining normal graft function in transplant recipients is patient adherence.
Materials and Methods: The study involved 89 patients after renal transplantation: 48 from a living donor (LD) and 41 from a deceased donor (DD). Basic sociodemographic characteristics, as well as laboratory parameters: creatinine-based eGFR, haemoglobin, fasting blood sugar, and proteinuria were collected. All participants filled out a questionnaire measuring three dimensions of adherence: Information (how informed the patient feels about the treatment), Motivation (how motivated the patient feels to adhere to treatment) and Behaviorual Skills (how the patient perceives his/her skills for keeping good adherence). IBM SPSS version 22 statistical software was used for the analysis (including Student's t-tests, as well as Pearson's r coefficient).
Results: In comparison to DD kidney recipients, LD kidney recipients were younger [49 vs 40 years ($p < 0.01$)]; both groups comprised mostly men (61% and 83.3%). The health status of the recipients assessed by indirect methods (history) and direct methods (laboratory tests results) was comparable in both groups. One major factor contributing to adherence to treatment was graft type. Greater motivation for treatment adherence was shown in the LD group vs the DD group ($p=0.01$). Non-first-time graft recipients showed significantly greater skills ($p=0.01$) and higher level of information ($p=0.001$), contributing to adherence to treatment. Women demonstrated more information on transplantation-related subjects, which also contributed to adherence ($p=0.02$). Other sociodemographic characteristics or the type of renal replacement therapy had no significant effect.
Conclusions: Greater motivation for treatment adherence was observed in LD kidney recipients vs DD kidney recipients, and in women from both groups. Non-first-time graft recipients showed higher motivation for adherence to treatment due to their greater knowledge and skills.
References:
[1] Denhaerynck K, Schmid-Mohler G, Kiss A, Steiger J, Wüthrich RP, Bock A, De Geest S. Differences in Medication Adherence between Living and Deceased Donor Kidney Transplant Patients. Int J Organ Transplant Med. 2014; 5: 7-14
[2] Arunachalam C, Garrues M, Biggins F, Woywodt A, Ahmed A. Assessment of living kidney donors and adherence to national live donor guidelines in the UK. Nephrol Dial Transplant. 2013; 28:1952-1960

P.1592

Characteristics of alert pathogens isolated from inpatients of a Department of Transplantation and Nephrology at one of Warsaw hospitals in 2014

Jolanta Gozdowska[1], Łukasz Chabros[2], Magdalena Czerwińska[1], Grażyna Młynarczyk[2], Andrzej Chmura[3], Magdalena Durlik[1].
[1]Department of Transplantation Medicine, Nephrology and Internal Deseases, Medical University of Warsaw, Warsaw, Poland; [2]Department of Microbiology, Medical University of Warsaw, Warsaw, Poland; [3]Department of General and Transplantation Surgery, Medical University of Warsaw, Warsaw, Poland.

Background: Inpatients are particularly susceptible to colonization and infection with multidrug-resistant bacterial strains that have undergone selection in hospital settings. Multidrug-resistant microorganisms spread easily, especially among immunosuppressed patients.
Methods: A total of 636 bacterial isolates were obtained from positive cultures of blood, urine, sputum, fluid from body cavities, throat and rectal swabs (screening for carriers) from 111 patients (F=57, M=54) hospitalized between October 2013 and October 2014. These were organ transplant recipients: kidney (n=70), liver (n=17), kidney-pancreas (n=3), and heart (n=2), as well as patients with chronic kidney disease (CKD) stage 5D (n=12) and CKD stage <5 (n=7). For further analysis, 186 isolates were selected based on their resistance to at least 2 drug classes and presence of the following important mechanisms of resistance: ESBL, VRE, MRCNS, MBL, HLAR, MRSA, AmpC, MLSb.
Results: The most commonly isolated multidrug-resistant strains were extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae (n=78), which constituted 12.26% of all the 636 original isolates. Other multidrug-resistant pathogens included vancomycin-resistant enterococci (VRE) n=20 (3.4%), methicillin-resistant coagulase-negative staphylococci (MRCNS) n=8 (1.26%), Pseudomonas aeruginosa and metallo-beta-lactamase (MBL)-producing Gram-negative Enterobacteriaceae n=7 (1.1%), HLAR n=6 (0.94%), AmpC n=2 (0.31%), MRSA n=2 (0.31%), and MLSb n=1 (0.16%). No KPC, NDM-1 or OXA-48-producing pathogens were isolated in the analysed time period. A significant proportion of multidrug-resistant pathogens was isolated from carriers (n=49; 7.7%).
Conclusions: The high prevalence of ESBL and MBL-producing bacilli as well as VRE in rectal swabs from transplantation ward patients suggests high carrier rates and demands thorough epidemiologic surveillance in order to eliminate foci of infection.
References:
[1] Kawecki D, Wszola M, Kwiatkowski A, Sawicka-Grzelak A, Durlik M, Paczek L, Mlynarczyk G, Chmura A. Bacterial and fungal infections in the early post-transplant period after kidney transplantation: etiological agents and their susceptibility. Transplant Proc. 2014; 46:2733-2737
[2] Kawecki D, Pacholczyk M, Lagiewska B, Sawicka-Grzelak A, Durlik M, Mlynarczyk G, Chmura A. Bacterial and fungal infections in the early post-transplantation period after liver transplantation: etiologic agents and their susceptibility. Transplant Proc. 2014; 46: 2777-2781

P.1593

New onset diabetes after transplantation in a single center at Cali - Colombia

Guillermo E.Guzman[4], CarlosE. Duran[1], Laura Gonzalez[3], Eliana Manzi[2], Maria C.Sanchez[3], Leydi J.Plaza[3], Johanna Schweineberg[1], Liliana Mesa[1], Juan G.Posada[1], Oscar J.Serrano[1], Gabriel J.Echeverri[1], Luis A.Caicedo[1].
[1]Transplant Unit, Fundacion Valle del Lili, Cali, Colombia; [2]Clinical Research Center, Fundacion Valle del Lili, Cali, Colombia; [3]Faculty of Health Sciences, Universidad ICESI, Cali, Colombia; [4]Endocrinology Unit, Fundacion Valle del Lili, Cali, Colombia.

New Onset Diabetes Mellitus after transplantation (NODAT) is a common complication in recipients kidney transplantation, its onset is associated with increased morbidity. Given the consequences and little information about the impact on our country and institution, a study was made to determine the incidence of the NODAT in our center.
Materials and Methods: We examined information from the institutional Renal Transplant Registry (TRENAL). From 1995 to 2014, 1141 renal transplants were performed in adult's patients. We excluded the patients were lost to follow up in the first thirty days. We analyzed this data using STATA 12. Kaplan-Meier and Log Rank test were used for bivariate and survival analysis.
Results: Out of 1,141, 123 had NODAT (11%). Acumulated incidence to 5 years post transplant was 9.1%. Of the receptors, 57% were men, mean age at diagnosis was 53±12 years, median time between transplant and NODAT was 3.23 months (RIQ: 1- 35), afroamerican 17%, 13% were above 30 BMI, and 83% had a deceased donor. At time of transplant, 27% received Tacrolimus and 25% prophylaxis for CMV. CMV infection was 15% and acute graft rejection was 20%. In bivariate analyses we found, betwen other factors, that age at transplant (HR:1.05, IC95%[1.03 -1.06]), afroamerican (HR:2.05, IC95%[1.28 - 3.3]), tacrolimus (HR:2.36, IC95%[1.57 -3.57]), CMV prophylaxis (HR:1.78, IC95%[1.17 -2.7]) and infection (HR:2.07, IC95%[1.26 - 3.38]) were identified as possible factor associated with NODAT development.
Conclusion: We found an incidence similar to the reported in the literature. We report an 11% incidence of NODAT and a cummulative incidence at 5 years of 9.1%. In our bivariated analysis we showed an increase on the incidence of NODAT with the risk factors above mentioned, all of them described in prior studies. This is an initial report from a transplant center in Cali Colombia, and is the first report of a descriptive analysis on NODAT in a cohort of kidney transplant in Colombia.

P.1594

Skin cancer incidence in renal transplant patients in Fundacion Valle del Lili: 20 years experience

Carolina Cabrera[2], Claudia L.Montoya[3], Andres Vidal[3], Eliana Manzi[2], Liliana Mesa[1], Juan G.Posada[1], Carlos E.Duran[1], Johanna Schweineberg[1], Jorge I.Villegas[1], Oscar J.Serrano[1], Gabriel J.Echeverri[1], Luis A.Caicedo[1].
[1]Transplant Unit, Fundacion Valle del Lili, Cali, Colombia; [2]Clinical Research Center, Fundacion Valle del Lili, Cali, Colombia; [3]Dermatology Unit, Fundacion Valle del Lili, Cali, Colombia.

Introduction: Skin cancer is the most frequent malignancy in organ transplant recipients (OTRs). It is recognized that immunosuppression promotes cancer development in this population, although physiopathology is still unknown. Since few studies have described the clinical characteristics of skin cancer of OTRs in tropical countries, we depict the presenting nature of skin cancers in renal transplant recipients (RTRs) in Fundación Valle del Lili (FVL).
Materials and Methods: First we examined information from 1493 patients in the Renal Transplant Registry of FVL(TRENAL) from 1995 to 2015. We included 1334 patients in the analysis using STATA 12 and excluded 72 who were lost to follow up plus 86 patients who received a second transplant. We then examined clinical charts of 216 patients who consulted a dermatologist in FVL from 1 June 2015 to 30 June 2015.
Results: Out of 1,334 patients who were followed up for a period longer than 30 days, total cancer incidence in RTRs was 100/1334 (7.4%), 60 (4.49%) were diagnosed with skin cancer, which corresponds to 60% of total cancer diagnoses made. Of these 60 patients, 63% were men, median age at time of diagnosis was 57 years (RIQ: 47 65), median time between transplant and cancer diagnosis was 51 months (RIQ: 22 98) and 80% had a deceased donor. Squamous cell carcinoma was the most frequently diagnosed cancer (46.3%). At time of cancer diagnosis, 85% of patients were receiving Cyclosporin A and 78% Mycophenolate mofetil. Forty patients were switched to mTOR but 8 presented adverse events and were switched back. Fitzpatrick IV was the most common skin type. Incidence of skin cancer 10 years post transplant was 5.6%.
Discussion and Conclusions: Although incidence of skin cancer in RTRs of our institution is not as high as that reported in other countries, other characteristics such as skin cancer predominance, BCC to SCC ratio, gender, type of donor and age at transplant are what is expected in comparison to other reports.

P.1595

HLA identical kidney transplantation from live and deceased donor

Luis A. Caicedo[1], Juan C.Gomez[2], Mauricio Duque[1], Eliana Manzi[2], Liliana Mesa[1], Johanna Schweineberg[1], Calos E.Duran[1], Juan G.Posada[1], Jorge I.Villegas[1], Oscar J.Serrano[1], Gabriel J.Echeverri[1].
[1]Transplant Unit, Fundacion Valle del Lili, Cali, Colombia; [2]Clinical Research Center, Funndacion Valle del Lili, Cali, Colombia.

Introduction: Kidney transplantation is the treatment of choice for patients with endstage renal disease(ESRD). Graft rejection is much lower in terms of acute rejection and improved graft survival in renal transplantation with HLAidentical compared to nonidentical HLA receptors. The aim of this work is to describe the experience of HLA identical kidney transplantation from live and deceased donor that have been performed at Valle de Lili Foundation since 1995 to 2014.
Materials and Methods: HLA identical were identified from 1,462 kidney transplants that had been performed. Molecular techniques PCRSSOP/PCRSSP were used to typify HLA. Sociodemographic characteristics, received immunosuppression, graft function, acute rejection, complications and survival rates of both graft and patient were analyzed. A descriptive statistical analysis for selected variables and subgroups was made. Categorical variables were presented in proportions and graft survival was made with KaplanMeier curve.
Results: A total of 29 HLA identical kidney transplants were performed (25 living donor). 58.6% were male, 72.41% corresponded to Africandescendent individuals and 86.21% had pretransplant dialysis. 48.2% underwent posttransplant biopsy and 6.9% were diagnosed with at least one episode of acute rejection. All patients received initial triple immunosuppression therapy. In regards to renal function, average pretransplant creatinine was 9.00 mg/dl(±4.8), 6 months later average was 1.3 mg/dl(±0.3) and annual control average was 1.3 mg/dl(±0.3). The probability of being free of acute rejection in first, third month and in the first year was 100%, 100% and 92.8% respectively. Graft survival was 90.5%, 72.4% and 72.4% at the fifth, tenth and fifteenth year respectively and patient survival was 93.7%, 84.3% and 84.3%.
Discussion and Conclusions: HLA identical receptors have prolonged graft survival with minimal complications and favorable rates of renal function that remains over time. Obtained results are favorable and comparable with those reported worldwide and according to our knowledge the first report of a series of HLA identical kidney transplants in our country.

P.1596

Cytomegalovirus infection after kidney transplantation in a population with high pretransplant seroprevalence

Liliana Mesa[1], Fernando Rosso[2], Johanna Schweineberg[1], Carlos E.Duran[1], Juan G.Posada[1], Jorge I.Villegas[1], Oscar J.Serrano[1], Gabriel J.Echeverri[1], Eliana Manzi[2], Luis A.Caicedo[1].
[1]Transplant Unit, Fundacion Valle del Lili, Cali, Colombia; [2]Clinical Research Center, Fundacion Valle del Lili, Cali, Colombia.

Introduction: CMV remains one of the most important infections in kidney transplant recipients. It can affect allograft function and increase patient morbidity and mortality[1]. We want to describe our experience with patients affected with CMV infection in a population of kidney receptors with high seroprevalence of CMV.
Materials and Methods: We examined information from the Renal Transplant Registry of Fundación Valle del Lili(TRENAL). From 1995 to 2014, 1210 renal transplants were performed in adults. We excluded the patients were lost to follow up in the first thirty days. Two immunosuppression schemes were used, without and with Thymoglobulin induction and universal prophylaxis for CMV for six months(from Jul-2013 until now). We analyzed this data using STATA 12. KaplanMeier were used for survival analysis.
Results: The prevalence of CMV_IgG titers in receptors was 95%. Out of 1,210, 111 had CMV(9.2%). Cumulated incidence to 6 and 12 months was 8.1% and 8.6% respectively. Of the receptors,62% were men, mean age at transplant was 47±12 years, median time between transplant and CMV was 2 months(IQR: 1.53.1), mismatches≥3 61%, 16% had pretransplant DM and 87% had a deceased donor. Nine patients were D+R- and one D-R-. 31/111 patients had CMV disease, more frequent in gastrointestinal tract(15/31) and acute graft rejection was 13%. Graft and patient survival at 1, 5 and 10 years was 92%, 87% and 86%; and 95%, 94% and 94 respectively, no statistical differences were observed compared with patients without CMV infection(p>0.05).
Conclusions: Despite having a high CMV seroprevalence, CMV infection is an important cause of infection on kidney transplant receptors[2]. It is more frecuent on males around their fifth decade of life with more than 3 HLA mismatches. Patients with CMV disease had more frecuent gastrointestinal and renal complications. No significative differences were found between patients with CMV infection regarding acute rejection, graft and patient survival. After universal profilaxis no CMV infections were detected[3],[4].
References:
[1] The impact of infection on Chronic allograft dysfunction and allograft survival after solid organ transplantation Martin Gandul ; Muellller, Pascual Am J Transplant Epub 2015 Oct
[2] Cytomegalovirus prevention strategies in seropositive kidney transplant recipients. Fernandez Ruiz M. Transpl Int 2015 SEp
[3] Impact of cytomegalovirus on long term mortality and cancer risk after organ transplantation. Desai R Transplantation 2015
[4] Current characteristics and outcomes of cytomegalovirus infections after kidney transplantation. Helantera. Transpl Infect Dis 2014.

P.1597

Liver transplant in patients with hepatitis c virus induced cirrhosis: 15 year experience in Fundacion Valle de Lili, Cali, Colombia

Luis A. Caicedo[1], Alejandro Delgado[2], Diego F.Jimenez[1], Mauricio Sepulveda[1], Jairo A.Garcia[1], Eliana Manzi[2], Jorge I.Villegas[1], Oscar J.Serrano[1], Gabriel J.Echeverri[1].
[1]Transplant Unit, Fundacion Valle del Lili, Cali, Colombia; [2]Clinical Research Center, Fundacion Valle del Lili, Cali, Colombia.

Introduction: Hepatitis C Virus induced cirrhosis is the main cause of orthotopic liver transplant world wide. In the last 5 years remarkable advances in the management of liver transplanted patients have been made with a positive impact in their survival.
Materials and Methods: A case series study was perfomed with the aim to describe clinical characteristics, lab results, clinical follow-up and outcomes of adult patients with HCV induced cirrhosis who underwent liver transplant between the years 1996-2015. Patient survival was made using the Kaplan-Meier method.
Results: 71 out of 701 liver transplants receptors where chosen. Median receptor age was 57 (IQR:49-63). Pretransplant clinical characterestics where: Alkaline Phosphatase, Total Bilirubin, Aspartate Aminotransferase, Alanine Aminotransferase was 154 (±79), 2.3 (±1.8), 108 (±10), 87 (±119) respectively. All where deceased donor transplants with median age of 27 years (IQR:20-37). Cold ischemia time had a mean of 8.79 hours (±2.4) and a Red Blood Cell transfusion median of 4 units (2–6.7). Intensive Care Unit stay had a median of 5 days (RIC:3-8). Clinical follow up had a median of 20.4 months (IQR:2-61), the following complications occurred: Acute Graft Rejection within 1st year 43%, Acute Renal Failure 38%, and Bile Duct Stenosis 29%. 25 out of 69 patients died due to: Infection 43%, HCV Recurrence Complications 22%, Intraoperatie death 13%, postoperative hemorrhage 8.7% and None-Primary graft function 8.7%. Transplanted patients between the years 1996-2005 had a survival of 54%, 44%, 44%, 44% during first, third, fifth and tenth year postransplant respectively and those transplanted between the year 2005 and 2015 had a survival of 83%, 77%, 71%, 71% during first, third, fifth and tenth year postransplant respectively.
Conclusion: Pacient survival has improved due to surgical experience, fewer graft cold ischemia time, and new HCV posttransplant treatment. Fundacion Valle de Lili is a reference center for liver transplantation in the southwestern Colombian territory and the observed data is comparable with those reported in other transplant groups.

P.1598

Kidney transplantation: Twenty years of experience in a single center

Luis A. Caicedo[1], Juan C.Gomez[2], Jimmy A.Gallego[1], Eliana Manzi[2], Liliana Mesa[1], Johanna Schweineberg[1], Carlos E.Duran[1], Juan G.Posada[1], Jorge I.Villegas[1], Oscar J.Serrano[1], Gabriel J.Echeverri[1].
[1]Transplant Unit, Fundacion Valle del Lili, Cali, Colombia; [2]Clinical Research Center, Fundacion Valle del Lili, Cali, Colombia.

Introduction: Medical advances have improved graft and patients survival in kidney transplant. The aim of the study is to report the experience of 20 years in kidney transplantation performed in our institution.
Materials and Methods: A retrospective study was carried out with patients who underwent kidney transplantation, with a 30 days minimum follow-up between 1995-2013. A descriptive-multivariate statistical analysis was made for selected variables and subgroups. Kaplan-Meier method was used for patient analysis, graft survival and acute rejection probability.
Results: 1316 kidney transplants were included in our study. Mean receptor age was 41 years(±16), 84.5% were 18-65 years. 75.5% were deceased-donor-transplants(DD), 6.1% were re-transplants and 11% did not required dialysis before transplantation. Delayed graft function(11.9%) was the most common medical complication. Most frequent neoplasia was Skin cancer(3.8%). Schemes of immunosuppression between 1995-1998 were steroid induction (E) and maintenance with cyclosporine(CsA), azathioprine, prednisolone(P); between 1999-2005 E/Monoclonal antibodies(mAb) induction and maintenance CsA Mycophenolate(M), P; finally, between 2006-2013 induction E/mAb and maintenance CsA/Tacrolimus, M, P; for the last half of 2013 thymoglobulin induction was used. Acute Rejection(AR) probability of all the transplant recipients was 25% at year 1, patient survival was 93.6% after 5 years, 89.9% in year 10, and 87.7% at year 15. Graft survival was 81.6% at 5 years, 72.3% at 10 years and 65.9% after 15 years, with an improvement in the last period ($p<0.001$). Multivariate analysis of the variables associated with graft loss were cold ischemic time greater than 17 hours(HR 1.52,CI 1.14-2.01,p=0.004), DGF(HR 2.05,CI 1.53-2.75,p=<0.001), AR(HR 2.24,CI 1.77- 2.83,p=<0.001), recipient age(HR 0.98,CI 0.97-0.99,p=0.001) and DD(HR 1.95,CI 1.2-3.0,p=0.004).
Discussion and Conclusions: Patient and graft survival improved over time, possible due to recipients age, deceased donor, cold ischemic time >17 hours, DGF and AR. Fundacion Valle de Lili is a reference center for kidney transplantation in the southwestern Colombian territory and the observed data is comparable with those reported in other transplant groups.

P.1599

Duodeno-duodenostomy enteric drainage in simultaneous pancreas and kidney transplantation. A single center experience

Luis A. Caicedo[1], Juan C.Gomez[2], Oscar J.Serrano[1], Eliana Manzi[2], Johanna Schweineberg[1], Carlos E.Duran[1], Liliana Mesa[1], Juan G.Posada[1], Jorge I.Villegas[1], Gabriel J.Echeverri[1].
[1]Transplant Unit, Fundacion Valle del Lili, Cali, Colombia; [2]Clinical Research Center, Fundacion Valle del Lili, Cali, Colombia.

Introduction: Simultaneous kidney and pancreas transplantation (SPR) is an effective therapy for patients with type I diabetes mellitus(DM-I) and chronic kidney disease(CKD). Within these last one, there is a difficulty in monitoring due to the absence of a strong serological marker and dificult access for biopsies. In order to solve this problem, enteric derivation has been described recently through performance of a duodenoduodenostomy, which makes the possibility of a comfortable access for endoscopic biopsy feasible.
Materials and Methods: A descriptive analysis was made for patients undergoing SPR transplant, who underwent duodeno-duodenostomy enteric drainage, evaluating safety in this type of intervention and outcomes.
Results: Since 2014 the surgical technique used so far in our institution was modified, therefore a total of 6 patients were selected for our study. Median age was 41.5 years(IQR 28.75-43.75), 5 were men, their median body mass index was 21.4 kg/m2(IQR 20.5-23.3). All patients received insuline management. 4 patients were in pre-transplant dialysis, 4 had retinopathy, 2 neuropathy, and 2 heart diseases. Portal bypass to the superior mesenteric vein was performed in one patient and remaining cases to the vena cava; arterial anastomosis in the infrarenal aorta was performed in all cases with exocrine drainage by manual laterolateral duodenoduodenostomy in two planes. Cold ischemic time median for pancreas graft was 10 hours (IR 9.87-10.62) and 11 hours in kidney graft(IR 10-11.25); All patients received the same immunosuppression scheme. Normoglycemia was reached in the first 24 hours postoperatively and median creatinine at 7 days posttransplant was 1.17 mg/dl(IR 0.90-1.39). One patient had pancreatic graft loss secondary to vascular thrombosis and another secondary to a venous thrombosis-abdominal infectious event. Remaining patients are insulin free and with stable renal function.
Discussion and Conclusions: Fundacion Valle de Lili has become a regional and national reference center in SPR transplant. Duodenal exocrine drainage will allow the evaluation and monitoring of accessible endoscopic pancreatic graft.

P.1601

Urinary tract infections in renal transplant recipients

Melike Hamiyet Demirkaya[1], Aysegul Yesilkaya[1], Ozlem Kurt Azap[1], Hande Arslan[1], Mehmet Haberal[2].
[1]Infectious Diseases and Clinical Microbiology, Baskent University, Ankara, Turkey; [2]General Surgery, Baskent University, Ankara, Turkey.

Introduction: Urinary tract infection (UTI) is the most common infectious complication seen after renal transplantation. Emergening antibiotic resistance particularly in gram negative enteric bacilli is a great concern for the management of these infections. The aim of this study is to determine the ratio of multi-drug resistant (MDR) bacteria that causes UTI following renal transplantation and also to evaluate the periods of infectious episode; as the early period, the second period and late period after transplantation.
Patients and Methods: We evaluated renal transplant recipients with confirmed bacterial urinary tract infection followed up from January 2015 to December 2015 at Baskent University Ankara Hospital. Medical reports of the patients were reviewed retrospectively.
Early period was defined as occurence within the first month after transplantation, the second period was defined as the period of the second month to sixth month, and the late period was defined as occurence at the time after sixth month.
MDR bacteria was defined as being resistant to ceftriaxone, trimetophrim sulfamethoxazole and ciprofloxacin for gram negative bacteria and vancomycin resistance for gram positive bacteria.
We also evaluated antibiotic resistance rates for trimetophrim sulfamethoxazole, ciprofloxacin and carbapenem resistance each against gram negative bacteria.
Results: In the study period, 87 renal transplant recipients had 215 bacterial UTI episodes. Twenty eight renal transplant recipients (32%) had 3 and more UTI episodes in a year, defined as recurrent UTI. Thirty seven UTI episodes (17%) were seen in the two weeks after the treatment of the previous episode and defined as relapse.
26 (12%) isolated bacteria was gram positive, and 191(88%) was gram negative.
The total ratio of MDR bacteria was 27.6% (60) in this study. MDR bacteria ratio was 27% (5/18) in the early period, 41% (25/61) in the second period and 21% (30/142)in the late period.
The trimetophrim sulfamethoxazole, ciprofloxacin and carbapenem resistance separately for gram negative bacteria is shown in Table 1.

Table1: Antibiotic resistance rates for gram negative bacteria

Antibiotic	Early Period % (18)	Late Period % (142)
TMP-SMX	100% (18)	72% (103)
Ciprofloxacin	33% (6)	52% (74)
Carbapenem	0% (0)	2.4% (3)

Conclusion:
1. Antibacterial resistance is a major concern for the management of urinary tract infections in renal transplantation recipients limiting antimicrobial choices.
2. TMP-SMX usage for prophylaxis may cause high resistant UTI in renal transplant recipients in the early period.

P.1602

Late-onset BK viruria in renal transplant recipients

Mediha Boran[1], Tolga Yildirim[2], Ertay Boran[3], Mertay Boran[4].
[1]Nephrology, Hemodialysis, and Transplantation, Turkiye Higher Education Hospital, Ankara, Turkey; [2]Nephrology, Ankara Diskapi Yildirim Beyazit Training and Research Hospital, Ankara, Turkey; [3]Anesthesiology and Reanimation, Duzce University Faculty of Medicine, Duzce, Turkey; [4]Thoracic Surgery, Duzce University Faculty of Medicine, Duzce, Turkey.

Background: Most cases of BK virus (BKV) infections emerge within the 1st years of kidney transplantation. We aimed to determine the prevalence of late-onset BKV infection and whether there are any differences between risk factors in early and late BKV infections.
Methods: In this single-center retrospective study, we reviewed 300 kidney transplant recipients that were under regular follow-up and selected recipients with BKV infection and recorded associated risk factors, connection with immunosuppression, and responses to modification of treatment.
Results: BKV was detected within the 1st 5 years after transplantation in 20 patients (6.6%, group 1) and after 5 years in 15 patients (5.0%, group 2). There were no significant differences between the 2 groups regarding age, sex, sex mismatches, donor type, BKV elimination time, serum creatinine, and estimated glomerularfiltration rate at the times of BKV detection and last follow-up visit. In group 1, 2 recipients had biopsy-proven BKV associated nephropathy (BKVAN), 3 recipients had BK viruria and viremia without BKVAN (biopsy proven), and 15 recipients (75%) had only BK viruria. In group 2, all of the patients had only BK viruria. In this group, on detection of BK virusria and immediate modification of immunosuppressive regimens prevented BK viremia.
Conclusions: Routine screening of renal transplant recipients for BKV was indicated not only during the 1st 5 years, but also for the full follow-up period after transplantation.
References:
[1] Hirsch HH. Prospective study of polyomavirus type BK replication and nephropathy in renal-transplant recipients. N Engl J Med 2002;347:488-96
[2] Randhawa P. K virus infection in transplant recipients: an overview and update. Am J Transplant 2006;6:2000-5.

P.1603

Disseminated Cryptococcosis initially presenting as lower limb cellulitis in a renal transplant recipient

Katrina Chakradeo[1,2], Y.Y. Paul Chia[1,3], Cheng Liu[4], David W.Mudge[5], Janath De Silva[1].
[1]Department of General Medicine, Mackay Base Hospital, Mackay, Australia; [2]School of Medicine, Griffith University, Gold Coast, Australia; [3]College of Medicine and Dentistry, James Cook University, Townsville, Australia; [4]Department of Anatomical Pathology, Royal Brisbane and Women's Hospital, Brisbane, Australia; [5]Queensland Renal Transplant Service, Princess Alexandra Hospital, Brisbane, Australia.

Disseminated cryptococcal infection is a serious infection seen predominantly in immunocompromised hosts. Initial presentation is rarely described as lower limb cellulitis[1]-[3]. We report a renal transplant patient who presented initially with bilateral lower limb cellulitis without other symptoms or signs. After failure of conventional therapy for cellulitis with flucloxacillin and progression of cellulitis a skin biopsy was performed. This confirmed cryptococcal cellulitis.
Presence of cryptococcal cellulitis prompted additional evaluation for extra-cutaneous disease. Computed tomography (CT) of the chest showed mediastinal lymphadenopathy without presence of any focal pulmonary lesions. CT of the head revealed no signs of elevated intracranial pressure or cryptococcoma. Lumbar puncture was performed with initial India ink staining of cerebrospinal fluid (CSF) showing organisms resembling cryptococci which was subsequently confirmed on culture to be *Cryptococcus neoformans*. Blood cultures also grew *C.neoformans*. Both CSF and serum cryptococcal antigen titre were high at >1:1024. These findings confirmed the diagnosis of disseminated cryptococcal infection with high burden of disease.
Our case was treated with 6 weeks induction therapy of intravenous liposomal amphotericin B and flucytosine followed by fluconazole consolidation and maintenance therapy. Management was concordant with Infectious Diseases Society of America (IDSA) guidelines[4], and achieved a good clinical response.
This case illustrates cryptococcal cellulitis may serve as a portal of entry for disseminated disease or alternatively serve as a marker of disseminated disease. It highlights the need for thorough investigation of immunosuppressed patients with cryptococcal cellulitis for evidence of disseminated disease to enable prompt and effective treatment.
We would like to acknowledge the assistance of Dr. Geoffrey Playford and the Infectious Diseases department at Princess Alexandra Hospital, Brisbane for their assistance with the patients' management
References:
[1] Srivastava G, Tilak R, Yadav J, Bansal M. Cutaneous Cryptococcus: marker for disseminated infection. *BMJ Case Reports* 2015; bcr2015210898.
[2] Horrevorts A, Huysmans F, Koopman R, Meis J. Cellulitis as First Clinical Presentation of Disseminated Cryptococcosis in Renal Transplant Recipients. *Scandinavian Journal of Infectious Diseases* 1994; 26(5): 623-626.
[3] Anderson D, Schmidt C, Goodman J, Pomeroy C. Cryptococcal Disease Presenting as Cellulitis. *Clinical Infectious Diseases* 1992; 14(3): 666-672.
[4] Perfect J, Dismukes W, Dromer F et al. Clinical Practice Guidelines for the Management of Cryptococcal Disease: 2010 Update by the Infectious Diseases Society of America. *Clinical Infectious Diseases* 2010; 50(3): 291-322.

P.1604

Parvovirus B19 associated pure red cell aplasia after renal transplantation

Takashi Fujita[1], Masashi Kato[1], Yasuhito Funahashi[1], Fumitoshi Sakamoto[1], Tsuyoshi Majima[1], Norihisa Matsukawa[1], Takayuki Katsuno[2], Shoichi Maruyama[2], Momokazu Gotoh[1].
[1]Urology, Nagoya University Graduate School of Medicine, Nagoya, Japan; [2]Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Japan.

Parvovirus B19 (PVB19) is a single-strand DNA virus and has a propensity to infect erythroid progenitor cells. Patients receiving immunosuppressive therapy can develop pure red cell aplasia (PRCA). A few reports describe severe anemia as a result of PVB19 infection after renal transplantation. We describe a 58-years-old man with type 2 diabetes mellitus and end-stage renal disease. The patients underwent ABO incompatible living related renal transplantation from his wife. He received rituximab and 4 sessions of plasmapheresis as desensitization therapy. As maintenance immunosuppressive agents, tacrolimus (TAC), mycophenolate mofetil (MMF), prednisolone (PSL), and basiliximab (BXM) were used. Kidney graft immediately functioned and he was discharged with serum creatinine level (S-Cr) 1.27mg/dl. However he required an emergency hospitalization, because his hemoglobin level reduced to 5.8g/dl postoperative day 44. We identified seriously reticulocytopenia, positive cytomegalovirus (CMV) antigen and cytomegalovirus ulcer in the duodenum. We immediately decreased MMF dosage and started antiviral agent. Nonetheless, anemia requiring blood transfusion was persistent. We decided to perform a bone-marrow biopsy to investigate the other causes of anemia. Then PVB19 infection was revealed from bone-marrow biopsy findings. From histological sign of PVB19 infection and PVB19 DNA in peripheral blood, we diagnosed PRCA caused by PVB19. After therapy with IVIG and dosage adjustment of MMF, anemia resolved. Retrospectively, detailed medical interview had revealed that this patient contacted with his grandchild with erythema infectiosum postoperative day 13. PVB19 is a rare but clinically significant infection that causes severe anemia during the posttransplantation period. PVB19 infection should be included in the deferential diagnosis of severe anemia in the posttransplant patients.

References:

[1] Anca V. Florea. Parvovirus B19 infection in the immunocompromised host. 2007; 131:799-804
[2] Albert J. Parvovirus B19 Infection after Transplantation: A Review of 98 case. 2006; 43: 40-48

P.1605

Spectrum of pneumonia in renal transplant recipients in a tertiary care centre

Krishan Gupta[1], Sukanya Govindan[1], Ashish Sharma[2], Ritesh Agarwal[3], Arunaloke Chakrabarti[4].
[1]Department of Nephrology, Post Graduate Institute of Medical Education and Research, Chandigarh, India; [2]Department of Renal Transplant Surgery, Post Graduate Institute of Medical Education and Research, Chandigarh, India; [3]Department of Pulmonary Medicine, Post Graduate Institute of Medical Education and Research, Chandigarh, India; [4]Department of Microbiology, Post Graduate Institute of Medical Education and Research, Chandigarh, India.

Background: Respiratory tract infections are common in solid organ transplant recipients and they carry high morbidity and mortality[1]. Invasive tests are often required for accurate diagnosis of infection and prompt management of patient. Knowledge about clinical and local microbiological spectrum in these patients would help in preventing unfavourable outcome.The present study aimed to study the clinical and microbiological spectrum of severe respiratory tract infections in renal transplant recipients.
Methods: Total of 62 consecutive renal transplant recipients with pneumonia who were included, were admitted to our hospital over 1 year (Nov 2014 to Dec 2015). Investigations including cultures, radiology and invasive tests like bronchoscopy when necessary were done. Clinical findings and microbiological spectrum were analysed and compared with outcomes like duration of hospital stay, mechanical ventilation and death.
Results: The median time of presentation of our patients was 14 months (1-174 months) post transplant. Majority of our patients (79%) were on maintenance immunosuppression with tacrolimus, mycophenolate mofetil (MMF) and steroids. Induction agents were used in 38.7% of patients. Chronic hepatitis C and hepatitis B infections were found in 11.2% and 3.2% of patients. New onset diabetes after transplant (NODAT) was found in 24.2% of patients. Fever and cough were the commonest presenting symptoms. Forty percent of patients had 2nd organ involvement. Diarrhea was the commonest accompanying symptom, found in 11.3% of patients. Hypotension and hypoxia at presentation was seen in 43.5% and 63% of patients. Cytopenias and acute kidney injury were present in 38.7% and 85.5%. Bronchoscopy was done in 43.4% of patients with diagnostic yield of 37%. Fine needle aspiration (FNA) was done in 30% of patients with yield of 57.8%. Fungal infections were the commonest (25.5%) followed by TB (16.1%), bacterial (12.9%) and viral (9.6%) infections. Mixed infections were found in 16.1% of patients. Etiology of infection could not be found in 38.7% in spite of invasive tests. Mortality rate among our patients was 25.8%. It was highest among bacterial infections (50%) followed by viral and fungal infections (33% and 31%). Presence of hypoxia and hypotension at presentation was associated with increased risk of death while use of induction agents, NODAT and AKI did not correlate with death or increased duration of hospital stay.
Conclusions: Pneumonia carries high risk of mortality in renal transplant recipients. Fungal and tubercular infections are found in most patients. Bacterial, fungal and viral infections carry high risk of mortality. In spite of invasive investigations, etiology may not be found in significant number of patients and hence a high index of suspicion may be required to treat such cases.

References:

[1] Fishman JA, Rubin RH. Infection in organ-transplant recipients. N Engl J Med. 1998 Jun 11. 338(24):1741-51

P.1606

Efficacy of valganciclovir for prevention of cytomegalovirus in high–risk renal transplantation

Taiji Hayashi[1], Yoshitaka Saitou[2], Yasunori Mori[3], Kazuhiro Nose[2], Masaaki Imanishi[4], Tsukasa Nishioka[1], Hirotsugu Uemura[2], Takahiro Akiyama[5].
[1]Urology, Kindai University Sakai Hospital, Osaka, Japan; [2]Urology, Kindai University School of Medicine, Osaka, Japan; [3]Urology, Kaizuka Municipal Hospital, Osaka, Japan; [4]Urology, Saiseikai Tondabayashi Hospital, Osaka, Japan; [5]Urology, Sakai Onshinkai Hospital, Osaka, Japan.

Background: Cytomegalovirus(CMV) is a major cause of morbidity and mortality in renal transplantation. The patients at the highest risk for CMV disease are seronegative recipients (R-) of an organ from a seropositive donor (D+). CMV prophylaxis in high risk cases is now widely performed in renal transplantation and has been associated with reduced incidence of CMV disease, mortality, and graft rejection. Previously, we performed Valganciclovir(VGCV) prevention for 3 months in D+/R- transplant patients, but all cases developed a CMV infection within 1 month after the VGCV prevention. Therefore VGCV prevention was extended for 6 months from January 2010.This is a single center, retrospective analysis evaluating the efficacy of 6 months prophylactic regimens of VGCV on the prevalence of CMV disease.
Materials and Methods: VGCV prevention was performed in 9 cases of the D+/R- renal transplantation from January 2010 to December 2014. The average age of the recipient was 31.1 years old (25-44), and a donor was 67.4 years old (61-74). Induction and maintenance immunosuppression were calcineurin inhibitor, mizoribine, steroid, and basiliximab. Patients received oral VGCV 450mg daily for 6 months. The dose was adjusted for renal function. CMV diagnosis was positive if antigenemia (5-10 cells/2×105 PBMC of cutoff) at any time after transplantation.
Results: Average dosage of VGCV was 193mg daily. CMV viremia was occurred in 4cases after prevention therapy. There is only 1 case developed viral enteritis in 1 month later of VGCV prevention. Acute rejection was occurred in 4cases within one year after transplantation. All cases were recovered by steroid pulse treatment. There were no hematologic adverse events during the treatment phase.
Conclusion: This study demonstrates that low dose and 6month VGCV prophylaxis may reduces the incidence of CMV disease and viremia in high-risk renal transplantation.

P.1607

Usefulness of valacyclovir prophylaxis for preventing cytomegalovirus infection after anti-thymocyte globulin treatment as antirejection therapy

Eun Jeong Ko, Ji Hyun Yu, Bun Soon Choi, Cheol Whee Park, Yong-Soo Kim, Chul Woo Yang, Byung Ha Chung.
Transplant research center, Division of Nephrology, Department of Internal Medicine, College of Medicine, Seoul St.Mary's Hospital, The Catholic University of Korea, Seoul, Korea.

Background: Cytomegalovirus (CMV) infection is one of the most critical factor that affect graft and patient survival after kidney transplantation. Anti-thymoglobulin (ATG) treatment for acute T-cell mediated rejection can decrease cellular immunity, which can raise the risk of CMV infection. So re-initiation of CMV prophylaxis or preemptive therapy is usually recommended with ATG treatment. Therefore, Pprophylaxis for CMV infection along ATG treatment is crucial, but the regimen varies among transplant center. We purposed to evaluate the effect of maintenance valacyclovir universal prophylaxis against CMV infection after intravenous ganciclovir injection during anti-thymoglobulin (ATG) administration as anti-rejection therapy.
Methods: We retrospectively analyzed 59 kidney transplant recipients (KTR)patients single center, who receiving ATG treatment for steroid resistant acute rejection as a rejection therapy after kidney transplantation. All patients received intravenous ganciclovir (2.5mg/kg q 12hr) during ATG injection (1.5mg/kg/d) for a week, and serum CMV PCR test was follow-up on D7, 1, 3, 6, 9, and 12 months after ATG injection initiation. Fifteen patients received maintenance valacyclovir prophylaxis (per oral 2g qid) for CMV prophylaxis just after end of the ATG injection belonged to study group (VAL). Prior to July 2013, as previous protocol, hHistory control group (CON, n=34) consists of patients who discontinued antiviral agent just after end of the ATG injection. According to revised protocol since July 2013, 15 patients (the study group, VAL) received maintenance valacyclovir prophylaxis (per oral 2g qid) for CMV prophylaxis just after end of the ATG injection. The primary outcome was incidence of CMV infection. The secondary outcomes were incidence of subsequent acute rejection and graft survival rate.
Results: No significant differences were found among the groups in demographic characteristics at the time of ATG injection. CMV seropositivity status at pre-transplantation or immunosuppressant regimen before ATG treatment also showed no difference. In VAL group, median duration of valacyclovir administration was 25.5 ± 0.7 days. The incidence of CMV infection was lower in VAL group (2/15, 13.3%) compared to CON group (20/34, 58.8%). Prophylaxis with maintenance valacyclovir after ATG treatment reduced the incidence of CMV infection, significantly. (p=0.003). A one-year-CMV-free survival rate was higher in VAL group compared to control group. (80% Vs 38.6%, p=0.009). In CONT group, all CMV infection occurred within first 3 months after ATG treatment, whereas all the case of CMV infection occurred later, after discontinuation of valacyclovir on VAL group. (CONT 34.8 ± 19.2 Vs VAL 101 ± 33.9, p=<0.001). Graft failure also occurred more less in VAL group (5/15, 33.3%), compared to 67.6% (26/34) in CON group (p=0.025). But there was no difference of incidence of subsequent rejection after ATG treatment. (CONT 29.4% (10/34) Vs VAL 26.7% (4/15), p>0.999). There was no difference the incidence of other than CMV infection, repeated rejection, and graft survival.
Conclusion: Valacyclovir maintenance prophylaxis after ATG treatment is effective in reducing CMV infection, also improve CMV-free survival. CMV prophylaxis with valacyclovir after ATG therapy can reduce CMV infection.
References:
[1] Kotton et al *Transplantation*. 2013; 96: 333-360
[2] Lowance et al. *N Engl J Med* 1999;340:1462-70
[3] Reischig et al. *Transplantation* 2005;79:317-324
[4] Reischig et al. *Kidney Blood Press Res* 2005;28:218-225

P.1608

Non-tuberculous mycobacterial infections in transplant patients in Singapore – an emerging cause for concern

Lionel Lum[1], Isaac Low[1], A. Vathsala[1], K.K. Madhavan[2], P.A. Tambyah[1].
[1]Medicine, National University Health System, Singapore, Singapore; [2]Surgery, National University Health System, Singapore, Singapore.

Introduction: Non tuberculous Mycobacterium (NTM) infections in transplant patients are increasingly recognised. These are a diagnostic and therapeutic challenge especially in terms of the drug interactions. We reviewed our consult databases to report a cases series of NTM infections in transplant patients from our 1000 bed tertiary institution.
Materials and Methods: A retrospective analysis of transplant patients with NTM infections from 2008 to 2013 in the National University Health System in Singapore was conducted. We described the demographics of the patients, transplant details, diagnosis of NTM infection, clinical presentation and the treatment instituted as well as the outcomes, in particular, mortality and rejection rates.
Results and Discussion: 4 renal transplant patients and 1 liver transplant patient (four males and one female) were included in the study. The median age was 59 (56-71). All NTM diagnoses were made at least 5 years after transplant, interestingly two after graft loss. 3 of the patients had previous CMV viremia. Two were intra-abdominal and two pulmonary, the last in the hand. The major pathogen identified was M abscessus which was highly resistant to most common antibiotics. Targeted treatment was provided based on susceptibility results and immunosuppression modified. There was 1 death.
Conclusions: NTM infections remain a challenge in Transplant patients. Risk factors, duration of treatment and optimal treatment agents minimizing drug interactions with immunosuppressants are still not known. It is also not clear if the ATS criteria used to diagnose NTM infections in immunocompetent patients can be applied to the transplant population and if NTM infections affect overall mortality or rejection rates. Larger multicenter or multinational studies should be conducted to further answer these questions.

P.1609

Pneumonia in renal transplant patients: A new insight into the risk factor spectra

Anil Mani, Mukut Minz, Ashish Sharma, Sarbpreet Singh, Deepesh B.Kenwar, Sunil Kumar, Nikhil Mahajan.
Renal Transplant Surgery, PGIMER, Chandigarh, India.

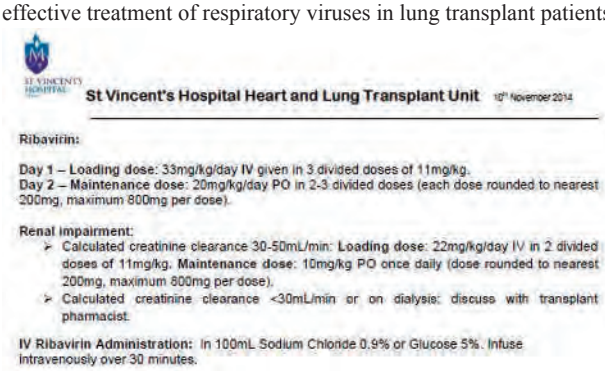
Background: Infections continue to be a major cause of mortality and morbidity in renal transplant recipients, amongst which pulmonary infections are the most dreaded.
Knowledge on infection risk, the temporal relationship between immunosuppression and infectious etiology, as well as the prompt diagnostic pursuit of organism identification together with empiric therapy; are required to reduce the consequences of this complication.
Aim: To study the clinical profile of patients with post transplant pneumonia and to compare risk factors across the case and control groups.
Material and Methods: The study was done during a 6 month period from February 2015 to July 2015 in the Department of Renal Transplant Surgery, PGIMER, Chandigarh. The study cohort included 1513 transplanted patients performed between 2006 and 2015. A total of 25 patients who required admission during the study period for pneumonia and had positive findings on HRCT were taken as cases and was evaluated prospectively. Patients who underwent transplant one day prior and after the cases, served as control and their data was analyzed retrospectively. Their demographic and clinical profiles were compared and were statistically analyzed. The standard immunosuppression protocol included tacrolimus/MMF/Steroids. Unrelated patients selectively received induction therapy with either ATG (3- 3.5 mg/kg given in 3 divided doses) or Basiliximab (20mg on POD0 and POD4) based on risk stratification.
Results: There were 25 patients (Mean age 40.52 yrs M:F 21:4) who presented after 25.28 ± 29.62 (range 1 to 105) months after transplant. In the control group of 50 pateints mean age was 39.18 yrs (M:F 43:7). 80 %age of patients in case group and 86% in control group were on tacrolimus/MMF. Fungal pneumonia 8/25 (32%) and TB pneumonia 8/25 (32%) were the most common pathologies. Bacterial pathogens were isolated in 7 (28%) cases. In 3 cases (12%) no definitive diagnosis could be made. CMV was detected in 2 cases and PCP was isolated in 1 case. 3 patients had multiple infections. 28% in the case group and 22% in the control group received induction immunosuppression. Hepatitis, PTDM and induction therapy (p-.56) was not associated with increased risk. Diabetic nephropathy (p-.003) and treatment of acute rejection (p-.001) conferred a risk with likelihood ratio of 8.9 and 10.6 respectively. Overall mortality was 36%. Fungal pneumonia had the maximum mortality (44%). 64% had favorable outcome.
Conclusion: Diabetic Nephropathy as basic disease and acute rejection during post transplant period results in higher incidence of post transplant pneumonia. Wider use of induction therapy can reduce incidence of acute rejection, which can paradoxically help in preventing pneumonias in transplant patients.

P.1610

A predictive pharmacokinetic model of ribavirin plasma concentration in lung transplant recipients with active respiratory syncytial virus or human metapneumovirus infection

Eliza Milliken[1,2], Stephanie Reuter Lange [2], Deborah Marriott[2], Ana Schteinman[2], Indy Sandaradura[2], Lilibeth Carlos[1,2], Fay Burrows [1,2], Allan Glanville[1,2].
[1]Faculty of Medicine, The University of Notre Dame, Sydney, Australia; [2]St Vincent's Hospital, Sydney, Australia; [3]Lung Transplant and Thoracic Medicine, St Vincent's Hospital, Sydney, Australia.

RBV is a guanosine analogue used for treatment of respiratory syncytial virus (RSV) pneumonitis in vulnerable populations[1]. RBV is standard of care for RSV in global lung transplant (LTx) centres to prevent acute pneumonitis and BOS[2]-[12]. Despite this the pharmacokinetic profile of RBV in the transplant population is unknown and is likely to be altered due to immunosuppressant regimens, renal and hepatic impairment and cystic fibrosis in affected individuals. At St Vincent's Hospital, the empirically-determined treatment protocol for ribavirin in lung transplant patients is three initial IV loading doses (11mg/kg tds) followed by maintenance dosing with oral ribavirin (10mg/kg bd); however, whilst the treatment protocol has demonstrated efficacy, the ribavirin exposure for the two treatment formulations has not been established.[5] To examine the pharmacokinetics of oral and IV ribavirin in the lung transplant population, a prospective, single-centre cohort study was conducted. Twelve lung transplant patients with PCR-confirmed RSV or HMPV infection were recruited for study participation (7 male/5 female; Age: 45.3 ± 13.9 years; BMI: 23.8 ± 4.82 kg/m2; CrCL: 69.2 ± 26.7mL/min). Patients were administered ribavirin according to the standard hospital protocol, and blood samples were collected throughout the IV and oral treatment periods. Plasma ribavirin concentrations were quantified using a validated HPLC-UV analytical method. Patient concentration-time data, combined with previously published ribavirin pharmacokinetic data, were used to develop a population pharmacokinetic model, using NONMEM® VII software, incorporating inter-individual and residual unexplained variability. Patient factors contributing to parameter variability (such as renal function, patient status, body weight) were modelled using standard forward-inclusion/backward-deletion methods. Model selection was based on the objective function value and standard diagnostic plots. The developed model was then used to conduct Monte Carlo simulations examine alternate dosing regimens, in particular if comparable drug exposure can be achieved with oral only dosing regimens as opposed to mixed regimens, thereby reducing patient bed days and increasing convenience. Additional research is needed to establish effective plasma ribavirin concentrations for viral eradication; however, it is anticipated that the developed pharmacokinetic model will allow predictions of optimal dosing regimens to meet therapeutic pharmacokinetic/pharmacodynamic endpoints as more is learned about effective treatment of respiratory viruses in lung transplant patients.


St Vincent's Hospital Heart and Lung Transplant Unit 10th November 2014

Ribavirin:

Day 1 – Loading dose: 33mg/kg/day IV given in 3 divided doses of 11mg/kg.
Day 2 – Maintenance dose: 20mg/kg/day PO in 2-3 divided doses (each dose rounded to nearest 200mg, maximum 800mg per dose).

Renal impairment:
- Calculated creatinine clearance 30-50mL/min: **Loading dose**: 22mg/kg/day IV in 2 divided doses of 11mg/kg. **Maintenance dose**: 10mg/kg PO once daily (dose rounded to nearest 200mg, maximum 800mg per dose).
- Calculated creatinine clearance <30mL/min or on dialysis: discuss with transplant pharmacist.

IV Ribavirin Administration: In 100mL Sodium Chloride 0.9% or Glucose 5%. Infuse intravenously over 30 minutes.

Dr Nisha Menon; Dr Cara Platts ; Assoc. Professor Ross Norris Scientific Head SydPath ; Professor Richard Day AM MD FRACP

References:

[1] Barr, F. et. al. Treatment of Respiratory Syncytial Virus, UpToDate, http://www.uptodate.com/contents/respiratory-syncytial-virus-infection-treatment?source=search_result&search=RSV+ribavirin&selectedTitle=1~150#H10Jul 28 2015.
[2] Burrows, F. et al. Department of Microbiology and Clinical Infectious Diseases, St Vincent' s Hospital, Sydney, Australia;Lung Transplant Unit, St Vincent' s Hospital, Sydney, Australia. Oral Ribavirin Is a Cost-Effective Alternative to Intravenous Ribavirin for Respiratory Syncytial Virus (RSV) Infection after Lung Transplantation. Abstract Presentation. ISHLT Conference 2013.
[3] Danziger-Isakov, L. et. al. RSV prevention and treatment in pediatric lung transplant patients: A survey of current practices among the International Pediatric Lung Transplant Collaborative Pediatric Transplantation16:638-644, 2012.
[4] Fuehner, T. et. al. Single-centre experience with oral ribavirin in lung transplant receipients with paromyxovirus infections.
[5] Glanville, etl. Al. Intravenous Ribavirin is a safe and cost effective treatment for respiratory syncytial virus infection after lung transplantation. The Journal of Heart and Lung Transplantation, Vol.24, Number 12. 2005
[6] Grayson, et. al. Kucer' s The Use of Antibiotics, Sixth Edition. CRC Press, 2010. Transplant website.
[7] Hynicka L, Ensor C. Prophylaxis and Treatment of Respiratory Syncytial Virus in Adult Immunocompromised Patients The Annals of Pharmacotherpay. 558-566, Vol. 46, 2012.
[8] Lily Li, BA,a Robin Avery, MD,b Marie Budev, DO, MPH,c Sherif Mossad, MD,b and Lara Danziger-Isakov, MD, MPHd. Oral versus inhaled ribavirin therapy for respiratory syncytial virus infection after lung transplantation. The Journal of Heart and Lung Transplantation, Vol 31, No 8, August 2012.
[9] Liu V. G.S. Dhillon, D. Weill. A multi-drug regimen for respiratory syncytial virus and parain£uenza virus infections in adult lung and heart/lung transplant recipients. Transplant Infectious Disease12:38-44
[10] McCurdy L, MD,a Aaron Milstone, MD,b,c and Stephen Dummer, MDa,c Clinical Features and Outcomes of Paramyxoviral Infection in Lung Transplant Recipients Treated With Ribavirin. The Journal of Heart and Lung Transplantation 1053-2498, July 2003
[11] Pelaez A, MD,a,e G. Marshall Lyon, MD,b Seth D. Force, MD,c,e Allan M. Ramirez, MD,a,e David C. Neujahr, MD,a,e Marianne Foster, NP,e Priyumvada M. Naik, MD,a Anthony A. Gal, MD,d Patrick O. Mitchell, MD,a and E.Clinton Lawrence, MDa,e Efficacy of Oral Ribavirin in Lung Transplant Patients With Respiratory Syncytial Virus Lower Respiratory Tract Infection, The Journal of Heart and Lung Transplantation. Vol 28, No 1. January 2009.
[12] Plit, M. et. al. Specific Community Acquired Virus Infections and the Risk of Bronchiolitis Obliterans Syndrome after Lung Transplantation. St Vincent' s Hospital Sydney. Submitted for Publication, 2015.

P.1611

Antibiotic prophylaxis for ureteral stent removal after kidney transplantation: to use, or not to use, that's the question

Jennifer Lee[1], Esther Liu[1], Mohamad Alkadi[3], John Lee[3], Anthony Watkins[2], Jim Kim[2], Jun Lee[3], Darshana Dadhania[3], Choli Hartono[3], David Serur[3], Meredith Aull[2], Sandip Kapur[2], Manikkam Suthanthiran[3], Thangamani Muthukumar[3].
[1]Department of Pharmacy, Weill Cornell Medicine, New York, United States; [2]Department of Surgery, Division of Transplantation, Weill Cornell Medicine, New York, United States; [3]Department of Medicine and Transplantation Medicine, Weill Cornell Medicine, New York, United States.

Introduction: There are no recommendations for antibiotic prophylaxis prior to removing ureteral stents placed at the time of kidney transplantation. At our center, all patients who tolerate sulfamethoxazole/trimethoprim (SMX/TMP) prophylaxis receive it for one year, but the decision for additional antibiotics prior to stent removal is physician dependent. The purpose of our observational study was to determine whether antibiotic prophylaxis prior to ureteral stent removal is associated with reduced incidence of bacterial urinary tract infections (UTI).
Methods: We defined UTI associated with stent removal as the first episode of urine culture with ≥30,000 cfu/mL within 4 weeks of stent removal regardless of the symptoms. We reviewed the charts of 120 consecutive adult kidney transplant recipients with ureteral stent placement between 01/2015-07/2015 period. All patients had bacterial culture of urine done during follow up visits prior to or after ureteral stent removal. The primary outcome measure was the incidence of UTI in patients who received (+) and who didn't receive (-) antibiotics prophylaxis.
Results: Forty two of the 120 patients (35%) received antibiotic prophylaxis prior to stent removal. The two groups, Antibiotic (+) and Antibiotic (-), were similar in demographic characteristics. The incidence of UTI associated with stent removal was 21% (UTI prior to stent removal: 27%, P=0.36).
There was no statistically significant difference in the incidence of UTI between Antibiotic (+) and Antibiotic (-) groups.

Variables	Prophylactic Antibiotic Prior to Ureteral Stent Removal		
	Antibiotic + N=42 (35%)	Antibiotic - N=78 (65%)	P
Age of transplant recipient, years, mean ± SD	52 ± 13	53 ± 14	0.65
Female	19 (45)	51 (65)	0.11
Black	7 (17)	16 (21)	0.81
Prior transplant	3 (7)	7 (9)	0.99
Thymoglobulin induction	35 (83)	65 (83)	0.99
Corticosteroids maintenance	11 (26)	16 (21)	0.50
UTI prior to stent removal	10 (24)	22 (28)	0.67
UTI associated with stent removal	**10 (24)**	**15 (20)**	**0.64**
Days from transplant to stent removal, mean ± SD	41 ± 5	44 ± 6	0.03
Days from stent removal to urine culture, mean ± SD	5.6 ± 3.2	5.9 ± 4.6	0.77
Type of bacteria			
E. Coli	2 (20)	6 (40)	0.40
Klebsiella spp.	2 (20)	1 (7)	0.54
Enterococcus spp.	3 (30)	6 (40)	0.69
Other	3 (30)	2 (13)	0.36
Treated UTI	7 (70)	9 (60)	0.66
Required hospitalization	2 (20)	1 (7)	0.54
SMX/TMP prophylaxis at the time of stent removal	13 (31)	57 (73)	0.0001
	SMX/TMP + N=70 (58)	SMX/TMP - N=50 (42)	
	Antibiotic + N=13 (19) / Antibiotic - N=57 (81)	Antibiotic + N=29 (58) / Antibiotic - N=21 (42)	
UTI associated with stent removal	2 (15) / 8 (14)	8 (27) / 7 (34)	
	P=0.99	P=0.76	

All values expressed as n (%) unless otherwise noted

For patients who were already on SMX/TMP prophylaxis (n=70, 58%), UTI associated with stent removal was not statistically different between the two groups (15% vs. 14%, P=0.99). For those not on SMX/TMP prophylaxis (N=50, 42%), UTI was higher in the Antibiotic (-) group (34% vs. 27%), but this difference was not statistically significant (P=0.76).
Conclusion: There is no benefit to antibiotic prophylaxis prior to ureteral stent removal in transplant recipients already on SMX/TMP prophylaxis. Routine use of antibiotic prophylaxis prior to ureteral stent removal is not needed.

P.1612

Furunculosis and its complications: a cause of morbidity in renal transplant recipients?

Asma Naseem[1], Sunil Kumar[1], Anwar Naqvi[2].
[1]Infectious Diseases, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [2]Urology and Transplantation, Sindh Institute of Urology and Transplantation, Karachi, Pakistan.

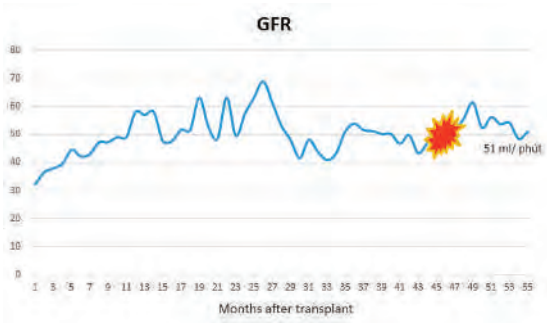
Introduction: Infectious skin diseases are common in Pakistan. Furunculosis is invariably caused by Staphylococcus aureus. Furunculosis and recurrent furunculosis is associated with poor personal hygiene and overcrowded living conditions. The renal transplant population at our center is mainly from lower socioeconomic strata. Furunculosis and its complications like bacteremia and recurrent furunculosis may be common in this population and may lead to increase morbidity, mainly because of their compromised immune status. Objectives: To find out the frequency of furunculosis, its complications and their effect on morbidity in renal transplant recipients.
Methods: Patients were identified from microbiology laboratory data during the period of January 2014 to December 2014. All patients with isolation of Staphylococcus aureus from skin and soft tissue infections were included. Patients' records were reviewed regarding history of furunculosis, its recurrence and complications.
Results: A total of 30 patients presented with staphylococcal aureus skin and soft tissue infection during the study period. There were 24 (80%) were males and 6 were females (20%). Gluteal abscesses (26.6%) and multiple boils all over the body (23.3%) were the most common presentation. S.aureus bacteremia associated with furunculosis was seen in 4 (13.3%) of the patients. Recurrent furunculosis was observed in 12 (40%) of the patients.
Conclusion: Furunculosis and its complications are observed in transplant recipients. A considerable number of patients develop bacteremia with staphylococcus aureus, leading to hospital stay and intravenous antibiotics. Recurrent furunculosis is common in this population which can cause increase morbidity.

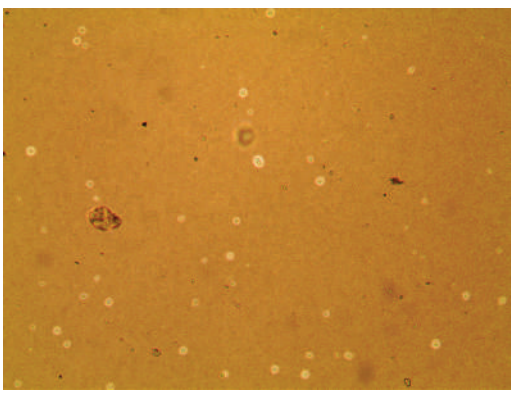
P.1613

Cryptococcal meningitis after kidney transplantation: Two cases report

Hien Nguyen[1], Tan Mai Viet Nhat[1], Thong Dang Vu[2], Hung Le Quoc[3], Chuan Hoang Khac[1], Thu Du Thi Ngoc[1], Sam Thai Minh[1], Sinh Tran Ngoc[1].
[1]The Urology and kidney transplantation, Cho Ray hospital, Ho Chi Minh, Viet Nam; [2]The Respiratory, Cho Ray hospital, Ho Chi Minh, Viet Nam; [3]The Tropical diseases, Cho Ray hospital, Ho Chi Minh, Viet Nam.

Objective: Cryptococcal meningitis is an opportunistic infection mainly occurring in immunocompromised hosts. In general, the most common fungal infection in kidney transplant patients is candidiasis, followed by aspergillosis and cryptococcosis. The infections largely result from inhaling the spores of fungi or yeasts.
Materials and Methods: From 1994 to 2015, there were two cryptococcal meningitis cases diagnosed at Cho Ray hospital, in which more than 700 kidney transplant patients have being followed, thanks to positive cerebrospinal fluid culture, approximately 40 months after transplantation. Treatment strategies included antifungal agents, lowering immunosuppressive medicines, together with enhancing patients' status.
Results: Two patients had been discharged from the hospital healthily. Moreover, five months later, the first patient died of severe pneumonia. The second patient lives with her healthy transplanted kidney.
Conclusions: Not only does cryptococcal meningitis show atypical or minor symptoms regarding headache or blurred vision, but it may advance severely and fatally. Therefore, it is vital to understand that cryptoccocal meningitis in renal transplant patients need to be diagnosed as early as possible so as to offer them the proper treatment. Healthy patient and well-functioning transplanted kidney act as a catalyst for evaluation a successful treatment strategy.
Keywords: Cryptococcal meningitis, MPA: mycophenolic acid, MIT: maintenance immunosuppressive therapy, Kidney transplantation.





Du Thi Ngoc Thu; Thai Minh Sam; Tran Ngoc Sinh

References:

[1] AU Franzot SP. J Clin Microbiol. 1999;37(3):838
[2] Beyt BEJ. N Engl J Med 1978; 298:825–6
[3] Dromer F. AIDS 2004; 18:555–62
[4] Garcia-Hermoso D. J Clin Microbiol 1999; 37:3204–9
[5] Haugen RK. Am J Clin Pathol 1954; 37: 1381–90
[6] Husain C. Emerg Infect Dis 2001; 7:375–381
[7] Lortholary O. AIDS 2005;19:1043–1049
[8] Nina S. Immunocompromised Hosts • CID 2008:47
[9] Nosanchuk JD. Ann Intern Med 2000; 132:205–8
[10] Ooi HS. Transplantation 1971; 11:428–9
[11] Silveira EP. Transplant Infect Dis 2007;9:22–27
[12] Singh N. Clin Infect Dis 2005;40:1756–1761
[13] Vilchez R. Am J Transplant 2002; 2:575–80

P.1614

The effect of direct acting antiviral medications in kidney transplant recipients with hepatitis C virus: A case report

Chiaki Kawabata[1], Satoshi Hamanoue[1], Mariko Toyoda[1], Yuji Hidaka[2], Shigeyoshi Yamanaga[2], Soichi Uekihara[1], Seishi I[3], Hiroshi Yokomizo[2], Sho Nishida.
[1]Internal Medicine, Japanese Red Cross Kumamoto Hospital, Kumamoto, Japan; [2]General surgery, Japanese Red Cross Kumamoto Hospital, Kumamoto, Japan; [3]Emergency, Japanese Red Cross Kumamoto Hospital, Kumamoto, Japan.

Introduction: Chronic Hepatitis C virus (HCV) decreases allograft and patient survival rates in kidney transplant recipients. Until recent years, we did not have treatments free of interferon (IFN), which has risks of allograft rejection. IFN-free therapy with direct acting antivirals (DAAs) in the last few years have less frequent and less severe adverse effects, shorter durations of therapy, and higher cure rates. We report herein on a case where the patient achieved a sustained virologic response (SVR) due to DAAs.
Case: A 67-year-old female received a kidney transplant from a deceased donor in 2003. She had already been infected with HCV at this time. She was diagnosed with HCV (Genotype 1) and started on a therapy of Daclatasvir and Asunaprevir in June 2015. HCV-RNA was negative two weeks later. She developed cough, palpitation, edema of the limbs, her creatinine level (Cr 1.1mg/dl) slightly deteriorated (Cr 1.6mg/dl) and she had mild ascites. We were able to manage her over-hydration with diuretics. Seven months have passed since the DAAs therapy regimen started, the patient has maintained an SVR and preserved a relatively normal creatinine level.
Discussion: Previous IFN-based regimens limited HCV treatment to pre-transplantation, and resulted in only a minority of eligible patients for the therapy; moreover, poor tolerability often resulted and only a small proportion of patients were able to complete the treatment and thus achieve SVR. Recent studies show that liver transplant recipients can be safely and effectively treated with the new DAA regimens. There is still little data on DAA regimens for kidney transplant recipients. We consider this case is precious in this new era.
Conclusion: DAAs can attain early SVR of HCV in kidney transplant recipients but creatinine levels may require monitoring.

P.1615

Chikungunya infection in a kidney transplant recipiente: A case report

Ligia Pierrotti[1,2], Odeli Nicole E.Sejas[1], Ana Patricia Nascimento[2], Elias E.David-Neto[3], Luis Sérgio L.S.Azevedo[3].
[1]Infectious Diseases Division, Hospital das Clínicas, School of Medicine, University of São Paulo, São Paulo, Brazil; [2]Clinical Research Center, DASA Medicina Diagnóstica, São Paulo, Brazil; [3]Kidney Transplantation Service, Hospital das Clínicas, School of Medicine, University of São Paulo, São Paulo, Brazil.

Introduction: Chikungunya virus (CHIKV) is a mosquito-borne alphavirus transmitted to humans by Aedes mosquitoes, the same vectors that also transmit dengue and zika virus. Since December 2013 local transmission of CHIKV has been reported in several regions of Caribe and Americas. In Brazil the first two autochthonous CHIKV infection cases were reported in Sept 2013; since them, more than 20,000 suspected cases have been reported, mostly in the Northeast region.
CHIKV infection is most often symptomatic, characterized by acute onset of fever and polyarthralgia, sometimes associated with rash, ocular pain, and myalgia. Arthralgia can be severe and debilitating and can persist for months. Risk of severe atypical presentation is related to older age and presence of comorbidities. Mortality is rare.
Case Presentation: A 41-year-old Brazilian woman, affected by end stage renal disease secondary to systemic arterial hypertension, received living donor kidney transplantation on March 2006. The patient had an uneventful post-transplant course and was receiving prednisone (5 mg/day), tacrolimus (2 mg every 12 h) and azathioprine (100 mg/day), with normal renal function.
On January 27th, 2016, she presented with severe joint pain on coxofemoral joint, with difficult waking because of the pain. The migratory arthralgia has started 20 days before, involving different joints (including bilateral knees, ankles, and hands). At the beginning of the picture she also presented with ocular hyperemia, myalgia, and a discrete rash with pruritus, lasting about 4 days. She denied fever and any other symptoms.
The patient lives in São Paulo, Brazil, but she was visiting her relatives in Paraiba, the Northeast of Brazil, when she got sick; she reported many people there with similar symptoms. She reported mosquito bites during her travel and presence of mosquito like Aedes in Paraíba.
Physical examination showed discrete inflammation signs in both ankles with edema and tenderness. Laboratory tests showed a normal hemogram, creatinine of 0.89 mg/dL, C-reactive protein of 7.8 mg/dL, and normal liver enzymes. CHIKV immunoglobulin (Ig)M and IgG using enzyme-linked immunosorbent assay (ELISA) performed on DASA Medicina Diagnóstica Laboratory confirmed the diagnosis of CHIKV infection ; dengue virus IgM and IgG serology was negative.
Prednisone dose was increased to 20 mg/day with resolution of the join pain complaints in about 3 days. One month later the patient remains stable and the higher dose of prednisone (20 mg/day) should be keep during the next 6 months to avoid future joint pain relapses.
Discussion and Conclusion: To the best of our knowledge, this is the second case of CHIK infection in kidney transplant recipient. This case and the other previously reported had a favorable evolution. Treatment is palliative and includes anti-inflammatory drugs as prednisone to control joint pains. The recommendation to prevent arboviroses is avoid mosquito bites.
Clinicians need maintain a higher clinical suspicion for arboviroses in tropical regions where Aedes mosquitoes are largely distributed, including CHIKV, dengue, and zika virus infections. CHIKV infection should be considered in patients with acute onset of fever and polyarthralgia.
References:
[1] Dalla Gasperina D, Balsamo ML, Garavaglia SD, Rovida F, Baldanti F, Grossi PA. Chikungunya infection in a human

immunodeficiency virus-infected kidney transplant recipient returning to Italy from the Dominican Republic. Transpl Infect Dis. 2015 Dec;17(6):876-9.
[2] Azevedo Rdo S, Oliveira CS, Vasconcelos PF. Chikungunya risk for Brazil. Rev Saude Publica. 2015;49:58. doi: 10.1590/S0034-8910.2015049006219. Epub 2015 Sep 18. English, Portuguese.
[3] Nunes MR, Faria NR, de Vasconcelos JM, Golding N, Kraemer MU, de Oliveira LF, Azevedo Rdo S, da Silva DE, da Silva EV, da Silva SP, Carvalho VL, Coelho GE, Cruz AC, Rodrigues SG, Vianez JL Jr, Nunes BT, Cardoso JF, Tesh RB, Hay SI, Pybus OG, Vasconcelos PF. Emergence and potential for spread of Chikungunya virus in Brazil. BMC Med. 2015 Apr 30;13:102.

P.1616

Pre-emptive screening and management of BK polyoma virus in renal transplant recipients

Joyce Popoola[1], Mitul Patel[1], David Carrington[3], Michael Lapsley[2]. [1]Department of Nephrology and Transplantation, St George's University Hospitals Foundation NHS Trust, London, United Kingdom; [2]Pathology Services, Epsom and St Helier University Hospital NHS Trust, Carshalton, United Kingdom; [3]Department of Virology, St George's University Hospitals Foundation NHS Trust, London, United Kingdom.

BK polyomavrus nephropathy is an important cause of renal transplant dysfunction. Its incidence has grown with increased immunosuppresant regimen potency, awareness and improved reliability of testing methods. Treatment remains variable with minimal evidence base from validated studies. A survey carried out in the London transplant units to identify patients with BK Nephropathy and their outcomes between 2004-2005 showed over 60% of cases had significant graft dysfunction and a graft loss and only 50% managed to clear the virus. Noting the incidence and significant morbidity to grafts we have adopted routine screening for BK Nephropathy on our renal transplant recipients by SV40 large T antigen staining on protocol biopsies at 3 months and on all for cause biopsies carried out for transplant dysfunction between 2008 and 2015. Transplant recipients transplanted at our centre during this period were 893..Protocol biopsy uptake varied 77.5 - 96%.The immunosuppressant regimens at time of diagnosis were mycophenolate/tacrolimus for low immunological risk and with steroids for high immunological risk recipients. Sixteen cases had SV40 staining on biopsy. Our immunosuppressant protocol supports conversion from mycophenolate to azathioprine at 3 months and dropping tacrolimus concentrations from 8-12 ng/ml to 5-8 ng/ml reducing the overall immunosuppressant burden.Ten cases were diagnosed with BK nephropathy and viraemia. On diagnosis mycophenolate was stopped in all these patients and tacrolimus levels were allowed to fall closer to 5, and ciprofloxacin was started. The viral load was monitored until it became persistently absent over 3 months. Four patients with BK Nephropathy and rejection like appearance (C4d staining and cellular infiltration) started Leflunomide A77 1726 in addition due to its immunosuppressant/anti-viral properties.No graft lost so far from 9 cases with significant viraemia >10,000, 8 have cleared the BK viraemia. Recipient with persisting viraemia was treated for a lymphoma in the early post transplant period. Cidofovir added due to refractory Leflunomide response. Viral load has reduced from 1.1 million to 53,000 BKV DNA copies/ml. Cidofovir has been associated with initial further graft dysfunction. Latest creatinine 329micromols/L (3.72mg/dL). Creatinine range for other treated recipients (72-167 micromols/L) mean 122.25 micomols/L (1.39mg/dL). BK diagnosis/treatment requires further research in order to develop appropriate management protocols. We advocate a prospective randomised multinational study to answer questions of valuation of role of protocol biopsies, immunosuppression protocols, immune monitoring of the BK viral-specific and alloimmune response, also development of targeted and validated treatment protocols. Additionally, donor pretransplant BK virus serotyping prior to transplant to enable stratification for risk of developing BK nephropathy and tailoring immunosuppressant regimen in a planned fashion.
South Thames Network Transplant Team

References:

[1] BK virus Nephropathy in Renal Transplant Patients in London. LH White et al. Transplantation 2008; 85: 1008-15

P.1617

Profile of infections in renal transplant recipients in a tertiary care hospital from India

Sriram Sriperumbuduri, Karthik Kalidindi, Swarnalatha Guditi, Gangadhar Taduri.
Nephrology, Nizam`s Institute of Medical Sciences, Hyderabad, India.

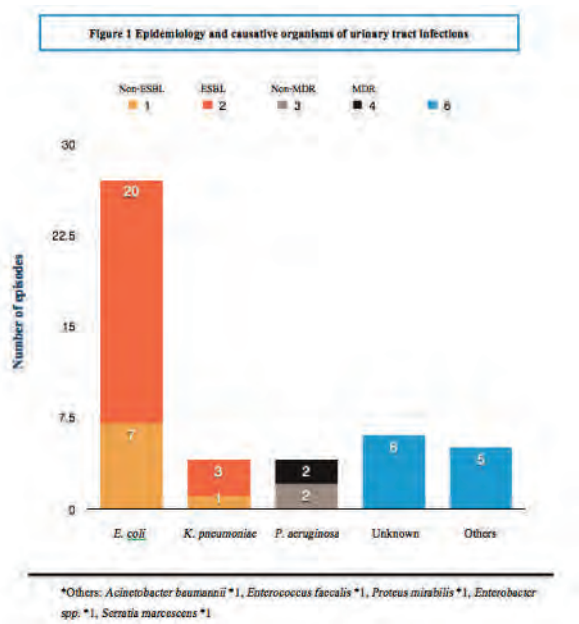
Introduction: Infections are an important cause of morbidity and mortality in renal transplant recipients. This retrospective study was done to study the infection profile in renal transplant recipients and analyze possible risk factors.
Materials and Methods: A total of 72 patients who underwent renal transplant surgery at a tertiary care centre in India between January 2010 and June 2014 were studied. Infections were analyzed in terms of incidence, time of onset after transplant, clinical presentation, diagnosis, graft dysfunction and patient and graft survival. Risk factors analyzed were diabetic status of patient (pre-existing diabetes and new onset diabetes after transplantation- NODAT), induction therapy, anti rejection therapy and high serum Tacrolimus levels. Statistical analysis was done with chi square test and Pearson correlation coefficient.
Results and Discussion: A total of 149 infection episodes were documented in the 72 patients studied. Majority (82.2 %) were males and females constituted 17.8 %. Predominant age group involved was 21-30 years (35 %). Most common donor was mother (31.5%). Induction therapy was given in 21(30%) patients. Most common infections were those of urinary tract (34.2%), followed by viral (28.8%), sepsis (11.4 %), mycobacterial (10%), fungal (6%) and pneumonia (4%). Parasitic infections (Giardiasis and Strongyloides hyperinfection syndrome) occurred in 2(1.4 %) patients. Cytomegalovirus accounted for 12.7% and BK virus for 5.3% of total infections. Majority (54 %) of the infection episodes occurred in the first 3 months of transplantation. There were 63(42.3%) episodes of graft dysfunction and 3 patients had graft failure. A total of 9 deaths were recorded due to these infections. Risk factor analysis revealed significant association of infections with anti-rejection therapy ($p=0.008$, 95% CI ranges from 0.24 to 0.46 & $r=0.9543$) and diabetic status ($p=0.0125$; 95% CI ranges from 0.29 to 0.51).Induction therapy and high serum tacrolimus levels did not have a significant ($p>0.05$) association with infections.
Conclusion: Urinary tract is the most common source of infection in renal transplant recipients, followed by viral infections. Majority of the episodes occurred in first 3 months of transplantation. Anti rejection therapy and diabetic status of patients are significant risk factors associated with infections.
Dr. Ramakanth for helping with biostatistics

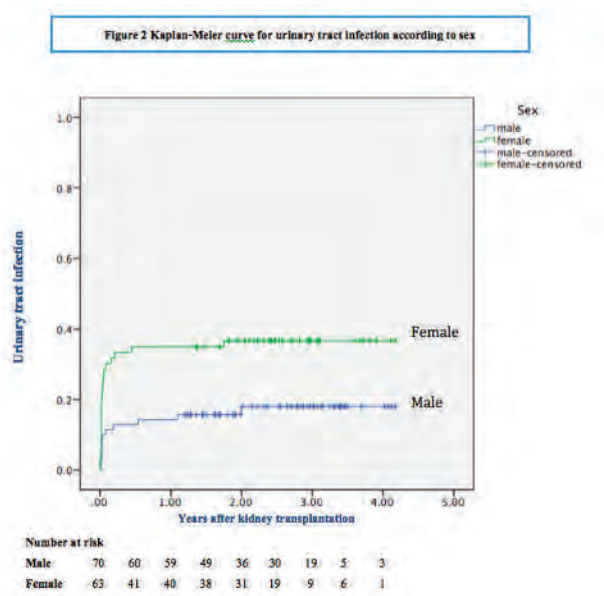
P.1618

Urinary tract infection in kidney transplant recipients in King Chulalongkorn Memorial Hospital

Sarita Thawanaphong[1], Leilani Paitoonpong[2], Kamonwan Jutivorakool[2], Yingyos Avihingsanon[3], Kearkiat Praditpornsilpa[3], Natavudh Townamchai[3].
[1]Department of medicine, Faculty of medicine, Chulalongkorn University, Bangkok, Thailand; [2]Division of infectious disease, Department of medicine, Faculty of medicine, Chulalongkorn University, Bangkok, Thailand; [3]Division of nephrology, Department of medicine, Faculty of medicine, Chulalongkorn University, Bangkok, Thailand.

Background: Urinary tract infection (UTI) is the most common infection in kidney transplant (KT) patients. But the incidence and causative agents are still unknown in Thailand. This study aims to investigate incidence, common infectious agents and risk factor of urinary tract infection in kidney transplant patient
Method: This is a single-center retrospective descriptive study at a tertiary referral hospital. Medical records of patients who underwent kidney transplant from January 2012 to December 2014 were reviewed. Full protocol was approved by Institutional Review Board. Statistical analysis with descriptive statistics, Kaplan-Meier estimation and Cox regression analysis were performed by SPSS version 22.0 software.
Result: Total of 134 patients were included in study, one was excluded due to primary non-function of renal allograft. There were 43 episodes (32.33%) of UTI in 35 patients (26.32%). Microbiological isolation was confirmed in 37 episodes. The most frequent organisms were ESBL-*Escherichia coli* 20 episodes (43.48%), non–ESBL *E. coli* 7 episodes (15.22%) and ESBL-*Klebsiella pneumoniae* 3 episodes (6.52%). The others were *Pseudomonas aeruginosa*, non-ESBL *K. pneumoniae*, *Acinetobacter baumannii*, *Enterococcus faecalis*, *Proteus mirabilis*, Enterobacter spp., *Serratia marcescens*. Septicemia rate was 10.53% (14 episodes). Neither graft loss nor death was occurred. Risk factor for UTI is female sex (Hazard ratio 2.457, 95% confidence interval [CI] 1.222-4.940, $p = 0.012$).





Conclusion: Incidence rate of UTI in KT patients was 32.33% which most of them occurred in the first six months after transplantation. ESBL-*E. coli* is the most common pathogen. Risk factor from this study is only female sex. There was no negative impact on graft function or survival rate.

Keywords: Urinary tract infection, kidney transplant, renal transplant, epidemiology, incidence, risk factor

References:

[1] Ito K, Goto N, Futamura K, Okada M, Yamamoto T, Tsujita M, et al. Death and kidney allograft dysfunction after bacteremia. Clin Exp Nephrol. 2015.
[2] Alangaden GJ, Thyagarajan R, Gruber SA, Morawski K, Garnick J, El-Amm JM, et al. Infectious complications after kidney transplantation: current epidemiology and associated risk factors. Clin Transplant. 2006;20(4):401-9.
[3] de Souza RM, Olsburgh J. Urinary tract infection in the renal transplant patient. Nat Clin Pract Nephrol. 2008;4(5):252-64.
[4] Abbott KC, Swanson SJ, Richter ER, Bohen EM, Agodoa LY, Peters TG, et al. Late urinary tract infection after renal transplantation in the United States. Am J Kidney Dis. 2004;44(2):353-62.
[5] Bodro M, Sanclemente G, Lipperheide I, Allali M, Marco F, Bosch J, et al. Impact of urinary tract infections on short-term kidney graft outcome. Clin Microbiol Infect. 2015.
[6] Espinar MJ, Miranda IM, Costa-de-Oliveira S, Rocha R, Rodrigues AG, Pina-Vaz C. Urinary Tract Infections in Kidney Transplant Patients Due to Escherichia coli and Klebsiella pneumoniae-Producing Extended-Spectrum β-Lactamases: Risk Factors and Molecular Epidemiology. PLoS One. 2015;10(8):e0134737.
[7] Noppakun K, Ingsathit A, Pongskul C, Premasthian N, Avihingsanon Y, Lumpaopong A, et al. A 25-year experience of kidney transplantation in Thailand: report from the Thai Transplant Registry. Nephrology (Carlton). 2015;20(3):177-83.
[8] Valera B, Gentil MA, Cabello V, Fijo J, Cordero E, Cisneros JM. Epidemiology of urinary infections in renal transplant recipients. Transplant Proc. 2006;38(8):2414-5.
[9] Senger SS, Arslan H, Azap OK, Timurkaynak F, Cağir U, Haberal M. Urinary tract infections in renal transplant recipients. Transplant Proc. 2007;39(4):1016-7.
[10] Lee JR, Bang H, Dadhania D, Hartono C, Aull MJ, Satlin M, et al. Independent risk factors for urinary tract infection and for subsequent bacteremia or acute cellular rejection: a single-center report of 1166 kidney allograft recipients. Transplantation. 2013;96(8):732-8.
[11] Camargo LF, Esteves AB, Ulisses LR, Rivelli GG, Mazzali M. Urinary tract infection in renal transplant recipients: incidence, risk factors, and impact on graft function. Transplant Proc. 2014;46(6):1757-9.
[12] Hooton TM, Bradley SF, Cardenas DD, Colgan R, Geerlings SE, Rice JC, et al. Diagnosis, prevention, and treatment of catheter-associated urinary tract infection in adults: 2009 International Clinical Practice Guidelines from the Infectious Diseases Society of America. Clin Infect Dis. 2010;50(5):625-63.
[13] Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev 2005;18:657-86.
[14] Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268-81.

P.1619

Tuberculosis before and after the modern era of kidney transplantation

Siriorn Watcharananan[1], Viroon Mavichak[2], Kaenchai Pipatpannawong[2], Kumthorn Malathum[1], Wisit Liwlompaisan[2], Pongpan Thanak[2], Nattakan Naitook[2].
[1]Medicine, Ramathibodi hospital, Mahidol University, Bangkok, Thailand; [2]Kidney Transplant Institute, Praram 9 hospital, Bangkok, Thailand.

Background: Little is known about the incidence, clinical characteristics and outcome of tuberculosis (TB) after the use of more intense immunosuppression for kidney transplantation (KT) in the TB endemic area.
Methods: A retrospective cohort study of adult KT recipients during 1992-2015. Patients' medical records were reviewed for underlying characteristics, type of KT and immunosuppressive regimen. Patients with TB were reviewed for clinical presentation, laboratory findings, anti-TB treatment and the outcome. None received isoniazid prophylaxis post KT. Induction therapy was used in 413 (70.8%), including ATG [60 (9.79%)] and anti-IL2 [374 (61.01%)].
Result: A total of 613 KT cases were reviewed. One who later received liver transplantation was excluded. The cumulative incidence (CI) of TB was 4.08 % (5.08 % and 3.2% among ATG, and anti-IL-2 group, respectively). The median time to the TB diagnosis was 36.3 (range; 0.7-220) months after the KT. Nine (36%) were diagnosed early (< 6 months) after KT. Patients' median age was 50 (range; 23-71) years and 17 (64%) were male. Most (92%) were Thais but 2 (8%) were Burmese. Majority (60%) were living related. Major type of maintenance immunosupression was mycophenolate mofetil (84%), prednisolone (72%), tacrolimus (48%) and cyclosporine A (48%). Pulmonary TB was the most common (52%). Disseminated TB occurred in 7 (28%). All (100%) of the 3 cases in ATG group were disseminated and occurred early after transplantation. Two of the TB cases in ATG group were possible donor-derived. Both cases began with TB of the kidney allograft which was later complicated with disease progression involving other organ. Four (25%) of the TB cases in the anti-IL2 group were disseminated, all of which occurred late post KT. All of the culture-proven cases (n=17) were pan-drug susceptible. Standard regimen (isoniazid, rifampicin, pyrazinamide, ethambutol followed by isoniazid and rifampin) was most commonly (60%) used. One (4%) who received ATG induction required transplant nephrectomy for uncontrolled TB of the allograft. One (4%) died from TB sepsis.
Conclusion: The incidence of TB after kidney transplantation is relatively high compared with those reports of South East Asian Region. Use of ATG induction appears to increase the incidence of TB, with notable severe presentation early after KT.

P.1620

An insight into the predisposing risk factors of pneumonia in renal transplant patients

NIkhil Mahajan, Anil Mani, Ashish Sharma, Mukut Minz, Sarbpreet singh, Deepesh Kenwar, Sunil Kumar.
Renal Transplant Surgery, Post Graduate Institute of medical education and research, Chandigarh, India.

Backgroun: Infections continue to be a major cause of mortality and morbidity in renal transplant recipients, amongst which pulmonary infections are the most dreaded.
Knowledge on infection risk, the temporal relationship between immunosuppression and infectious etiology, as well as the prompt diagnostic pursuit of organism identification together with empiric therapy; are required to reduce the consequences of this complication.
Aim: To study the clinical profile of patients presenting with post transplant pneumonia and to know risk factors predisposing to it.
Materials and Methods: The study was done during a 6 month period from February 2015 to July 2015 in the Department of Renal Transplant Surgery, PGIMER, Chandigarh. The study cohort included 1513 transplanted patients performed between 2006 and 2015. A total of 25 patients who required admission during the study period for pneumonia and had positive findings on HRCT were taken as cases and was evaluated prospectively. Patients who underwent transplant one day prior and after the cases were taken as controls and their demographic and clinical profiles were compared and were statistically analyzed. The standard immunosuppression protocol included Tacrolimus(Tac)/mycophenolate(MMF)/steroids. High risk patients who could afford induction therapy received either ATG (3- 3.5 mg/kg given in 3 divided doses) or Basiliximab (20mg on POD0 and POD4) based on the clinical judgment .
Results: There were 25 patients (Mean age 40.5+ yrs M:F 21:4) who presented after 25.28 ± 29.62 (range 1 to 105) months after transplant. Controls comprised of 50 patients [Mean age 39.2+ yrs(M:F 43:7). Majority (80% in case & 86% in controls) were on Tac & MMF. Fungal pneumonia 8/25 (32%) and TB pneumonia 8/25 (32%) were the most common pathologies. Bacterial pathogens were isolated in 7 (28%) cases. In 3 cases (12%) no definitive diagnosis could be made. CMV was detected in 2 cases and PCP was isolated in 1 case. 3 patients had multiple infections. 28% in the case group and 22% in the control group received induction immunosuppression. Hepatitis, PTDM and induction therapy (p-.56) was not associated with increased risk. Diabetic nephropathy (p-.003) and history of acute rejection (p-.001) conferred a risk with likelihood ratio of 8.9 and 10.6 respectively. Overall mortality was 36%. Fungal pneumonia had the maximum mortality (44%).
Conclusion: Diabetic Nephropathy as basic disease and acute rejection during post transplant period results in higher incidence of post transplant pneumonia. Wider use of induction therapy can reduce incidence of acute rejection, which can help in preventing pneumonias in transplant patients.

P.1621

A novel single port retroperitoneal approach of donor nephrectomy using non muscle cutting lombotomy incision

Ashish Sharma, Sarbpreet Singh, Deepesh Kenwar, Sunil Kumar, Nikhil Mahajan, Navdeep Singh, Shiva Kumar, Soham Dasgupta, Mukut Minz.
Renal Transplant Surgery, Post Graduate Institute of medical education and research, Chandigarh, India.

Surgical Technique: Kidney was approached through 6cm lumbotomy incision nearly parallel to sacrospinalis muscle with donor in lateral position. Lumbar fascia is identified after retracting sacrospinalis muscle and is incised. Gerota's fascia is opened and ureter and gonadal vein are identified by reflecting the peritoneum with the open approach. Alexis® port with rubber glove was used as a single port with two 10mm and two 5mm ports placed in the finger of gloves. Further dissection is carried out laparoscopically. Ureter is lifted off the retroperitoneal tissues and gonadal vein are dissected till its drainage into renal vein avoiding injury to the ureter and its adventitia and ligated. Gonadal artery if encountered can also be ligated. As one reaches renal hilum, pulsations of renal artery can be seen and lumbar vein is visualised in front of the artery. Lumbar vein is controlled after which renal artery is seen which is dissected till its origin. After that, tissue around the renal vein is dissected and adrenal vein is identified. At this stage, lower pole of the kidney is separated from the peritoneum when the kidney which was hanging from the peritoneum starts to fall down. Dissection is carried out on the surface of kidney to free it from the surround fat till renal vein is seen anteriorly. Adrenal vein is identified and divided, adrenal gland is dissected and separated from upper pole of the kidney and left in situ. Ureter is divided once the kidney and renal vessels are free. Renal artery and renal vein are separately ligated with two liga clips each and cut with scissors. Kidney is retrieved into the Alexis wound retractor, and taken out with the retractor.

P.1622

The outcomes of kidney transplantation in hepatitis B surface antigen (HBsAg) positive donor and/or recipients: A center experience

Berna Yelken[1], Cihan Karatas[2], Oya Yonal[3], Serpil Gorcin[1], Basak Akyollu[2], Burak Kocak[2], Aydin Turkmen[1].
[1]Nephrology, Sisli Memorial Hospital, Istanbul, Turkey; [2]Solid Organ Transplantation, Sisli Memorial Hospital, Istanbul, Turkey; [3]Gastroenterology, Sisli Memorial Hospital, Istanbul, Turkey.

Aim: The aim of this study was to determine the effects of hepatitis B surface antigen (HBsAg) positivity of the donors and/or recipients on patient/graft survival and hepatit B virüs (HBV) transmission/reactivation in renal transplant recipients.
Patients and Method: We included renal transplant recipients with HBsAg(+) or HBsAg (-) negative recipients receiving graft from HBsAg-positive donors among 1206 patients who underwent renal transplantation in our hospital between 2007 and 2015 years, retrospectively. We examined patient/graft survival rate and HBV transmission/reactivation, in these patients.
Results: There were 12 (11 male, 44±10 years) renal transplant recipients with HBsAg(+). One patient of them had chronic hepatitis B infection that was confirmed by liver biopsy, other patient had liver transplantation six days ago and another one patient had both HBsAg (+) and Anti HCV(+). Five of 12 renal transplant recipients with HBsAg(+) were HBV DNA positive, however HBV DNA levels of all of them were under 2000 IU/mL. Six patients were taking lamivudine, 5 patients were taking entecavir, and 1 patients were taking telbuvidin throughout the duration of transplantation. Only one patient had HBV DNA positive (1480 IU/mL) at the end of 9.4±8.2 months of follow-up period. We did not observed any HBV reactivation in HBsAg(+) recipients. One patient had graft failure due to thrombotic microangiopathy. The patient survival was 100% in recipients with HBsAg(+). There were 5 (4 male, 50±10 years) renal transplant recipients whom donors had HBsAg positivity. Only one renal transplant recipient had HBsAg positivity. Only one recipient's anti-HBs titer above 100 mIU/mL undergoing KT from HBsAg(+) donor. Hepatitis B vaccine were started in the other 4 receipents. Four of 5 donor had HBV DNA positivity and HBV DNA levels of 2 donor were over 2000 IU/mL. While the one of these two patients had slightly higher Alanin transaminase level and was treated with entecavir and HBV immunoglobulin for HBV prophylaxis. The other receipents were treated with lamivudin. Nobody had HBV transmission at the end of 9.2±6 months of follow-up. The patient and graft survival were 100% in recipients who had transplanted from HbsAg (+) donors.
Discussion: We experienced that, renal transplantation from HBsAg(+) donors and renal transplant recipients with HBsAg(+) is safe with antiviral treatment. Therefore, HBsAg (+) donors should be evaluated by enthusiasm for the donation especially in the organ shortage era.

P.1623

The effect of C1q est done before kidney transplantation on acute rejections: Center experience

Berna Yelken[1], Funda Yalcin[1], Serpil Gorcin[1], Cihan Karatas[2], Basak Akyollu[2], Burak Kocak[2], Emel DemiralpEksioglu[3], Aydin Turkmen[1]. [1]Nephrology, Sisli Memorial Hospital, Istanbul, Turkey; [2]Solid Organ Transplantation, Sisli Memorial Hospital, Istanbul, Turkey; [3]Immunology, Sisli Memorial Hospital, Istanbul, Turkey.

Introduction: C1q is a modified single antigen bead (SAB) test used to detect the HLA Antibodies which have the capacity to fixe or activate compleman. The aim of this study is to investigate the effect of C1q-fixing donor of specific antibodies (DSA) identified prior to transplantation (Tx) on postTx acute rejection.
Materials: A total of 20 patients who had DSA positivity and underwent C1q test before Kidney transplantation is included in to the study. C1q positive and negative patients were evaluated in terms of acute rejection after Tx.
Results: Of the 20 DSA positive patients (14 females; mean age: 50 ± 14), pre-Tx desensitization protocol was applied to 8 patients. Five of these 8 had a history of renal Tx. Seventeen DSA positive patients had negative C1q test, three were C1q positive but unable to connect to DSA. Post-Tx acute rejection was seen in 10 of 20 patients at a mean of 54 days (3-150 days). Eight of 10 patients developed acute antibody mediated rejection (AMR) and 2 had acute cellular rejection (ACR). C4d was positive in 5 of 8 patients with AMR and in 2 of them ACR was accompanied with. Six of 10 patients with acute rejection were desensitized. During pre-Tx period, acute rejection was detected in only 1 of the 3 C1q positive patients. Two patients with acute rejection lost graft and returned to dialysis. One patient developed graft dysfunction.

	C1q(+) (n=3)	C1q (-) (n=17)
Pre-Tx desensitization (n=8)	1	7
Second renal Tx (n=5)	1	4
AMR (n=8)	0	8
ACR (n=2)	1	1
AMR+ACR (n=2)	0	2
Graft loss (n=2)	0	2

Discussion: Although anti-HLA antibodies are considered to be harmful, some antibodies does not significant damage on kidney allograft. Activation of complement system has been considered to be key component of AMR. Thats why the capacity of anti-HLA antibodies binding to the C1q components of complement may be diagnostic for the harmful anti-HLA antibodies and predictive AMR. Some studies revealed that the C1q binding anti-HLA antibodies may be related to AMR. On the contrary we showed that the pretransplant C1q binding capasity is not predictive for AMR (33% vs 52%) Loupy et al showed that the posttransplant de-novo antibodies which binding to C1q is strictly related with AMR and graft survival. The major differences between the antibodies in these two cohort is the timing of occurrence of antibodies, pretransplant or posttransplant. These data support to the occurrence of posttransplant C1q binding antibodies may be harmful than the pretransplant one.
Conclusion: Negative C1q test in pre-Tx period, seems to be insufficent in predicting the acute antibody-mediated rejection early after Tx, however, C1q negative DSAs' might mediated to acute rejection in preTx period. More comprehensive are needed on this subject.

P.1625

Implementing a collaborative request model for organ donation in an Australian icu- the art of change management

Bettina Clark.
Intensive Care Unit- Organ and Tissue Donation Team, Austin Health, Melbourne, Australia.

Introduction: Until recently, organ donation has only been raised by senior intensive care doctors. The Austin Health Department of Intensive Care participated in a pilot study, undertaken by the Australian Organ and Tissue Authority, which evaluated a collaborative request model (CRM) for requesting organ and tissue donation.
The CRM aimed to provide families with information regarding donation to facilitate a fully informed decision that is right for them. This centred on the introduction of a dedicated and trained donation specialist requestor into family donation conversations alongside the treating team. Their role was to promote discussions that support the family and treating team, while providing donation information that allows informed decision-making. All donations requests were to be initiated by the specialist requestor. Austin Health was interested in participating as we had a strong interest in collaborative requesting, were keen improve the decoupling of conversations (particularly in DCD cases), and wished to normalise donation conversations as part of end of life care.
Methods: Prior to the implementation of the new model we conducted a risk analysis, which formed the development of a structured plan. Donation Specialists completed specific training, a hospital trigger for donation requesting was created and comprehensive education of all ICU clinical staff was completed.
During the 12-month study period ending June 2014, all organ donation requests were made through collaboration between the treating specialist and a donation specialist requestor. At Austin Health, the selected donation specialists included intensivists and donor coordinators. Each had received specialised communication training through the DonateLife Network Family Donation Conversations (FDC) program.
The treating clinical team remained responsible for patient management and communication with the family about clinical care, confirmation of brain death or the inevitability of death and palliative measures.
Results: Several challenges were identified during the implementation of the CRM. These included encouraging clinicians to change practice so as to enable time for pre-planning and post-meeting debriefs. It was also necessary to develop language and expertise that supported a prepared approach and yet did not appear rehearsed. Quite rapidly, The CRM became the accepted approach for raising donation, with participants feeling supported and able to more clearly identify their role.
Discussion: Through the implementation of the CRM, we identified a number of challenges and effective strategies that could be adopted by other hospitals wishing to implement this model. The pilot study enabled us to trial the new request model and gain useful feedback from clinical staff on the process.
Conclusion: The CRM has now been adopted as usual practice in all conversations when organ donation is being raised with families. Adoption of the CRM has also enabled a policy of universal referral within the unit whereby all patients for whom end of life care is planned are referred to be considered regarding donation potential. We have achieved 100% referral rate of such patients to the donation team over the past year, and had no unrealised potential donors.

P.1626

The organ preservation and enhancement of donation success ratio effect of extracorporeal membrane oxygenation in circulatory unstable brain death donor

Xiaoli Fan[1], Zhiquan Chen[1], David Nasralla[2], Xianpeng Zeng[1], Jing Yang[1], Shaojun Ye[1], Yi Zhang[1], Guizhu Peng[1], Yanfeng Wang[1], Qifa Ye[1].
[1]Transplant Center, Zhongnan Hospital of Wuhan University, Wuhan, People's Republic of China; [2]Transplant Center, Nuffield Department of Surgical Sciences, Oxford University, Oxford, United Kingdom.

Background and Aim: Between 2010 and 2013 our institution recorded 66 cases of failed organ donation after brain death due to the excessive use of the vasoactive drugs resulting in impaired hepatic and/or renal function. To investigate the effect of extracorporeal membrane oxygenation (ECMO) in donor management, from 2013 to 2015, ECMO was used to provide support for circulatory or respiratory failure DBD donors.
Methods: A retrospective cohort study between circulatory non stable DBD with vasoactive drugs (DBD-drug) and circulatory non stable DBD with ECMO (DBD-ECMO) was made to compare transplant outcome.
Results: 19 brain death donors have been supported by ECMO. The post-transplant liver PNF incidence was 10% (2/20) in DBD-drug group vs. 0 in DBD-ECMO group. Kidney function indicators including creatinine clearance and urine production were Significantly better in DBD-ECMO group, as well as decreased kidney DGF rate. Donation success rate increased steadily from 47.8% in 2011 to 84.6% in 2014 after ECMO intervention.
Conclusion: The use of ECMO in assisting circulatory and respiratory function of DBD can reduce liver and kidney injury from vasoactive drugs, thereby improving organ quality and reducing the organ discard rates.
The National Natural Science Foundation of Xinjiang joint fund key projects

P.1627

Hypothermic machine perfusion attenuate liver warm ischemia injury on a novel venous bypass supported pig liver auto-transplantation model

Xiaoli Fan[1], Zhiquan Chen[1], Cheng Zeng[1], Honglei Huang[2], Xiaoyan Hu[1], Tengyun Chen[1], Bingru Zhao[1], Yanfeng Wang[1], Qifa Ye[1].
[1]Transplant Center, Zhongnan Hospital of Wuhan University, Wuhan, People's Republic of China; [2]Transplant Center, Nuffield Department of Surgical Sciences, Oxford University, Oxford, United Kingdom.

Aim: To establish a novel liver auto-transplantation pig model supported by venous bypass and evaluate effect of hypothermic machine perfusion on liver underwent warm ischemia injury.
Method: 10 Landrace pigs were used to establish standardized auto-liver transplantation models (CS model group). Another 10 Landrace pigs were randomized into two groups after 30mins of warm ischemia, in one group, pig livers were preserved with cold storage (WI+CS) and another group underwent hypothermic machine perfusion (WI+HMP) before auto-transplantation. Blood samples of different time points were collected for biochemical analysis and animal survival rate was analyzed after auto-transplantation.
Results: The animals in the CS model group all survived over 30 days after the post liver auto-transplantation, which had significant differences with WI+CS group ($P<0.001$) and WI+HMP group ($P<0.001$). 24h and 48h survival rates of the WI+CS group were 0 and 80%, 0 and 20% for WI+HMP group. As a result, hypothermic perfusion prolonged the pig's survival time which undertook post liver auto-transplantation with 30min warm ischemia injury ($P=0.014$). HMP had decreased the bilirubin level at 4h post transplantation of warm ischemia liver compared with CS ($P=0.014$).
Conclusion: Venous bypass supporting pig liver auto-transplantation is a stable model for evaluation of liver functions. Hypothermic machine perfusion can improve warm ischemic animal's survival rate.
National Natural Science Foundation of China Xinjiang joint fund key project

P.1628

Tissue recovery activity from 2012 to 2014 in Eastern Japan and Tokyo area: More struggle than organ donation and much to learn from the United States

Yumi Akashi[1], Osamu Hattori[1], Yuji Sampei[1], Sayaka Nagashima[1], Michiko Seki[1], Shinichi Ozaki[2], Fuyuki Inagaki[3], Osamu Kinoshita[2], Haruo Yamauchi[2], Nobuhisa Akamathu[3], Aya Saito[4], Noboru Motomura[4], Minoru Ono[2], Norihiro Kokudo[3], Akihiro Masuzawa[1,2], Sumihito Tamura[1,3].
[1]University of Tokyo Tissue Bank, The University of Tokyo, Tokyo, Japan; [2]Department of Cardiothoracic Surgery, Faculty of Medicine, The University of Tokyo, Tokyo, Japan; [3]Artificial Organ and Transplantation Division, Department of Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; [4]Department of Cardiothoracic Surgery, Toho University Sakura Medical Center, Chiba, Japan.

Background: Organ donation in Japan from deceased donor remains at the lowest among developed countries despite legal revisions made effective in 2010. Tissue donation activity, however, has not been described in detail in English literature. We herein present our experience at the University of Tokyo Tissue Bank (UTTB), the core facility in Japan, in comparison with the United States (US) statics. UTTB is the information hub for East Japan Tissue Transplantation Network (EJTTN) that covers all tissue categories in Eastern Japan region and specializes in heart valve and vessel grafts.
Method: Retrospective analysis of potential donor information reported to EJTTN and activity of UTTB during the period of 2012-2014 was performed. US data and statistics were obtained from (AATB 2007)**.**
Results: Total number of potential tissue donor information reported to EJTTN between 2012 and 2014 was 260 cases, of which 176 were considered medically adequate. Of the 176, tissue recovery was made in 73 cases, 60 under cardiac death and 13 under brain death. UTTB recovered heart-valves and vessels from 29 donors in this period, resulting in 42 heart-valve grafts, and 203 vessels grafts. Adjusted by PMP, total recovery in Eastern Japan region was 0.38 cases pmp -year and heart-valves and vessels recovery by UTTB was 0.19 cases pmp -year, resulting in 0.15pmp -year heart valves. In comparison, in the US detailed report from 2007 describes that 30,380 tissue donors 93.5 pmp-year was recovered resulting in 7,216 heart valve grafts (22.3 pmp-year).
Conclusion: Revision of the Organ Donation Act enacted in 2010 intended in part to enlighten donation in a wider aspect has shown little effect in Japan. Tissue donation in Japan remains to lack legal supportive framework and continues to struggle at 0.58 percent of that in the US. Much remains to be learned from the US experience, not only in organ recovery but also in the field of tissue recovery and transplantation.

P.1629

The interaction between health care personnel and parents approached for organ and/or tissue donation and their adjustment to loss

Tamar Ashkenazi[1], Jonathan Cohen[1,2].
[1]Israel Transplant, Ministry of Health, Tel Aviv, Israel; [2]General Intensive Care, Rabin Medical Center, Petah Tikva, Israel.

Introduction: The effect of loss on those approached for organ and/or tissue donation, particularly in the years following the approach, has received little attention. We assessed whether adjustment of a parent to loss of a child is influenced by interactions with health care personnel.
Methods: A self-administered questionnaire was completed by the parents of 216 decedents. Interactions in the hospital were assessed by examining the experience in the hospital, physical separation from the child and the relationship with health care professionals. Adjustment to loss was defined by four components; grief, personal growth after loss, meaning of life after loss and the meaning of the organ donation.
Results: A positive experience in the hospital was significantly associated with the meaning of donation. Increased satisfaction with the separation process was associated with better adjustment of all the components studied. Finally, a better relationship with health care professionals was associated with less grief and with greater personal growth. Importantly, these responses were still evident years after the loss (median 4.9 years, range 6 months – 27 years).
Discussion. Interactions in the hospital appear to influence significantly adjustment to loss after approach for organ donation and were still apparent after the passage of many years.
Conclusion: Appropriate behavioral modifications by health care personnel may aid parents in their adjustment to life after organ donation.

P.1630

The effect of family accommodation on the organ donation process in Israel

Tamar Ashkenazi[1], Jonathan Cohen[1,2].
[1]Israel Transplant, Ministry of Health, Tel Aviv, Israel; [2]General Intensive Care, Rabin Medical Center, Tel Aviv, Israel.

Introduction: The Israel Brain - Respiratory Death Act allows for the accommodation of families who do not accept or understand the concept of death by neurological criteria (DNC). In these instances, DNC is either not determined or if determined, no approach is made for organ donation; in addition the family may request not to disconnect the deceased from the ventil ator following the declaration but wait for a cardiac arrest. We examined how this unique aspect of the Israeli Act affected the organ donation process.
Methods: We assessed all cases during 2014 who met criteria for DNC where family accommodation was applied. 2 groups were defined, namely the no-determination and no-approach groups. Demographic data collected included patient age and sex, cause of DNC, family ethnicity, religiosity and level of education, and outcome of the process after the accommodation decision, including days ventilated, therapies administered and site of therapy after accommodation decision, i.e. ICU or general ward).
Results: During the study period, we identified 173 patients with clinical evidence of DNC. DNC was not declared in 27 (15%) due to family opposition while an approach for donation after the determination was not made in a further 19 (11%). The no-declaration group was characterized by a higher level of religiosity and lower level of education than those in the no-approach group. Mean time to cardiac arrest in the no-declaration group following clinical signs of DNC was 4.8±3.3 days and 3.7±2.6 days following DNC determination in the no approach group. The following therapies were continued in the no-declaration and no-approach group, respectively: nutrition, 55.5 vs 42%; IV fluids, 100 vs 89%; antibiotics, 37 vs 36%; sedation, 18.5 vs 0%; and vasopressors, 77 vs 57%.
Conclusions: The Israeli Act is unique in providing options for accommodating families who do not accept DNC. However the result is that DNC is not declared or family not approached for organ donation in a significant number of potential donors.
Conclusion: These cases continue to occupy scarce resources in the ICU and by continuing to administer treatment, physicians may be drawing into doubt the concept of DNC.

P.1631

Comparative analysis of the last 5 edition of the Master degree in Donation and Transplantation of organs, tissues and cells of the University of Barcelona

Chloë Ballesté-Delpierre[1,2], Fritz Diekmann[1], David Paredes[1], Vicens Torregrosa[1], Ricard Valero[1,2], Gloria Páez[2], Marti Manyalich[1,2].
[1]Surgery, University of Barcelona, Barcelona, Spain; [2]TPM, Donation and Transplantation Institute, Barcelona, Spain.

Introduction: Since 2010 the Master degree in Donation and Transplantation of organs, tissues and cells has been held in the Faculty of Medicine of the University of Barcelona (UB) in its 2 modalities: professional and research. The aim of this study is to analyze the evolution of the student's profile as well as the results obtained to evaluate the possible improving measures to be applied.
Materials and Methods: Data have been obtained retrospectively and divided in 2 study groups: the student's profile and the scores obtained in each module. The variables chosen for the student's profile are: gender, nationality and academic background. For the modules evaluation the items chosen are: scores obtained per module and subject and the comparison between online and face to face outcomes.
Results and Discussion: The total number of participants has been 111 with a slight decreasing of registrations in the last editions (25; 30; 21; 20; 15).
Student's Profile: A trend is shown, first year excepted, of a major proportion of women (44%; 76,67%; 66,67%, 55%; 73,33%). The heterogeneity among academic backgrounds has remained high in all editions (with an average of 4 per course). The most common academic profile is Medicine (57,66% in total) and from the 11 specialties, the Transplant field is the most represented (65,63%) in all edition. Since 2010 students from 32 different nationalities have been trained from the 5 continents (Europe 46, America 50, Asia 10, Africa 4 y Oceania 1) with an average of 12 different origins per edition. The most frequent nationality is Spanish (39 students) being America the most represented continent with 50 students.
Scores Obtained: there are not major differences between the 5 editions, being the Organ Donation module with the lowest scores (7,32/10) and the Transplantation module with the highest (8,15/10). The differences observed between online and face to face courses vary among the editions (online scores/face to face scores per edition 6,86/5,7; 7,93/8,97; 8,05/8,4; 8,25/8,1; 7,4/8,92).
Conclusions: Taking into consideration that the main characteristics of this master are the internationality and heterogeneity of the participants involved, the improving measures have to be directed to enable more flexibility in the module selection and to promote online modality.

P.1632

European Mediterranean Postgraduate Program on Organ Donation and Transplantation (EMPODaT)

Chloë Ballesté-Delpierre[1,2], Melania Istrate[2], Ricard Valero[1,2], Ricardo Casaroli[1], Gloria Páez[2], Empodat Consortium[1], Marti Manyalich[1,2].
[1]Surgery, University of Barcelona, Barcelona, Spain; [2]TPM, Donation and Transplantation Institute, Barcelona, Spain.

Introduction: EMPODaT is a TEMPUS project (20132015) funded by the European Commission, Education, Audiovisual and Culture Executive Agency (EACEA). The consortium includes 11 partners from 4 European countries and 3 countries benefitting from the European Neighbourhood Policy Partnership Instrument (ENPI): Egypt, Lebanon and Morocco.
The objectives of the project are:1) To develop and implement a Postgraduate Program on Organ Donation and Transplantation (D&T) based on a common curriculum and certification degree, in accordance with the European Space for Higher Education.2) To train 12 ENPI tutors as faculty staff and coordinators of EMPODaT in their countries.3) To provide training to 90 ENPI students.4) To generate a consulting network.
Material and Methods: EMPODaT consists of three main phases as follows:
• Design. The project started with a diagnosis study, based on which the consortium drew up the plan on how to create a homogenized EMPODaT curriculum.
• Development. A two level training program was developed:
• "Learn to teach" program aimed at training the ENPI Faculty Staff
• "Postgraduate Program" (750 hours 30 ECTS) based on blended learning methodology: online modules, local seminars, practical hospital traineeships and international assessment seminars.
Implementation and follow-up, currently in progress. Working language is English and French.
Results and Discussion: 444 surveys were answered to identify the training needs on D&T in the ENPI partner universities. 12 ENPI tutors completed the ¨Learn to teach¨ course in Barcelona.
90 ENPI students were enrolled. Their results after the first online module are good.
First online module was assessed by participants as follows: English version with 4.12 and French version with 4.15 on a scale from 1 (poor) to 5 (excellent).

P.1633

The Trinidad and Tobago experience: A successful model to develop a donation system using SEUSA program

Gloria Paez[6], Chloë Ballesté-Delpierre[1], Maria Paula Gomez[1], Jose M.García-Buitrón[1], Antón Fernández[1], Manuel Wolf[1], Susan Gunderson[1], Bridget Elcock[2], Prisca Bradshaw[3], Chariande Gardiner[5], Lesley Roberts[4], Marti Manyalich[1].
[1]International Development, Donation and Transplantation Institute, Barcelona, Spain; [2]ICU, General Hospital of Port of Spain, Port of Spain, Trinidad and Tobago; [3]ICU, San Fernando Hospital, San Fernando, Trinidad and Tobago; [4]Direction, NOTU, Port of Spain, Trinidad and Tobago; [5]ICU, Eric Williams Hospital, Port of Spain, Trinidad and Tobago; [6]TPM-DTI Foundation, Barcelona, Spain.

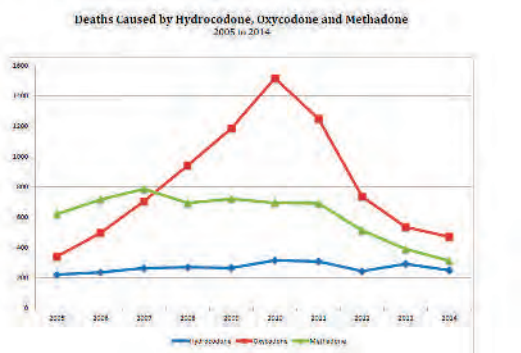
Introduction: In Trinidad and Tobago (T&T) there are more than 500 patients with chronic kidney failure. Kidney transplantation is their opportunity to recover their quality of life. World Health Organization (WHO) is advocating for the development of self-sufficiency in Donation and Transplantation (D&T) in all countries, as a practical alternative to combating transplant tourism and commercialism around the world. SEUSA is an international development program to increase the D&T rates. SEUSA methodology is based on three of the successful models in the world (Spanish–European–USA).
Material and Methods: SEUSA project was implemented in T&T in 2010 with the support of the National Organ Transplant Unit (NOTU) and the Ministry of Health of T&T. The SEUSA program included: a) diagnosis of the current situation using the ODDS (Organ Donation Diagnostic Surveys); b) creation of a human resources structure through Transplant Procurement Management (TPM); c) Detection of all brain and cardiac deaths in the hospitals implementing the DAS (Decease Alert System); e) In-hospital awareness based on the EODS (Essentials in Organ Donation) and f) External hospital audits. Continuous monitoring is performed.
Results and Discussion: Thanks to SEUSA program 94 health care professionals have been trained in D&T, the Living Kidney Program has been reinforced and the structure of the Deceased Donation network was defined. Since 2010, 328 potential organ donors have been detected, 6 of them had become actual organ donors. 20 patients have received a kidney transplant. In addition, Donation after Cardiac Death (DCD) program is, currently, being organized.
Conclusion: The SEUSA through the previous experience carried out in La Puglia (Italy) and Lebanon has showed, once more, to be a good option to consolidate the D&T Systems. SEUSA represents a valuable and replicable international collaborative strategy to improve organ donation worldwide.

P.1634

Closing the Flamingo Highway: heroin deaths in Florida

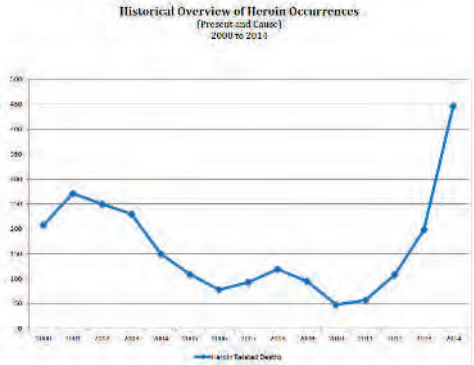
Jean Davis[1], Elizabeth Lehr[2], Charles Wright[2], Susan Rabel[2].
[1]LifeLink Foundation, Inc., Tampa, FL, United States; [2]LifeLink of Florida, Tampa, FL, United States.

Introduction: The CDC reported that opioid overdose deaths increased in Florida by 61% between 2003 and 2009[1]. In 2010, 98 of the top 100 prescribers of Oxycodone in the USA were located in Florida[1]. In response to this crisis, the Florida legislature and the DEA worked on a number of initiatives to put a stop to "pill mills" in the state[2]. Unfortunately in 2014, the state saw a 124% increase in heroin related deaths. The CDC and others are reporting that heroin use nationally is on the rise, perhaps in part, due to the increased availability of heroin relative to prescription opioids[3]-[5]. LifeLink of Florida OPO serves the central west coast of Florida, and evaluates the risk profile of donors. With the increasing prevalence of intravenous versus oral routes for drug intoxication, the risk profile of the donor changes. Infectious disease testing window considerations may exist and need to be effectively communicated to transplant centers, and may impact the number of organs ultimately accepted for transplant.



Materials and Methods: We reviewed the reports from the Florida Medical Examiners Commission showing opiate related deaths for 2009-2014 and did a comparison of donor statistics for the same time period. Reports were compared to donor statistics from the designated service area, with attention to detail on changing risk profile on recovered doors. A comparison was made between actual organs transplanted and SRTR expected (O to E).

Results: In 2014, 34, or 18% of our donors were classified as PHSIR, and in 2015, 58, or 28% of our donors were classified as PHSIR. In a 24 month period, organs transplanted for PHSIR donors in a testing window were 44% below expected by SRTR calculations.



Conclusion: There were 48 heroin deaths in Florida in 2010 and in 2014 there were 447. The dramatic rise in IV drug related deaths added an increased measure of risk to our donor population. Fewer organs were transplanted from this increased risk donor group than would have been expected by SRTR calculations. OPOs need to continue to pursue increased risk donors and transplant centers and OPOs need to work together to ensure testing window considerations are adequately communicated by the OPO and evaluated by the transplant center. Transplant centers need to educate and inform their recipients of potential testing window considerations at the time of listing to avoid problems when an organ is available.

References:

[1] H. Johnson, L. Paulozzi, C. Porucznik, K. Mack, B. Herter. "Decline in Drug Overdose Deaths After State Policy Changes-Florida, 2010-2012." www.cdc.gov/mmwr. Weekly. July 4, 2014/63(26);569-574
[2] P. Bondi. June 25, 2014. Report to the U.S. Senate Health, Education, Labor and Pension (HELP) Committee: Prescription Drug Abuse Working Group
[3] www.drugfree.org. October 29, 2015. "Heroin's Low Cost and Easy Access Attract People Addicted to Painkillers: Study."
[4] R. Rudd, N. Aleshire, J.Zibell, R.M. Gladden. Center for Disease Control and Prevention MMWR Weekly; Vol.64; Nos 50 & 51. January 1, 2016. "Increases in Drug and Opioid Overdose Deaths-United States, 2000-2014"
[5] W. Compton, C. Jones, G. Baldwin. January 14, 2016. "Relationship between Nonmedical Prescription-Opioid Use and Heroin Use." The New England Journal of Medicine. www.nejm.org

P.1635

Deciding to discuss organ donation with parents: An in-depth interview study with Chinese young adults

Timothy Fung[1], Isabella Ng[2], Kelvin Lee[3], Fai M.Lam[4].
[1]Department of Communication Studies, Hong Kong Baptist University, Kowloon Tong, Hong Kong; [2]Department of Asian and Policy Studies, Hong Kong Institute of Education, Tai Po, Hong Kong; [3]Academy of Film, Hong Kong Baptist University, Kowloon Tong, Hong Kong; [4]Faculty of Medicine, University of Hong Kong, Pokfulam, Hong Kong.

Government health departments and organ procurement organizations, with little success[1], have long been encouraged family discussion about organ donation because it plays an important role on the final donation status[2]. A family talk about organ donation is associated with higher willingness to register the donor card[3] and higher likelihood of familial consent to donate after one's death[4]. To promote the action of initiating such a discussion, in-depth studies are needed to account for factors of willingness to discuss donation with family members, we applied the Theory of Motivated Information Management to explore the factors that influence Hong Kong young adults' decision to talk with their parents about their plan to donate their organs after death.[2] We conducted 25 semistructured in-depth interviews with undergraduate students. The findings indicated that individuals' perceived need for discussion, anxiety to discuss, efficacy to communicate their will to donate, and efficacy to respond to parents' reactions were the factors to influence their decision to initiate a family discussion. Because of the Chinese traditional view that children are the "flesh and bone" of their parents, for cultural, psychological, and practical reasons, most of the prospective organ donors expressed their wish to know how their parents view their plan. The informants also said that discussing this would prevent any regret not to have shared their plan while their parents were still alive. These informants also wanted to ensure that their parents would act according to their will to donate their organs in case if they passed away. The results concluded that the majority of the informants considered discussing the topic with parents as essential in their decision-making process.
The results also suggest that media coverage of organ donation or third party mediation would help the informants broach the subject with their parents. Either method could lower the informants' anxiety level and help the communication process. Informants whose parents had not indicated their views on organ donation have expressed that to have TV advertisements or other media formats begin to address the topic would ease the sensitivity when potential donors approach the issue with their parents. Some expressed that having third party mediation, especially a medical professional or family member who has already signed up for donation, would help potential donors' parents to resolve their concerns. This study is significant for policymakers to encourage people to discuss openly about organ donation, an increasingly important but sensitive subject in the Hong Kong community.

References:

[1] 1. Morgan SE. The power of talk: African-Americans' communication with family members and its impact on the willingness to donate organs. J Soc Pers Relatsh 2004;21(1):112-24.
[2] 2. Afifi WA, Morgan SE, Stephenson MT, Morse C, Harrison T, Reichert T, Long SD. Examining the decision to talk with family about organ donation: applying the Theory of Motivated Information Management. Commun Monogr 2006;73(2):188-215.
[3] 3. Morgan SE, Miller J. Beyond the organ donation card: the effect of knowledge, attitudes, and values on willingness to communicate about organ donation to family members. Health Commun 2002;14(1):121-34.
[4] 4. Siminoff LA, Gordon N, Hewlett J, Arnold RM. Factors influencing families' consent for donation of solid organs for transplantation. JAMA 2001;286(1):71-7.

P.1636

Main factors affecting loss of the potential deceased donors for kidney transplantation

Luis García Covarrubias[1], Aldo Garcia[1], Hector Hinojosa[1], Arturo Reding[2], Alejandra Cicero[1], Edwin Ventura[1], Juan de los Santos[1], Pedro Fragoso[1], Jose Manuel Torres[1], Rodrigo Miyagui[1], Roberto Martinez[1], Hector Diliz[1].
[1]Transplantation, Hospital General de Mexico Dr. Eduardo Liceaga, Mexico, Mexico; [2]Research, Hospital General de Mexico Dr. Eduardo Liceaga, Mexico, Mexico.

Background: In Mexico the shortage of organs for transplant is increasing, due to the lack of timely detection of potential donors: patients with Glasgow Coma Score (GCS) less than 7, with neurological damage that may progress to brain death. Delaying the detection of these patients may contribute to the development of contraindications which could have been prevented and/or corrected.
Objective: To describe the main factors that influence the loss of potential donors in our hospital.
Methods: Retrospective, transversal (cross-sectional) study in which we reviewed information from charts of 44 possible kidney donors (26 were possible but not potential donors and 18 were potential kidney donors). A multiple logistic regression model was used to identify variables associated with the loss of potential donors after the patients were considered to be possible donors.
Results: A total of 150 medical records were reviewed from March 1 to July 1 2015. Regarding gender 27 (38.6%) PD were female and 17 (38.6%) were male; and 60 (62.3%) NPD were female NPD; and 40 (37were male. The main causes of neurological damage were: intra cranial hypertension in 15 (39.1%)PD and in 26 (69%) NPD, neoplasms of the central nervous system in 12 (27.3%) PD and in 2 (11.3%)NPD (53%), other metabolic disorders in 6 (13.6%) PD and in 50 (50.9%) NPD, this was found to be statistically significant (p0.0001) Mean age of patients included in the study was 51.1 ±17.2 years. After the multiple logistic regression model was applied we found that patients who had a medical/surgical procedure performed were 20 times more prone (OR= 20.2)to be lost as potential kidney donors after being considered as possible donors (p <0.05).
Discussion and Conclusions: The lack of timely identification of potential donors by donation services is a major cause of loss. The implementation of a warning system for the remission of all possible potential donors should increase the identification of these patients by coordinator. We conclude that, as reported in other studies neoplasms and infectious diseases were the leading cause of loss of potential donors. Systemic infectious diseases can sometimes be prevented and thus increasing the actual and potential donor pool.

Table 2. Logistic Regression Model for factors associated with the loss of potential kidney donors

Variable	OR	p-value
Age	0.99	0.619
Sex		
Female	1.00	
Male	2.54	0.230
Diagnosis		
Intracranial hemorrhage	1.00	
Head trauma	2.05	0.467
CNS tumor	2.91	0.219
Medical/surgical procedure	20.20	0.013*
Constant	0.66	0.754

OR: Adjusted odds-ratio
* Statistically significant OR estadísticamente with p-value <0.05

Table 1. Demographic characteristics of patients in the ICU classified as possible but not potential, possible and potential kidney donors.

Variable	Possible, but not potential donor (n=26)	Possible and potential donor (n=18)	p-value*
Age, Media (SD)	46,7 (17,8)	52,8 (18,5)	0.272
LOS, Media (SD)	8,5 (5,9)	10,1 (7,1)	0.420
	n(percentage)		
Variable	Possible, but not potential donor (n=26)	Possible and potential donor (n=18)	p-value**
Sex			
Female	14 (53,9)	13 (72,2)	
Male	12 (46,2)	5 (27,8)	0.218
Diagnosis			
Medical/Surgical procedures	9 (34,6)	1 (5,6)	
CNS Tumors	8 (30,8)	4 (22,2)	
Head trauma	4 (15,4)	3 (16,7)	
Intracranial hemorrhage	5 (19,2)	10 (55,6)	0.039
SEPSIS			
No	14 (53,9)	9 (50,0)	
Yes	12 (46,2)	9 (50,0)	0.802
Infection source			
No	13 (50,0)	8 (44,4)	
Lungs	5 (19,2)	4 (22,2)	
Urinary	4 (15,4)	3 (16,7)	
SNC or Abdomen	4 (15,4)	3 (16,7)	0.687
Antimicrobials			
No	9 (34,6)	6 (33,3)	
Yes	17 (65,4)	12 (66,7)	0.930
Renal injury			
No	25 (96,2)	13 (72,2)	
Yes	1 (3,9)	5 (27,8)	0.023
Altered LFT's			
No	22 (84,6)	18 (100,0)	
Yes	4 (15,4)	0 (0)	0.081
Neurological damage			
None/moderate	0 (0,0)	1 (5,6)	
Severe	26 (100,0)	17 (94,4)	0.224

Variables AGE and LOS are presented as mean (SD). Categorical variables are presented as absolute and relative frequency (percentage)

* t-Student test was used for age and LOS.

** Pearson's χ2 was used for categorical variables.

References:

[1] World J Surg. 2012; 36: 2909-2913.
[2] JAMA Neurol. 2015; 72(1):112-118.
[3] CAN J ANESTH. 2006; 53(7): 716-721.
[4] N ENGL J MED. 2015;373(5): 477-478
[5] Transplant Int. 2011; 24: 373-378.

P.1637

Correlation in a year of monitoring kidney donors glomerular filtration rate between dtpa renal scan vs renal creatinine clearance in 24 hrs urine, MDRD-4, CKD-EPI and Cockcroft – gault

Luis García Covarrubias[1], Juan de los Santos[1], Hector Hinojosa[1], Edwin Ventura[1], Garcia Aldo[1], Pedro Fragoso[1], Jose Manuel Torres[1], Diana Fernandez[1], Arturo Reding[2], Hector Diliz[1].
[1]Transplantation, Hospital General de Mexico Dr. Eduardo Liceaga, Mexico, Mexico; [2]Research, Hospital General de Mexico Dr. Eduardo Liceaga, Mexico, Mexico.

Introduction: The TFG is considered as the best index to determine kidney function in general. GFR can be determined by measuring renal clearance intrinsic or extrinsic agent which is freely filtered by the glomeruli. Inulin clearance is considered the gold standard for measuring GFR, invasive, expensive. Measurement of plasma clearance through renal scintigraphy with 99 Tc-diethylenetriaminepentaacetic acid (99m Tc-DTPA) is used because of its accessibility, sensitivity and specificity exceeding 90%.
Objective: To demonstrate that eGFR by CKD-EPI has better correlation with DTPA renal scan in a year after nephrectomy in healthy kidney donors compared to the results obtained by creatinine clearance in 24-hour urine, MDRD and CG.
Materials and Methods: Observational, analytical, transversal, retrospective, in healthy kidney donors, from 1 January 2014 to 31 December 2015, the age variables were collected, weight, height, Body Mass Index (BMI). Calculated eGFR standardize DTPA by an area of 1.73 m2 body surface area. serum levels of serum creatinine and creatinine clearance were determined pre donation and one year follow-up to determine that equation more accurately correlated with the results obtained by DTPA renal scan also determine the decrease in GFR at one year.
Results: From January 1, 2013 to December 31, 2014 58 donor nephrectomies were performed with inclusion criteria 44 patients (25 women, 19 men). The average age 36 years, with an average weight of 65.5kg with 0.6 Kg annual increase. Determination of creatinine was pre donation 0.7 mg / dL. The estimated glomerular filtration rates were pre donation: creatinine clearance 24hrs urine was 108.8 mL / minute, DTPA renal scan 114 mL / minute, CKD-EPI 106 mL / minute, MDRD 99 mL / minute, Cockcroft 115 mL / min. Year follow-up there was a decrease in the glomerular filtration rate of 29.6 mL / minute (urine creatinine clearance 24 hrs 22 ml / min, DTPA 27 mL / min, CKD-EPI 31 ml / min, MDRD -4 33 mL / min, Cockcroft 35 mL / min). When analyzing the linear correlation between the determination of DTPA renal scintigram eTFG by the highest correlation it was obtained with the estimate calculated by CKD-EPI. (p 0.003)
Conclusion: We were able to confirm a decrease in GFR of 29 ml / min in our patients. In addition we found that eGFR with CKD - EPI has a good correlation with the estimated GFR by renal scintigraphy DTPA, which is considered a level worldwide a suitable parameter for and estimate the long-term renal function in monitoring kidney donors

References:

[1] Kasiske BL, Ravenscraft M, Ramos EL, Gaston RS, Bia MJ, Danovitch GM. The evaluation of living renal transplant donors: clinical practice guidelines. J Am Soc Nephr 1996; 7: 2288 –2313.
[2] Norden G, Lennerling A, Nyberg G: Low absolute glomerular fi ltration rate in the living kidney donor: A risk factor for graft loss. Transplantation 2000; 70: 1360–1362
[3] Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976; 16: 31–41.
[4] Imai E, Horio M, Nitta K, Yamagata K, Iseki K, Hara S, Ura N, Kiyohara Y, Hirakata H, Watanabe T et al. Estimation of glomerular filtration rate by the MDRD equation modifies for Japanese patients with chronic kidney disease. Clin Exp Nephrol 2007; 11: 41-50.
[5] Peters AM, Glass DM, Bird NJ. Extracellular fluid volume and glomerular filtration rate: their relation and variabilities in patients with renal disease and healthy individuals. Nucl Med Commun 2011; 32: 649-53.

P.1638

Impact of the network of clinicians and school teachers on awareness and attitudes of adolescents toward organ donation and transplantation

Hee Jung Jeon[1], Jieun Oh[1], Jinsook Yu[2], Goeun Lee[2], Soon Il Kim[2,3], Curie Ahn[2,4], Wonhyun Cho[2,5].
[1]Department of Internal Medicine, Hallym University Kangdong Sacred Heart Hospital, Seoul, Korea; [2]Vitalink, Seoul, Korea; [3]Department of Surgery, Yonsei University College of Medicine, Seoul, Korea; [4]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea; [5]Department of Surgery, Keimyung University School of Medicine, Seoul, Korea.

Background: In Korea, the culture of organ donation and transplantation from deceased donor has not been settled yet. Despite the increasing numbers of patients in need of organ transplantation following the increasing prevalence of chronic diseases, disparities between the supply and demand of transplantable organs are getting increasingly widened. Increased awareness of organ donation and transplantation has been found to have a positive influence on organ donation rates. To educate and raise awareness for adolescents about the importance of organ donation and transplantation, transplant clinicians have built a network with middle and high school teachers and educational professionals. The network intended to explore the school-based educational program about the importance of sharing life for adolescents, and to seek the ways to foster family discussions
Methods: A total of eight conferences and one workshop were held in 2015. In the workshop, we performed pre- and post-surveys for teachers and educational professionals to evaluate changes in their awareness and attitudes toward organ donation and transplantation. An essay and slogan contest for organ donation and transplantation was held for middle and high school students in Korea.
Results: A total of 24 teachers and educational professionals participated in the workshop, and there is increasing percentage of correct answers (mean 22.7%) in the pre- and post-surveys. In the contest, a total 63 essays and 446 slogans were submitted and 44 middle and high school students won prizes. Several teachers had educated and discussed the organ donation and transplantation in their classroom sessions, and they reported students' improvement of knowledge and attitudes toward organ donation.
Conclusions: Our network's aim is to develop education program and curriculum resources about organ donation and transplantation for adolescent. If adolescents' awareness and attitude about organ donation and transplantation is improved, we expect the culture of sharing life would be spread from the students and be settled in our country. Evaluation of its impact on deceased organ donor wish registration and organ donation remains to be seen.

P.1639

The experience of donation coordinator

Anes Kim[1], Hyun Jung Kim[1], Kyung Ock Jeon[1], Sunyoung Son[2], Ji Eun Lee[1], Jayoung Yoo[1].
[1]The Research Institute for Transplantation, Yonsei University, Seoul, Korea; [2]Department of Surgery, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea.

Purpose: Donation coordinator in an organ procurement process is one of the highly stressful job. Little has been written about the experiences of donation coordinators. The purpose of this qualitative study was to understand the experiences in the donation coordinators' practice.
Method: The grounded theory methodology was used for this study. The data was collected through in-depth interview from six participants who were donation coordinators from two hospitals in Seoul. Data was collected between February and March. Theoretical sampling was used until the data reached saturation.
Results: As a result of the analysis, "The burden to be professional while managing emergency works" was identified as the core category. Eleven subcategories were identified and were integrated to the core category. 'Establishment of self-confidence as a donation coordinator' was identified as the consequence.
Conclusion: Lifesaving organ transplantation cannot be performed without donation coordinating. The donation coordinators played various roles, they had a conflict in role identity due to poor working conditions. The results of this study suggested that an increase in understanding of the coordinators' role and institutional support for better working conditions are needed for donation coordinatiors to be acknowledged as professionals.
Key words: Donation coordinator, Transplantation, Experience, Nurses, Qualitative research

P.1640

Measures to improve family discussion for deceased organ donation

Taehyun Lee[1], Yongmin Lee[1], Misung Lee[1], Hwajung Lee[1], Jaesook Oh[1], Kyungsook Jang[1], Hyunjin Kang[1], Sunhee Kim[1], Jongwon Ha[1,2].
[1]Korea Organ Donation Agency, Seoul, Korea; [2]Department of Surgery, Seoul National University College of Medicine, Seoul, Korea.

With the effort of government and transplant society, the number of potential donor reported increased from 1126 cases in 2012 to 1850 cases in 2015. However the transplanted donor has fallen. In an attempt to increase organ donation success rates, we have looked closely in the number of cases where their organs were suitable for transplant however called-off due to death in brain death patients on 2015.
Method: We have analyzed the steps in how medical staff explains brain death to family in which its basis was based on study conducted by Franz and others on 1997 "Family communication protocol for explaining brain death" that was modified to local situation.
Result: 1850 cases were reported in 2015 due to enforcement of new policy, 500 cases were successful in leading to organ donation which rest of 1350 cases the donation wasn't proceeded where 196 cases the donation wasn't possible due to death of the patient. In the successful organ donor group, 76% of patients were notified of brain death within 7 days of admission, with average being 7 days. The unsuccessful group showed a similar percentage where 79% of patients were notified of brain death within 7 days of admission. In group where 196 patients die before preceding to donation, average time it took from declaring brain death to patient death took average of 2.33 days where 61 cases (36%) of the patient died on the date of declaring brain death. However, majority of patients (97cases, 57%) died within the first 7 days. In the process of medical staff explaining brain death to patients' family, only 72 cases (37%) mentioned the process of organ donation and the rest of 124 cases (73%) failed to mention about the organ donation. Out of the patient's family that hadn't received any explanation of organ donation, 123 cases were explained with 1st stage of brain death, 60 cases of 2nd stage, 4 cases of 3rd stage. We can see most of the medial staff were able to explain to the patients' family about the first stage but weren't able to proceeded to explaining 1,2 and 3rd stages which notifies the OPO. Furthermore, group where patients' family where explained showed average time of 1.9 days from declaration date to expiration date and 4.97 days from admission date to expiration date whereas the non-explained group shows not much of a difference.
Conclusion: The difference in average time of admission to notification between the successful organ donor group and the unsuccessful group due to death of the patient showed insignificant results. Furthermore, in the unsuccessful group, no significant results showed between the explained group and non-explained group. Therefore, after diagnosis of potential brain dead patients, rapid communication process with family is vital to the process of organ donation. For organizing effective organ donation process, it is important to separate explaining brain dead state by medical staff and communication for organ donation by OPC.

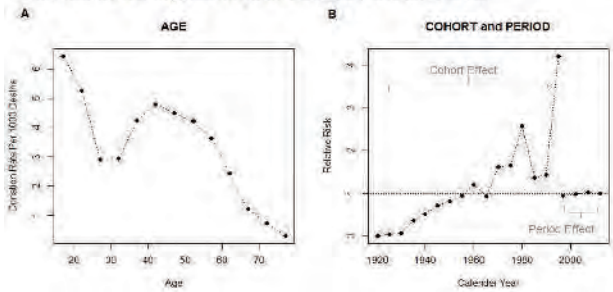
P.1641

Deceased organ donation in Hong Kong (1995-2014): An age-period-cohort analysis

Maggie Kam Man Ma[1], Mabel Chow[2], Ka Foon Chau[3], Bo Ying Choy[1], Samuel K.S.Fung[4], Philip K. T.Li[5], Jenny Koo[6], Mary Schooling[7], Tak Mao Chan[1].
[1]Department of Medicine, Queen Mary Hospital, The University of Hong Kong, Hong Kong, Hong Kong; [2]Transplant Coordination Service, Princess Margaret Hospital, Hong Kong, Hong Kong; [3]Department of Medicine, Queen Elizabeth Hospital, Hong Kong, Hong Kong; [4]Department of Medicine and Geriatrics, Princess Margaret Hospital, Hong Kong, Hong Kong; [5]Department of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong, Hong Kong; [6]Central Nursing Department, Queen Mary Hospital, Hong Kong, Hong Kong; [7]School of Public Health, The University of Hong Kong, Hong Kong, Hong Kong.

Introduction: Hong Kong has an opt-in organ donation system; the organ shortage is one of the greatest challenges to the Hong Kong transplant services. This study aims to evaluate the impact of socioeconomic development on organ donation in Hong Kong.
Materials and Methods: All deceased organ donors registered in the Organ Procurement System and Organ Transplant Coordinators' record between 1995 and 2014 were included in the analysis. The overall time trends in deceased organ donation rate and donor profile were assessed. To further understand trend of donor conversion, we decomposed the organ donation rate (per 1000 deaths) into the effects of chronological age, donation period and donor birth cohort by using a Poisson age-period-cohort (APC) model.
Results and Discussion: A total of 674 deceased organ donors (389 male and 258 female, mean age 45.9±15.1 years) were included. The deceased organ donation rate increased modestly from 3.7 donors per million populations (pmp) in 1995 to 5.4 donors pmp in 2014. During those 20 years, donor age ($p<0.001$), hypertensive donors ($p=0.049$) and expanded criteria donors (ECD) ($p=0.013$) increased over successive era (Table 1). The APC model (Figure 1) showed, as would be expected, donation rates after death dropped with increasing age, consistent with higher prevalence of co-morbidities, and cancer- and infection-related deaths among older people likely rendering them unsuitable for organ donation. Birth cohort contributed to the increased donation rate after death, in particular, upward inflections was demonstrated in the first generation to reach adulthood in Hong Kong (1930s birth cohorts) and the 1960s birth cohort. Conversely, period effects did not appear to contribute.
Table 1. Donor Characteristics According to Transplant Era

Figure 1. Plot of the Parameter Estimates of the Age, Period and Cohort Model. (A) Age-specific Donation Rate per 1000 Deaths. (B) Relative Risk of Organ Donation After Death According to Donor Birth Cohort and Donation Period.



	1995-1999 (n=124)	2000-2004 (n=156)	2005-2009 (n=182)	2010-2014 (n=212)	P
Mean donor age, n (%)	38.4±14.6	46.2±15.8	47.6±13.9	48.6±14.5	<0.001
Male Gender, n (%)	73 (58.9)	92 (59.0)	106 (58.2)	118 (55.7)	0.906
Hypertension, n (%)	17 (15.6)	38 (24.4)	55 (30.2)	53 (25)	0.049
Diabetes, n (%)	4 (3.7)	6 (3.8)	10 (5.5)	9 (4.2)	0.854
ECD, n (%)	16 (13.7)	35 (26.3)	48 (29.6)	50 (28.2)	0.013

Conclusion: The organ donation rate only increased modestly over the last 20 years in Hong Kong, partially driven by more donations from older and marginal donors. The first generation to reach adulthood in the relatively developed setting of Hong Kong had a higher donation rate (1930s birth cohort), as did the next generation (1960s birth cohort), suggested that socioeconomic change over generations could account for the change in acceptance of organ donation in the general population. However population wide influences affecting the population (period effects) did not appear to affect the donation rate. This suggests that organ donation might be connected with attitudes that change slowly over generations, so that more active policies to promote organ donation may be needed to ensure that organ donations are increased to meet the current organ shortage.

The authors would like to gratefully acknowledge the substantial contributions of the entire Organ Donation Coordination Team for their effort in organ procurement and promotion of organ donation, and the Organ Registry and Transplantation System (ORTS) team to maintain the ORTS database

P.1642

Organ and tissue donation in Emergency Clinical Hospital of Oradea Romania

Carmen Pantis, Pataki Stefan, Magyar Ioan, Mohan M.Aurel, Sabau Tudorel Florian.
ICU, Emergency Clinical County Hospital, Oradea, Romania.

Introduction: Emergency Clinical Hospital Oradea is situated in nordwest Romania; there are 1112 beds and one ICU with 30 beds in order to rezolve the emergency for a population of 550 000 inhabitants. Our study make a statistics about organ and tissue donation in the most performant hospital from Romania.
Quantitative descriptive analysis was used to describe the main features of the data. In Romania family consent is always requested before organ and tissue donation and each family have to consent for organ (heart, liver, kidneys) or tissue (skin, corneea, bones and tendons).
Material and Methods: Transplantation is a treatment for end-organ failure. We study 8 years of activity in ICU between 2008-2015 for organ and tissue donation. During this period our team (ICU doctors and nurses) identify 407 potential organ donors in ICU. The medical team from Oradea have worked very hard to ensure development of an ambitious program. Donor data are recorded by two trained hospital transplant coordinators according to precise rules and definitions. All brain dead patients referred for requesting authorization from families for organ and tissue donation were examined.
The interview setting was measured by location,doctors or nurse doing the interview,total numbers of interview and number of family members attending the interview,family rural or urban origin,family religion catholic, orthodox, protestant or agnostic, patient consent during his life time,the family respected the wishes of their relative,adequate family satisfaction with hospital care ,family member signing written informed consent to organ donation and it was the family who opted for donating the organs and tissue or only the organs, family member siging consent, age ,gender,length of interview,burial or cremation,consent to organ donation,consent to tissue donation,reasons :patient consented during life time,socio-economic factors,coroner autopsy, others, family refusal.
Results: From 1 January 2008 to 31 December 2015 there were 407 consecutive brain-dead patients and 226 family interviews for requesting organ and tissue donation for transplantation took place in ICU. 147 families accept organ donation and from them only 77 families accept organ and tissue donation. 201 brain dead donors were hemodinamically unstable, 47 brain dead donors were infected, with C virus and 30 brain dead donors were infected with Atg Hbs virus, 2 patients were malignant brain tumour and 1 patient was coroner refusal because suspicion of violent murder. 76 families from 226 refuse organ and tissue donation.
The concept that cerebral death is death must be explained extremely simple and clear.Often the position for donation is immediate during the first request.
Conclusion: Every family and every situation is different.Continual improvement in family interviews is necessary in all hospitals to increase the number of donors and the quality of organs and tissue available for transplant.

P.1643

Unexpected donors after circulatory death: Coagulation profiles after prolonged cardiac arrest in a porcine model

Stephanie Rakestraw[1], Nicole Werner[1], Fares Alghanem[1], Ryan Coute[3], Robert H.Bartlett[1], Robert W.Neumar[3], Alvaro Rojas-Pena[1,2]. [1]Surgery, University of Michigan Health System, Ann Arbor, United States; [2]Surgery, Section of Transplantation, University of Michigan Health System, Ann Arbor, United States; [3]Emergency Medicine, University of Michigan Health System, Ann Arbor, United States.

Introduction: Organ donation after circulatory determination of death (DCD) could be a major source of organs for transplantation, but it is limited by the logistics of procuring organs immediately after death. Intravascular complications of full-body hypoxia (agonal time) and ischemia (cardiac arrest), including microvascular coagulation, leukocyte adhesion, and endothelial injury, cause "no-reflow", affecting organ function. Blood samples where analyzed by thromboelestography (TEG) to study the dynamics of the coagulation system in our swine model of 60 minutes of cardiac arrest (unexpected DCD).
Methods: 10 healthy anesthetized swine were used in the study. Cardiac arrest (CA) was induced by ventricular fibrillation. Animals were allocated in two groups: **uDCD** (unexpected DCD, n=5), where animals were left untreated for 60 minutes in a no flow state; **uDCD+CPR** (n=5), untreated cardiac arrest for 10 minutes, followed by 50 minutes of CPR to simulate a low-flow state with mechanical cardiopulmonary resuscitation. Whole blood serial central venous blood samples from the inferior vena cava were obtained and analyzed with TEG for 60 minutes. TEG was measured prior CA (baseline), after 30 minutes, and 60 minutes of CA.
Results and Discussion: There was no significant difference between **uDCD** and **uDCD+CPR** groups at pre-arrest baseline. TEG diagrams for each group are shown for baseline, 30 minutes and 60minutes (Figure1). When compared to baseline (9.4±1. min) R values in the **uDCD** group were shorter after 30 minutes and 60 minutes (4.2±0.6 and 4.4±1.2min, respectively). In the **uDCD+CPR** (low-flow) this difference was not observed and R values at baseline, 30 minutes, and 60 minutes were within a similar range. All other parameters were similar between groups and CA time. Results illustrated in Figure 1, demonstrate an initial hypercoagulable state detected at 30 minutes of cardiac arrest followed by what appears to be a consumptive coagulopathy by 60 minutes of cardiac arrest in the **uDCD** group only.
Conclusion: A uDCD no-flow state results in shorter clot initiation than a uDCD+CPR low-flow state. CA develops into a hypercoagulable state followed by consumptive coagulopathy, indicating thrombosis that may affect organ viability.

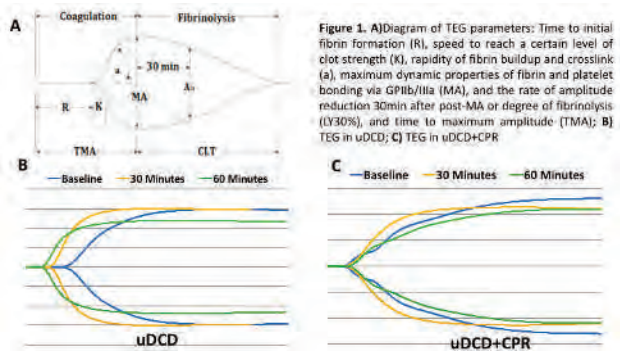


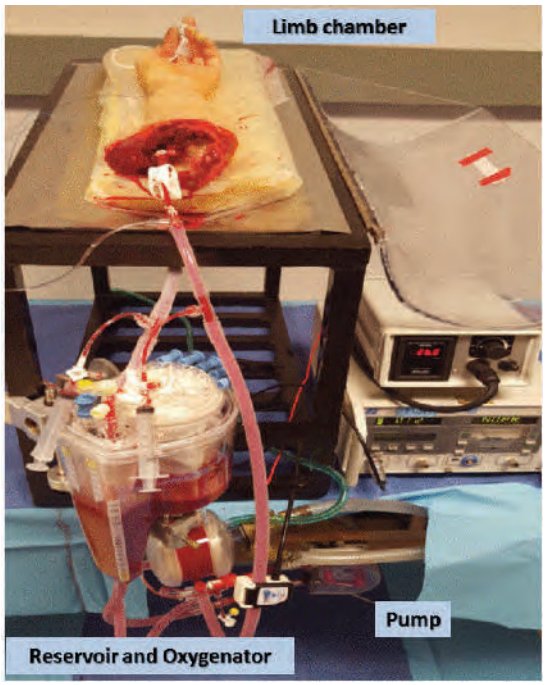
Figure 1. A) Diagram of TEG parameters: Time to initial fibrin formation (R), speed to reach a certain level of clot strength (K), rapidity of fibrin buildup and crosslink (a), maximum dynamic properties of fibrin and platelet bonding via GPIIb/IIIa (MA), and the rate of amplitude reduction 30min after post-MA or degree of fibrinolysis (LY30%), and time to maximum amplitude (TMA); **B)** TEG in uDCD; **C)** TEG in uDCD+CPR

P.1644

Vascularized composite allografts: Prolonged (24hr) Ex situ perfusion of human limbs

Alvaro Rojas Pena[1,2], Nicole L.Werner[2], Fares Alghanem[2], Stephanie L.Rakestraw[2], Dylan Sarver[4], Paul L.Lange[3], Steve M.Rudich[3], Christopher Mendias[4], John C.Magee[1,2], Robert H.Bartlett[2], Kagan Ozer[2,4].
[1]Department of Surgery, Section of Transplant Surgery, University of Michigan Health System, Ann Arbor, United States; [2]Department of Surgery, Extracorporeal Life Support Laboratory, University of Michigan Health System, Ann Arbor, United States; [3]Gift of Life Michigan, Ann Arbor, United States; [4]Department of Orthopedic Surgery, University of Michigan Health System, Ann Arbor, United States.

During the last 3 years our laboratory developed a novel *ex situ* perfusion system to prolong limb allograft viability in a porcine forelimb amputation/transplantation model. Using this animal model we were able to demonstrate the potential to preserve vascularized composite allografts (VCA) for 24hr. In our current translational study, we present our experience with the perfusion of human limbs for up to 24hr with our near-normothermic (31±1°C) *ex situ* perfusion system.
Methods: 5 human forearms were procured from brain-dead adult donors under tourniquet control. Following elbow disarticulation, the brachial artery was cannulated. The limb was flushed with heparinized saline and connected to a temperature controlled (31±1°C) *ex situ* perfusion system (**Figure 1**) for 24hr. Blood derived perfusate (fresh plasma and red blood cells targeting a hemoglobin (Hb) concentration of 4-6 g/dL) was used. Muscle biopsies (flexor carpi radialis) were obtained. *Ex situ* perfusion was pressure controlled to <110 mmHg systolic pressure, and perfusion flow was 6-8% of the estimated donor cardiac output.
Results: Average warm ischemia time was 72.2 min (range 40-88 min). Average perfusion pressure of 98±2 mmHg, perfusion flow of 318±18 mL/hr, and calculated vascular resistance of 158±18 mmHg/L/min. Perfusate had an average pH of 7.43±0.04, pCO_2 32±1 mmHg, paO_2 317±18 mmHg, and Hb 4.8±0.4 g/dL. All electrolytes remained within physiological range, except for lactate that which increased steadily throughout the experiment. However, neuromuscular electrical stimulation revealed ongoing contraction throughout the experiment, and hand histopathology showed no muscle injury. At the end of the *ex situ* perfusion the limb gain weight was <5% compared to baseline values.
Conclusion: All limbs appeared viable after 24hr of *ex situ* perfusion. In this model the long-term function of the extremity cannot be evaluated. However, this approach could help extent and improve VCA preservation by minimizing cellular injury associated with cold (5°C) storage.



R.E. Pietroski. Gift of Life Michigan, Ann Arbor, MI -USA

References:

[1] Ozer K, Rojas-Pena A, Mendias CL, Bryner BS, Toomasian C, Bartlett RH. The Effect of Ex Situ Perfusion in a Swine Limb Vascularized Composite Tissue Allograft on Survival up to 24 Hours. Hand Surg Am. 2016 Jan;41(1):3-12 ; PMID: 26710728

[2] Ozer K, Rojas-Pena A, Mendias CL, Bryner B, Toomasian C, Bartlett RH. Ex Situ Limb Perfusion System to Extend Vascularized Composite Tissue Allograft Survival in Swine. Transplantation. 2015 Oct;99(10):2095-101. PMID: 25929606

P.1645

The effectives of an organ donation process in Chiangrai Prachanukroh Hospital

Thanyapat Phongwiwat, Jeamjitt Shevitthai, Porntiwa Camwan. Organ donation center, Chiangrai Prachanukroh Hospital, Chiang Rai, Thailand.

Introduction: Organ Transplantation is used for treating patients with chronic incurable diseases, so organ transplantation recipients can live longer and have better quality of life. However, the major problem is the shortage of organs donors. As one says "When there is no organ, there is no organ transplantation recipient", and then help for and hope of patients decrease. Statistics from the Organ Donation Center Thai Red Cross 2013, the numbers of registered donor cases were158; whereas, 4081 cases were on a waiting list and three of them died every week. Particularly, Chiangrai Prachanukroh hospital has joined in the organ donation hospital network since 2007. From June 2007 - July 2014, the number of decease donors were 9 cases, but number of waiting cases for a kidney transplant were 25 cases which is higher than supply two and a haft time. This is critical. Thus, this study was conducted to understand contexts and motivation of donation so as to increase the number of donors.

Purpose: The purpose of this study was to study effectsof organ donation based on an organ donation process of Chiangrai Prachanukroh hospital.

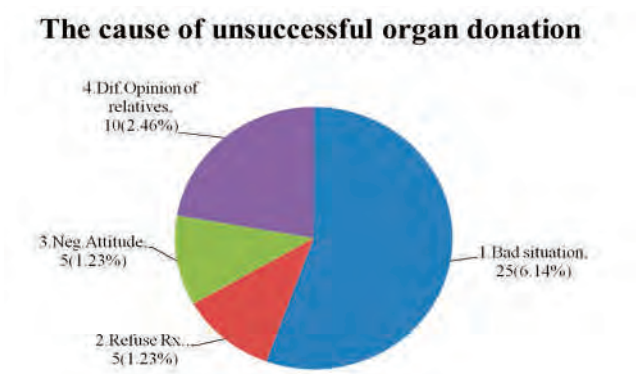
Research Design: Descriptive research: Cohort design.

Method: The sample included 20 relatives who were negotiated to donate organs of decease donors based on the organ donation process of Chiangrai Prachanukroh Hospital. This process (SPIKES) comprises 6 steps: leaflet of brain dead knowledge, organ donation, and its pictures. Data were collected by structured interview andquestionnaires. Research data were analyzed by content analysis and descriptive statistics (percentage).

Results: The results revealed that 80 % of relatives of decease donors were female, lived in other districts, and never had knowledge about brain death. They never have knowledge (40%) or experience (75%) about organ donation. They did not donated organs because 1) the relatives of donor have different opinion (40%), 2) they afraid of incomplete body organs (40%), 3) they could not accept the death of donors (15%), and 4) they refused treatment (5%).

Those subjects (84.61%) who had knowledge about brain death, organ donation, or experience of organ donation showed positive thinking to organ donation than those who had not., Subjects(28.57%) who had positive thinking to organ donation had influence to organ donation in the future.

Conclusion: Information of organ donation such as knowledge of brain death, organ donation, and organ transplantation is very important especially for people who live in other districts. When they have enough and correct information, they will have positive decision for organ donation.



No	Subject			Opinion to organ donation	Influence to organ donation
	Knowledge about brain death	Knowledge about organ donation	experience of organ donation		
1	-	+	+	+	+/-
2	-	+	-	+	+
3	-	-	-	-	-
4	-	-	+	-	+/-
5	-	+	-	+	+/-
6	-	+	-	+	+/-
7	-	+	-	+	+
8	-	-	-	+	+/-
9	-	-	-	-	-
10	+	-	-	-	+/-
11	-	-	-	-	-
12	+	+	-	+	+
13	-	-	-	-	+/-
14	+	+	-	+	+/-
15	-	+	+	+	+/-
16	-	+	+	+	+/-
17	-	-	+	+	+/-
18	+	-	-	+	+/-
19	-	-	-	+	+/-
20	-	-	-	+	+

Prof. Drs. Jayanton Patumanond; Chiangrai Prachanukroh Hospital

References:

[1] Ellen Bridget Linde ,(2009).Speaking up for organ donors.
[2] Marian Ryan.(2004) More public education needed on organ donation.
[3] David Harley (2002). Public education is the way to increase organ donation.
[4] Rogers, Everett M. (1983). Diffusion of Innovations. New York, A Division of Macmillan.

P.1646

Is the kidney donor risk index (KDRI) applicable to all populations? The Puerto Rican experience

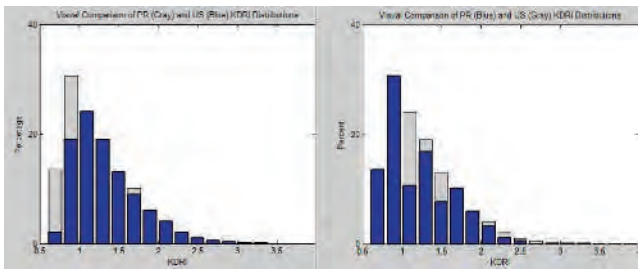
RafaelG. Ramos[3], Juan F.Ruiz[1], Eduardo Michelen[7], Luis A.Morales[2,5], Carlos del Coro[4], Marien Saade[6], Esther Torres[1,5]. [1]Medicine, University of Puerto Rico School of Medicine, San Juan, Puerto Rico; [2]Surgery, Hospital Auxilio Mutuo, San Juan, Puerto Rico; [3]Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA, United States; [4]Surgery, Las Palmas Medical Center, El Paso, TX, United States; [5]LifeLink of Puerto Rico, LifeLink of Puerto Rico, San Juan, Puerto Rico; [6]Continuing Education, UPR School of Nursing, San Juan, Puerto Rico; [7]Natural Sciences, University of Puerto Rico Rio Piedras Campus, San Juan, Puerto Rico.

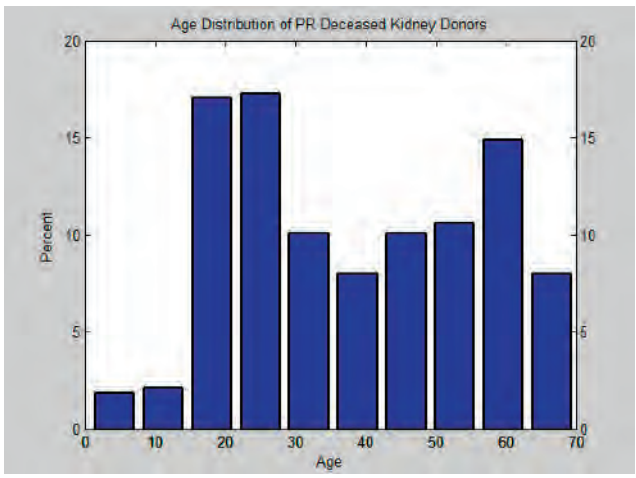
Introduction: The purpose of this study was to evaluate the effectiveness of the Kidney Donor Risk Index (KDRI) as a predictor of graft failure risk in Puerto Rican deceased donor kidneys. Previous studies have established the validity of the KDRI in the US population. However, Puerto Ricans are Hispanics of admixed racial background with different demographic, genetic and health factors and the applicability of the KDRI has not been evaluated in our population. We developed a systematic process to test the KDRI model in the Puerto Rican population as a tool for the clinical assessment of donor kidneys. This initial report highlights differences between the distribution of KDRI values in the USA and PR populations. We propose potential causes that can explain this difference.

Methods: 376 kidney donors from LifeLink of Puerto Rico between 2009-2011 were evaluated. Of these, 240 kidneys were transplanted locally and the data was collected from the transplant center electronic medical charts. KDRI values were calculated using the same method of computation used for US deceased donor kidneys. The resulting data was analyzed and contrasted with corresponding US data published in the original study. Demographic details of Puerto Rican deceased donors were also obtained and analyzed. The study was approved by the IRBs of the Auxilio Mutuo Hospital and the UPR Medical Sciences Campus.

Results and Discussion: The median KDRI value for the Puerto Rican population was found to be 1.07 which is comparable to the US median KDRI of 1.05. However, the distribution of KDRI values in the Puerto Rican population deviates significantly from that of the US. A two-peak distribution pattern describes Puerto Rican KDRI values, with two distinguishable clusters of kidneys having KDRI values of 0.85 and 1.32. This two-peak distribution pattern is mirrored by the age distribution of deceased donors obtained from the Puerto Rican population, hinting a potential explanation for this phenomenon. The two-peak distribution highlights how a population's median KDRI value fails to accurately describe the state of its donor kidneys and the importance of evaluating the entire population's KDRI distribution.

Conclusion: Our results highlight how distinct Puerto Rican demographics affect the distribution of KDRI values. In particular, the effect young donor age seems to have of our KDRI distribution may be a feature shared by other populations with a high incidence of youth mortality. A study evaluating the outcome of the recipients of these kidneys and its relation to the KDRI is ongoing. The impact of other variables in our population not included in the KDRI formula on the outcome of the transplants is unknown, and warrants further investigation. The identification of variables specific to a geographically distinct group may result in better donor selection, allocation and transplant outcomes.





References:

[1] Port, F.K. et al. Donor characteristics associated with reduced graft survival: an approach to expanding the pool of kidney donors. *Transplantation*. 2002; 74: 1281-1286.

[2] Rao, P.S., et. al. A comprehensive risk quantification score for deceased donor kidneys: the kidney donor risk index. *Transplantation*. 2009; 88: 231- 236.

[3] Rao, P.S. & Ojo, A. The alphabet soup of kidney transplantation: SCD, DCD, ECD—fundamentals for the practicing nephrologist. *Clinical Journal of the American Society of Nephrology*. 2009; 4: 1827-1831.

[4] Watson, C.J., et. al. A simplified donor risk index for predicting outcome after deceased donor kidney transplantation. *Transplantation*. 2012; 93: 314-318.

[5] Wolfe, R.A., et. al. Calculating life years from transplant (LYFT): methods for kidney and kidney-pancreas candidates. *American Journal of Transplantation*. 2008; 8: 997-1011.

[6] Wolfe, R.A., McCullough, K.P., & Leichtman, A.B. Predictability of survival models for waiting list and transplant patients: calculating LYFT. *American Journal of Transplantation*. 2009; 9: 1523-1527.

P.1647

Demographic and patient profiles in an area of low organ donation rate

Ming-Hui Shih[1], Kuei-Chun Chou[1], Dai-Yu Chung[2], Ming-Che Lee[2,3], Yen-Cheng Chen[2,3], Ching-Chun Ho[2], Chuan-Hsiu Tsai[1,4]. [1]Department of Nursing, Tzu Chi General Hospital Hualien, Hualien, Taiwan; [2]Department of Surgery, Tzu Chi General Hospital Hualien, Hualien, Taiwan; [3]School of Medicine, Tzu Chi University, Hualien, Taiwan; [4]Department of Nursing, Tzu Chi University, Hualien, Taiwan.

Introduction: When it was established in 2004, Taiwan Organ Registry and Sharing Center (TORSC), a non-profit organization funding by the government, is in charge in the promotion of organ donation, organ recovery, organ allocation and registration in Taiwan. The organ donation rate arises from 7.2 per million population (PMP) in 2006 to 10.3 PMP in 2015. As a member of Organ Procurement Organization supervised by TORSC, Tzu Chi General Hospital is the only one medical center and transplant center that obligates to manage the work regarding to organ donation and transplantation. Since eastern Taiwan is considered to be a rural area with multiple culture in influence, thus, the purpose of this study was to analyze the demographic and patient profiles in this area.

Materials and Methods: We retrospectively reviewed the medical records of 121 organ donors between 2005 and 2015 in our hospital. Descriptive data analysis was utilized to examine the profile of the donor characteristic.

Results: Totally 121 potential donors have been persuaded into process of organ recovery during this period. There was 34 patients successfully donated their organs. The mean age of the donors was 40.6±12.2 years. The male to female ratio was 79.4% to 20.6%. The major causes of death of those organ donors included cerebrovascular disease (52.9%) and traumatic cerebral hemorrhage (32.3%). The mean duration of hospitalization was 4.4±5.2 days. For those 87 potential donors we failed to convert the families' will for organ donation (failure group), the mean age of the patients was 44.9±14.9 years, and 70.1% were male. The most common causes of death were traumatic cerebral hemorrhage (49.4%). The factors associated with the refusal were listed as followings: family disagreement (67.8%); lack of understanding of illness progression (17.2%), uncontrolled medical conditions (12.6%) and patient related factor (2.3%). Cause of death between success donation group and failure group was significantly different ($p<0.05$).

Discussion: The leading cause of dead was traumatic cerebral hemorrhage (49.4%) in donation failure group, comparing to 39.4% in the success group. Apparently, the families of trauma cases may be psychologically unprepared, so they tend to disagree with the organ donation idea. In respond to their loss, the main reason of refusal was family disagreement (67.8%) and deviation of concerns about donation. Family decision plays a crucial role on major decision making. Family has a determined power in signing consent of donation. In Taiwan, a close relative of the patient is the key person for consent donation.

Conclusion: The deceased organ donation rates of PMP were around 10.3 cases in Taiwan in 2015. This was much lower than in the western countries. Based on our study, enabled the family numbers using enough time to discuss was an important arrangement toward fully discussion. We should promote the consent that discussed with the family about one's willingness of donation and registration. Through public education, donation rate improvement should be expected.

P.1648

Doubts and questions – Differences in the attitude of healthcare staff and lay people towards organ donation

Aniko Smudla[1], Timea Horvath[1], Emilia Korpas[1], Imre Fehervari[1], Sandor Mihaly[2], Zoltan Mathe[1], Janos Fazakas[1].
[1]Department of Transplantation and Surgery, Semmelweis University, Budapest, Hungary; [2]Organ Coordination Office, Hungarian National Blood Transfusion Service, Budapest, Hungary.

Introduction: The success of transplant programmes is determined by the attitude towards donation[1],[2]. The aim of this study was to investigate the knowledge and the attitude of healthcare staff and lay people regarding transplantation.
Materials and Methods: The results of previous investigations carried out with the same method were compared. The knowledge and the attitude of lay people (European Donation Day 2012; n=257) concerning transplantation and brain death were compared to those of students of Semmelweis University Faculty of Health Sciences (BSc, MSc, ambulance care assistants; n=111), congress of anaesthesiologist assistants and intensive care nurses (AITSZME 2013; n=98), and emergency care nurses (2015; n=78). The date were analysed with SPSS 20.0.
Results and Discussion: There was a higher percent of male and elder participants in the lay group (42.8% vs. 15%; p=0.000; 36.3 vs. 45.1 years; p=0.000). Compared to lay people, professionals were able to define brain death in a higher percentage (86.1% vs. 71.2%; p=0.000), but no difference was found regarding the acceptance of brain death: 41.5% accepted the fact without reservation that the brain-dead is identical with death, 44.5% with reservation, while the others refused it. Although 71.0% of the healthcare staff and 59.1% of the lay people (p=0.004) were familiar with the legal requirement of the strong version of presumed consent principle, a significant number of the healthcare staff (84.1% vs. 66.9%; p=0.000) supported the everyday practice demanding family consent. More young people (p=0.028; OR: 1.027), women (p=0.049; OR: 2.060), and lay people (p=0.004; OR: 3.273) were willing to donate their own organs, while the donation of their relatives' organs was supported in a higher percentage by lay people (p=0.000; OR: 3.235), participants familiar with the relevant legislation (p=0.020; OR: 1.715), and participants accepting brain death (p=0.000; OR: 2.545). A higher percent of the lay people gained information about donation in newspapers (40.6% vs. 21.8%; p=0.000), on internet (48.6% vs. 33.7%; p=0.000), and on television (65.8% vs. 40%; p=0.000), whereas 70.2% of the healthcare staff were informed about it in the medical care system (70.2% vs. 28.8%, p=0.000). Only a limited number of the participants were provided information by leaflets (9.0%) and patients associations (15.7%).
Conclusions: Although the surveys cannot be considered representative, they draw the attention to that fact that professionals have a more profound acquaintance of transplantation proceedings, but this knowledge does not influence the acceptance of brain death and the attitude towards donation in a positive way. While society can be reached through social media, the healthcare professionals' attitude can be changed only with targeted training. To determine the most effective educational methods for enhancing donation representative and comprehensive inquiries are needed.
References:
[1] Roels L. Critical Care staffs' attitudes, confidence levels and educational needs correlate with countries' donation rates: data from the Donor Action® database. *Transplant International* 2010; 23: 842–850.
[2] Smudla A. The attitude and knowledge of intensive care physicians and nurses regarding organ donation in Hungary – It needs to be changed. *Ann Transplant* 2012; 17: 93-102.

P.1649

Deceased organ donation in a public hospital - A model programme

Sujatha Suriyamoorthi, Sunil Shroff, Sumana Navin, Hemal Kanvinde.
MOHAN Foundation, Chennai, India.

Organ transplantation is one of the greatest advances of modern science that has resulted in many patients getting a renewed lease of life. In 1994 the Government of India passed the Transplantation of Human Organs (THO) Act that legalized the concept of brain death, making possible organ transplants from brain dead donors. An amendment in the law in 2008 made it mandatory for the hospitals to nominate a transplant coordinator before they are registered as transplant centres. As a result a considerable increase in the number of deceased donations occurred - from 0.16 per million population (pmp) in 2012[1] to 0.34 pmp in 2014[2].
Transplant coordinators play a pivotal role in deceased organ donation and transplantation. Their presence has been a key factor in augmenting the deceased donation programme (DDP). This has been established in Rajiv Gandhi Government General Hospital (RGGGH), Chennai - the apex public sector hospital in the state of Tamil Nadu, India. Only a handful of public hospitals in the country are participating in DDP and RGGGH is one of them. It has had the largest number of multi-organ donors in the country in a public sector hospital. In 2013, RGGGH had the fourth highest number of deceased donors in Tamil Nadu.
In February 2010 MOHAN Foundation entered into a Memorandum of Understanding (MoU) with RGGGH to place its trained transplant coordinators to facilitate DDP[3]. There was also active participation and support from the hospital administration, neurosurgeons and intensivists.
From February 2010 to June 2015, 187 brain deaths were certified in the hospital. Families of 185 brain dead patients were counseled for organ donation. 116 families said "yes" to organ donation (conversion rate 62%). 2 cases of brain death were not approached for organ donation because of medicolegal reasons. 472 organs and tissues were retrieved and shared by different hospitals through the state registry of Tamil Nadu. 206 corneas were also retrieved during this period as a result of sensitization of paramedical staff of RGGGH by transplant coordinators.
Rajiv Gandhi Government General Hospital (RGGGH), Chennai, India
References:
[1] Indian Transplant Newsletter (ITN) - Volume 12 - Issue 37 (November 2012 - February 2013)
[2] Indian Transplant Newsletter (ITN) - Volume 14 - Issue 43 (November 2014 - February 2015)
[3] BMJ 2013;346:f2155 doi: 10.1136/bmj.f2155 (Published 12 April 2013)

P.1650

Barriers to early post-operative discharge in living liver donors

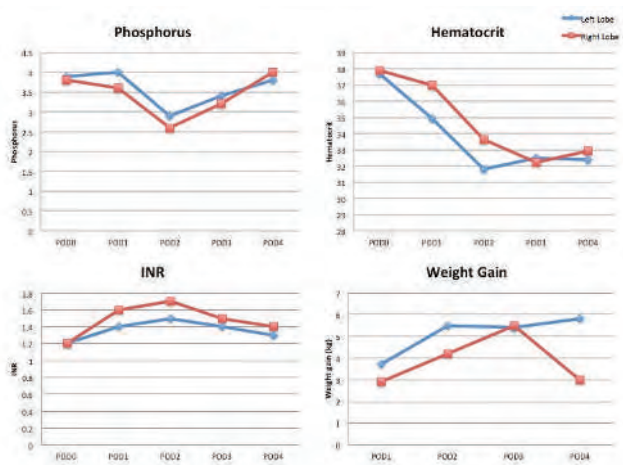
Hillary Braun[1], Garrett R.Roll[2], John P Roberts[2], Nancy L.Ascher[2].
[1]School of Medicine, University of California, San Francisco, San Francisco, United States; [2]Department of Surgery, University of California, San Francisco, San Francisco, United States.

Introduction: Hospital length of stay (LOS) has emerged as a quality, safety, and resource utilization metric over the last decade, and prolonged LOS is associated with increased risk of nosocomial infection and iatrogenic injury. Resource utilization and avoidance of complications are important elements in containing costs in live donor liver transplantation (LDLT). Enhanced recovery after surgery (ERAS) protocols are designed to minimize LOS but have not been implemented in living donors. We implemented several measures to improve post-operative recovery: 1) early oral nutrition, 2) left lobe (LL) donors receive epidural analgesia, which is removed when INR<1.4, and 3) intravenous phosphorus (phos) repletion to serum level > 3 to avoid adverse effects of oral phos on bowel motility. The purpose of this study was to identify the barriers to discharge (other than those related to postoperative complications) on post-operative day (POD) three following liver donation.

Materials and Methods: We conducted a retrospective chart review of LL and right lobe (RL) living donors from January 2015-February 2016. Basic demographic data and clinical details including pain management, diet, and bowel function were obtained, along with INR, hematocrit (Hct), phos, and daily weights from admission to POD4. Data are presented as median (interquartile range (IQR)) and differences were evaluated using Wilcoxon rank sum tests.

Results: 32 adult to adult LDLT (18LL, 14RL) were performed during the study period. Donors were 59% female (19/32), 34.5 (18.7-50.3) years of age, and discharged on POD 6 (4-8). There was no significant difference in median LOS between RL and LL donors (6 vs. 6, p=0.694). There was one readmission within 30 days for nausea and constipation that resolved with an oral bowel regimen.

The median duration of epidural analgesia in LL donors was 3 (1.7-4.3) days, and the time between epidural removal and discharge was 2 (0-4.3) days. Between RL and LL donors, there was no significant difference in days to return of bowel function (3(2-4) vs. 3(1-5), p=0.125) or days to regular diet (2(0.7-3.3) vs. 2(0-4), p=0.116). Between RL and LL donors, there were significant differences in POD1 phos (3.6 vs. 3.9, p=0.005), POD2 phos (2.6 vs. 2.8, p=0.018), and POD3 INR (1.5 vs. 1.4, p=0.009). Weight gain was substantial for RL and LL donors, but there was no significant difference on POD3 (5.5 vs. 5.4 kg, p=0.649). Serum phos, Hct, INR and weight change trends are shown in Figure 1. Barriers to discharge on POD3 are presented in Table 1.



Donor	Lobe	Discharge Day (POD)	Clinical Barriers (POD3)	Lab Barriers (POD3)
1‡	Right	5	Awaiting return of bowel function	None
2	Right	5	Awaiting return of bowel function	None
3	Left	5	Epidural, foley	None
4	Left	7	Epidural, poor pain control	None
5	Left	6	Epidural, foley, nausea	Hypophosphatemia
6	Left	6	Epidural, foley, awaiting return of bowel function, nausea/vomiting	None
7	Left	5	Epidural, awaiting return of bowel function	None
8	Left	8	Epidural	Hypophosphatemia
9	Left	4	Epidural, awaiting return of bowel function	None
10	Left	5	Epidural, foley	None
11	Right	7	IV pain meds, nausea, awaiting return of bowel function	None
12	Right	7	Nausea	None
13	Left	5	Epidural	None
14*	Left	6	Edema, IV pain meds, awaiting return of bowel function	None
15	Right	4	Edema, awaiting return of bowel function	None
16	Right	6	Edema, IV pain medication, awaiting return of bowel function	None
17*	Right	7	Urinary retention	None
18	Left	5	Epidural, foley, awaiting return of bowel function	Hypophosphatemia
19	Left	4	Epidural, foley, nausea	None
20	Left	4	Epidural, foley, volume overload	None
21	Right	7	Foley, nausea, awaiting return of bowel function, fluid overload	None
22	Right	5	Awaiting return of bowel function	None
23	Right	4	Awaiting return of bowel function	None
24	Right	6	Awaiting return of bowel function	None
25	Left	7	Nausea	Hypophosphatemia
26	Right	9	Nausea, vomiting, awaiting return of bowel function	None
27	Left	6	Epidural, foley	Hypophosphatemia
28	Right	4	None	None
29	Left	5	Epidural, awaiting return of bowel function	None
30	Left	7	Epidural, nausea	None
31	Left	6	Epidural	None
32	Right	6	IV pain meds	None

Table 1: Barriers to POD3 discharge in living liver donors (‡ denotes readmission within 30 days of discharge, * denotes post-operative complication prolonging hospital stay)

Discussion and Conclusion: Living liver donors are otherwise healthy patients exposed to hospital-associated risks with increasing LOS. Our results indicate that critical lab values such as phos, INR, and Hct have stabilized by POD3. Barriers to early discharge are pain control (with attendant nausea/vomiting) and fluid overload (likely secondary to IV phos repletion), and lab work is no longer required in stable patients. We are undertaking the study of the utility of epidural vs. nonnarcotic analgesia for LL donors and the study of the need for phos replacement. Modification of these elements should help us achieve the goal of routine 3 day hospitalization for live liver donors.

P.1651

Endourological management of live donors with urolithiasis at the time of donor nephrectomy: a single centre experience

Sandeep Guleria, Surjeet Kumar, Anshuman Agarwal, Praveen Pushkar, Pradeep Champawat.
Department of Transplant Surgery, Indraprastha Apollo Hospitals, New Delhi, New Delhi, India.

Live Donors with single sided sub centimeter ,asymptomatic renal calculi are usually made fit for renal donation in a two staged procedure. This includes Extra corporeal shock wave lithotripsy, Percutanaeous Nephrolithotomy or Retrograde intrarenal surgery. To reduce the cost and morbidity of a two staged procedure we carried out a study removing subcentimeter renal calculi by bench surgery at the time of donor nephrectomy.
Twenty Two live related renal donors with asymptomatic single sided asymptomatic renal calculi were included in this study. All donors underwent extensive metabolic work up in addition to the standard donor work up. After the donor nephrectomy the kidney was flushed with Histidine -Tryptophan -Ketoglutarate solution. With the kidney immersed in ice slush the ureter was first spatulated and by bench retrograde ureteroscopy the stone was retrieved. A pyelotomy was done once the stone was in the pelvis and the pyelotomy closed with 5-0 vicryl. In cases of an intra renal pelvis a holmium laser or pneumatic lithoclast was used for stone fragmentation and the fragments washed out through the ureter.
The donor age ranged from 27 to 64 years. (Mean 44.8 years). The stone size ranged from 3.4 mm to 10 mm. Five patients had multiple stones. Stone clearence was achieved in twenty one of the twenty two patients. The time taken ranged from 22 minutes to 49 minutes (Mean 28 minutes). In one patient the stone could not be retrieved as the infundibulum was very narrow and the stone could not be retrieved. The pneumatic lithoclast was used in four patients and the laser in six patients.
The renal translantation was carried out in a standard manner and the foleys catheter was removed on day five.Three patients had haematuria which resolved on day three. Twenty patients were discharged on day seven and two had a stay of eleven days as there recovery was complicated by an acute rejection in one and excessive lymphatic drainge in the other. The mean serum creatinine at discharge was 1.2$\pm$0.3 mg /dl.
No patient had recurrence of stones during follow up. Donors were followed up for 16 months (6-26 months) and recpients for the same. The single recpient in which ureteroscopy failed was also asymptomatic.
Endourological bench surgery for donors with single sided sub centimeter stones at the time of donor nephrectomy is a viable and effective option It is associated with minimal morbidity.

P.1652

Laparoscopic live donor nephrectomy: a single centre experience

Anwar Naqvi, Rehan Mohsin, Asad Shahzad, Gauhar Sultan, Altaf Hashmi, Manzoor Hussain, Zafar Hussain, Adib Rizvi.
Urology and Transplantation, Sindh Institute of Urology and Transplantation, Karachi, Pakistan.

Introduction: Since the initial report on Laparoscopic Donor Nephrectomy (LDN) in 1995, it has been performed at transplant center worldwide. The benefit of decrease pain, faster recovery and improved cosmetic conferred by LDN has been cited as potential reason for an increase in willing donor. Early concern of prolonged ischemic time, impaired graft function and donor safety have been addressed by several large series documenting excellent graft outcome and low donor morbidly. Because of this LDN has been edging towards gold standard in live donor nephrectomy.
Method: We collected retrospective live donor nephew crony data from single centre form 2014 - 2015. 198 laparoscopic donor nephrectomy cases were performed during this time period. Out of 198 patients, 23 had right sided laparoscopic donor nephrectomy. Prospective database were reviewed from both donor and recipient to record demographics, Intra and post operative events and complication. The aim was to introduce LDN as method of reducing the disincentives to live kidney donation by reducing the impact of the open nephrectomy operation on the donor patient.
Result: Left kidney was procured in 88.3% in LDN and 72.28% in ODN. Mean operative time was 182.6 and 194.5 minutes in ODN and LDN respectively with warm ischemic time of 2.97 in LDN. None of them were hand assisted and conversion rate was 3.0% Intraoperative complication was approximately 10% in LDN and predominantly was bleeding. Post operative complication in LDN was slightly more than 1% wound infection and few case of ileus. Post operative pain was almost nil after 72 hrs in LDN. Meantime of donor hospital stay was 3.6%. Recipient serum creatinine in LDN and ODN was comparable in both groups.
Conclusion: LDN can be performed with acceptable immediate morbidity and excellent graft function. Warm ischemic time was slightly high in LDN group. Bleeding was the most common intraoperative complication. Hospital stay was more common in ODN group. There was no difference in recipient serum creatinine at 4 weeks postoperatively.

P.1653

Decreased resistive index (RI) in renal transplant recipients–a treatable imaging finding

Rehan Mohsin[1], Asad Shahzad[1], Bux Ali[1], Tahir Aziz[2], Anwar Naqvi[1], Adib Rizvi[1].
[1]Urology and Transplantation, Sindh Institute of Urology and Transplantation, Karachi, Pakistan; [2]Nephrology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan.

Introduction: Transplantation is currently one of the accepted treatments of irreversible kidney disease. Improvements in surgical techniques and more sophisticated, potent immunosuppressive drugs have resulted in remarkable advances in survival of patients and renal grafts. Nevertheless, substantial complications occur in both the immediate postoperative period and later. Imaging has a critical role in the evaluation of these complications as decreased RI indicates compromised blood flow in the graft which may be managed successfully by surgical or radiological intervention which in majority of times is a saviour for graft.
The objective of study is to evaluate findings of decreased RI with graft function and to evaluate the outcome of different interventions performed in cases of decreased RI.
Material and Methods: It is a retrospective analysis which was done between time period of 2005 and 2015.Case notes of all patients who had renal transplant in this time period was analyzed for demographic data, angiographic findings and serum creatinine value at the time of decreased RI.Management which was offered was also recorded along with per operative findings. Finally the graft function and post operative RI was recorded.
Results: A total of 53 patients were included in this study. Majority of the patients were male with predominantly having single renal artery.79% of the patients were found to have their graft under tension because of abdominal muscle pressure. Graft dysfunction was found in 66% of patients. Decreased RI was noted on day zero in 13 patients. Angioplasty was performed in 13% of patients while repositioning of graft (kept in sub-coetaneous space) was done in 47% of patients. Renal functions post intervention was also recorded.
Conclusion: Post operative Color Doppler Imaging is mandatory in early as well as late post transplant period. If there is finding of decreased RI clinician should take a prompt action in terms of exploration especially in early post transplant period. However in late post transplant period an angiography should be performed and if there is any finding it should be managed.

P.1654

Donor comprehension of provided information during informed consent process in live donor nephrectomy; Does it matter what we tell donors? A pilot study

Kirsten Kortram[1], Emerentia Q.W.Spoon[1], Caspar WNLooman[2], Hendrikus J.A.N. Kimenai[1], Jan N.M.Ijzermans[1], Frank J.M.F.Dor[1].
[1]Surgery, Erasmus MC University Medical Center, Rotterdam, Netherlands; [2]Public Health, Erasmus MC University Medical Center, Rotterdam, Netherlands.

Background: Living kidney donors are a unique group of “patients”, undergoing surgery for the benefit of others. Safety and informed consent are even more important than in other surgical procedures. Current literature demonstrates great variations in informed consent practices. Donors report varying degrees of satisfaction with the information and preparation for live donor nephrectomy. But whether this stems from a lack of education, or comprehension remains unclear. It has been suggested that donors do not actually hear everything we tell them, but that they only use the information to confirm their decision, instead of weighing risks and benefits. But it is still vital that these donors receive all necessary details.
Methods: The preoperative surgical outpatient clinic visits of 46 potential living kidney donors were observed. Provided information was scored using standardized checklists, team members (N=9) received an “informer score” for each consultation by the observer, one point was awarded for each mentioned item. Immediately after giving consent for donor nephrectomy, and again on the day of admission for donation donors received a questionnaire testing their knowledge of the upcoming operation. Informers as well as donors could score a maximum of 20 points. Scores were compared between donors and informers. Outpatient scores were also compared with admission scores. Demographic data and baseline donor characteristics were documented for correlation purposes.
Results: Median informer score was 12 out of 20 points (range 2-20). Median donor score was 6 out of 20 (range 2-11). Donors scored best on duration of admission and convalescence, and worst on long-term complications. Risk of mortality was disclosed by 91% of informers, but only reproduced by 22% of donors at the outpatient clinic and 14% on admission. Donors living with children under 18, a higher educational level and registered (post-mortem) donors scored significantly better. Median donor satisfaction was 9 out of 10 (range 4-10).
Conclusion: There were marked variations between the information provided by different informers, important complications were not always disclosed. Overall donor scores were low, although satisfaction was high. Whether donors are actually well enough informed at the time of giving consent remains debatable.
References:
[1] Kortram, K., et al., The need for a standardized informed consent procedure in live donor nephrectomy: a systematic review. Transplantation, 2014. 98(11): p. 1134-43
[2] Gordon, E.J., Living Organ Donors’ Stories:(Unmet) Expectations about Informed Consent, Outcomes, and Care. Narrative Inquiry in Bioethics, 2012. 2(1): p. 1-6
[3] Valapour, M., The live organ donor’s consent: is it informed and voluntary? Transpl Rev, 2008. 22(3): p. 196-199

P.1655

Quality of life and socio-psychological issues in donors after kidney donation

Sakina Yousuf, Fatema Jawad, Mirza Naqi Zafar, Anwar Naqvi, Adib Rizvi.
Sindh Institute of Urology and Transplantation, Karachi, Pakistan.

Objectives: Kidney donation is a safe procedure and has minimal impact on quality of life of donors with low mortality. But less attention has been paid to determine psychological issues among donors after donation. Our aim to conduct this study on living related kidney donors was to evaluate the quality of life (QoL), identify the risk factors affecting QoL and to evaluate the social and psychological issues after donation.
Methods: During November 1985 to September 2014, a total of 4393 living related transplants were performed at SIUT. All donors are followed up in a dedicated donor clinic. We selected 350 kidney donors who attended the clinic during three months study period. The Short Form (SF-36) health survey form was used to evaluate the QoL. Second questionnaire based on 20 published studies on psychological assessments were used to assess the social and psychological issues. The data was analysed using SPSS at a= 0.05.
Results: There were 183 males and 167 females with mean age of 41.8±10.9 years and post donation period of 6.8±5.4 years. The results showed good QoL of donors who scored more than 80 in all dimensions of health except the vitality. Older donors and females scored low in all dimensions and had more complaint of pain. Similarly post donation period, education levels and recipient status have significant impact on QoL. The relationship of 12 (3.1%) have been effected after donation due to the family conflicts. Majority 97% were satisfied with their decision of donation, 95.4% were willing to donate again and 99% would encourage others to donate. Post donation 5.4% had health problems, 12 became jobless, 26 had financial problems, 4 got divorced and 5 experienced problems in getting married. Around 29% donors had mild to severe depression. Donor age, gender, post donation period, education level and recipient status were the risk factors of depression.
Conclusions: Our study concluded that our donors have good QoL however there are some psychological and social issues associated with donation. Regular donor follow-up can play an important role to reduce their psychological and social problems.

P.1656

Living organ donation high quality practices: LIDOBS network recommendations

Ana Menjivar[1], Christina Papachristou[2], David Paredes[1], Ignacio Revuelta[1], Constantino Fondevila[1], Fritz Diekmann[1], Jordi Colmenero[1], Xavier Torres[1], Entela Kondo[1], Scientific Committee LIDOBS Conference[3], Working groups Members LIDOBS Conference[3], Participant LIDOBS Conference[3].
[1]Hospital Clínic of Barcelona, Barcelona, Spain; [2]Charité - Universitätsmedizin Berlin, Berlin, Germany; [3]LIDOBS network, Barcelona, Spain.

Introduction: LIDOBS is a scientific network that shall function as a platform where international professionals actively involved in Living Organ Donation (LOD) may exchange knowledge, engage in discussions, and set priorities following the values of Safety, Quality and Transparency. Recommendations for best practices in LOD were developed during the "LIDOBS International Conference on Living Donation - High Quality Practices", the conference was held in November 2014 in Barcelona and supported by the European Commission enabled a considerate number of experts in donation and transplantation, but also patients and donors.
Objectives: 1- To achieve consensus and formulate recommendations for LOD practices in order to assure high quality practices.
2- To set up a community of experts in LOD Programs that will continue to expand and increase the knowledge on the donation and transplantation procedures.3- To disseminate the results of the European research projects on LOD.
Methods: The LIDOBS conference activities involved six Working groups and went through three main phases. Preconference actions: identification, discussion and update of the most relevant reference documents on Living Donation Practices. Formulation of recommendations on strategies and actions to be taken to assure High Quality Practices in LOD. The Impact and Feasibility of each recommendation was measured using the LIDOBS Canvas methodology, a tool designed specifically for this purpose.

Conference: Proposed recommendations were presented to the Conference attendees and underwent a televoter process.
Postconference: an international consultation process was developed to define the final conclusions.
Results: Conference attendance: more than 100 participants, 55 Institutions from 31 countries, 4 Continents (Europe, Asia, the Americas and Africa). Outcomes: Consensus on 22 recommendations for High Quality Practices, each recommendation was linked to one or more of the three values and was assigned one level of requirement (compulsory, advisable and excellence). Network Extension: 28 professionals from 20 Institutions from 13 countries were added. Established online platform for networking.
Conclusions: The establishment of international consensus in terms of ethical and legal aspects, protection practices, medical and psychosocial follow-up and the establishment of registries are advancements with

the potential to protect the living donors' health and safety and shall serve as a guiding framework. LOD demand a multidisciplinary approach with the involvement of all related national and international bodies which can enable the implementation of these recommendations. The safety of the living organ donors, the quality and transparency of LOD programmes are and will remain the main aims of LIDOBS.

P.1657

Psychosocial long-term impact of donation on donation on kidney living donors- a comperative study of two major European transplant centres

Entela Kondo[1], Christina Papachristou[2], Ana Menjivar[1], Marti Manyalich[1], Xavier Torres[3], Fritz Diekmann[4], Ignacio Revuelta[4], Fabian Halleck[5], Klemens Budde[5], Thomas Schachtner[6], Petra Reinke[6].
[1]Transplant support Unit, Hospital Clinic of Barcelona, Barcelona, Spain; [2]Department for Psychosomatic Medicine, Charité - University Medicine Berlin, Berlin, Germany; [3]Clinical Neuroscience Institute, Hospital Clinic of Barcelona, Barcelona, Spain; [4]Kidney Transplant Unit, Hospital Clinic of Barcelona, Barcelona, Spain; [5]Department for Nephrology, Campus Mitte, Charité - University Medicine Berlin, Berlin, Germany; [6]Department for Nephrology, Campus Virchow, Charité - University Medicine Berlin, Berlin, Germany.

Introduction: The long-term psychosocial outcome of kidney living donors has still not been studied thoroughly. Thus, important information regarding the decision-making, the preoperative information of the donors, their selection and their postoperative care is missing. Our study aims to fill in this gap by conducting a detailed follow-up about the psychosocial wellbeing of donors up to 16 years after donation in two major European transplantation centres, in the Hospital Clinic of Barcelona and the Charité-University Hospital in Berlin.
Methods: All kidney living donors who underwent nephrectomy between 1998-2014 were contacted. Data from 706 donors were collected. The donors completed a battery of psychometric questionnaires (HADS, PHQ, SOC, SF-36, ACSA, life events, questions from the EL SA), as well as questions regarding satisfaction, decision to donate and the donor-recipient relationship in a total of 274 questions. This makes our study one of the most thorough long-term psychosocial follow-up studies of kidney living donors.
Results: Results regarding the long-term psychosocial wellbeing after kidney donor nephrectomy are presented and compared to the general population of the two countries. The results of the two centers are being contrasted and comparisons for groups regarding age, gender, donor-recipient relationship, personality characteristics and recipient variables are made and risk and protective factors for kidney living donors are presented.
Discussion: The results are being critically discussed regarding the donor evaluation practices and their postoperative care, as well as in terms of organisational and cultural differences in the two hospitals. Suggestions for further optimization of the donor selection and care are formula.
All the donors who participated in the study

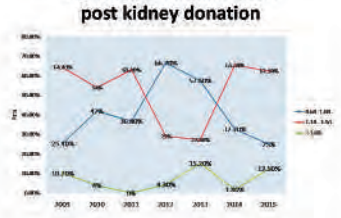
P.1658

Long term outcome of post living kidney donation monitored by the international foundation for organ transplant incorporated

Rose Marie Rosete-Liquete[1], Pedro L.Mejia[2], Albert Capitle[2].
[1]Organ Transplantation, National Kidney and Transplant Institute, Quezon, Philippines; [2]IInfort Inc., Quezon, Philippines.

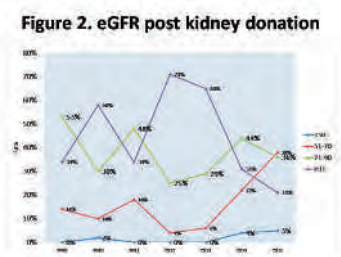
Background: There is growing concern on the development of ESRD on living healthy kidney donors. To provide living kidney donor protection, an "Oath of Undertaking" by the recipients to render follow up of their kidney donors in the early post donation period and yearly thereafter for 10 years was required by the National Transplant Ethics and the Implementing Rules and Regulations of the Anti-Human Organ Trafficking Law. The International Foundation for Organ Transplant, Inc. (InFORT, Inc. since 1998) was among the few foundations who rendered post donation follow ups of donors from different hospitals in Metro Manila, Philippines.
Objective: This study aims to determine if there is a significant deterioration of creatinine and eGFR in post kidney donation.
Materials and Methods: This is a retrospective cohort of 145 living kidney donors from 2007 to 2009. Data were retrieved from the records of the foundation. Donor data include date of operation, blood type, age, sex, urinalysis, creatinine level and eGFR computed using CKD-EPI .
Results: Out of 145 donors, 59 (40.7%) to 69 (47.6%) were followed up from 1 year to 7 years post donation. Year 4 had the highest number of follow up (69 donors). There were more male donors 119 (82%) than female donors 26 (18%). All underwent open donor nephrectomies and there were no perioperative complications. On yearly basis, they were required to attend seminars and lectures on maintaining health and livelihood programs. Two donors died of non-renal causes (trauma- 6 months and 6 years post donation, respectively). There was a trend towards increased creatinine (more than 1.6 ml/dl) in 7 (12.5%) donors at the end of 7 years.

Figure 1. Creatinine level post kidney donation



Donors who followed up on the 4th year exhibited the highest number of eGFR more than 90ml/min. Towards the 7th year, 3 (5%) donors had less than 50ml/min eGFR.

Figure 2. eGFR post kidney donation



Conclusion: There is a need for further studies to investigate the apparent trend in the deterioration of creatinine and eGFR towards the 7th year post donation.
Keywords: Living kidney donors, long term monitoring, creatinine, eGFR, outcome

P.1659

Robotic-assisted versus laparoscopic hand-assisted live donor nephrectomy

Pamela Sun[1], Julien Renard[3], Maurice Matter[2], Monika E.Hagen[1], Karine Hadaya[4], Manuel Pascual[2], Jean-Pierre Venetz[2], Nicholas Demartines[2], Leo Buhler[1], Philippe Morel[1], Christophe Iselin[3].
[1]Transplant Surgery, Hôpitaux Universitaires de Genève, Geneva, Switzerland; [2]Centre of Transplantation, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; [3]Urology, Hôpitaux Universitaires de Genève, Geneva, Switzerland; [4]Nephrology, Hôpitaux Universitaires de Genève, Geneva, Switzerland.

Background: Live donor nephrectomy allows preemptive and timely renal transplantation with optimal clinical outcomes for patients with end stage renal failure (ESRF). Currently living donor transplantation accounts for over a third of all renal transplantations in the US and UK. In view of a growing living donor pool, and its maximal benefit for patients with ESRF, surgical technique must be optimized to protect donor safety and welfare. Herein, we compare two minimally invasive surgical approaches, robotic-assisted versus laparoscopic hand-assisted, for live donor nephrectomy (LDN).
Methods: A retrospective study comparing robotic-assisted (n=25) versus laparoscopic hand-assisted (n=36) approaches utilized in 2 different centres for LDN over a 2-year period in 2014-2015.
Results: All LDNs, robotic or laparoscopic, were completed without conversion to open donor nephrectomy. Patient demographics were comparable in both groups in age (robotic: 53yrs±10 vs laparoscopic: 53yrs±12; p=ns), BMI (25kg/m2±5 vs 25kg/m2±4; p=ns) whilst female donors dominated (64%F vs 50%F; p=ns). Left sided nephrectomy was favoured in the robotic group (23L:2R, 92%L vs 18L:18R, 50%L, p=0.01).
Operative time of robotic surgery was longer with additional docking time than laparoscopic surgery (309mins±50, range 244-435 vs 135mins±23, range 94-187; p<0.01) nevertheless the warm ischaemic time was comparable in both groups (214secs±66 vs 218secs±74; p=ns). There were no perioperative complications necessitating reoperation.
Length of hospital stay tended to be shorter in the robotic group (median 4days, range 2-13 vs 6days, range 3-8; p=0.09) with majority of patients discharged within 4 days (15/25, 60% vs 10/36, 27%; p=0.02) compared to a smaller portion of patients in the laparoscopic group. Reduction of donor renal function was measured as a ratio of creatinine at discharge vs. preoperative, which was higher in the robotic group (1.58±0.32 vs 1.40±0.22; p=0.01); probably due to earlier discharges. Notably, there was no difference in actual donor creatinine at discharge (robotic: 114µmol/l±21 vs laparoscopic: 111µmol/l±24; p=ns). The recipient creatinine at discharge was acceptable at 132µmol/l±34 (range 89-227) in the robotic group. Postoperative complications developed in 2 patients in each group; 2 Clavien II (chest infection, wound infection) in the robotic and 2 Clavien III (abdominal wall abscess, incisional hernia) in the laparoscopic groups.
Conclusion: Robotic-assisted live donor nephrectomy was safe and feasible. Operative time was longer in robotic surgery nevertheless the warm ischaemic time was comparable to the laparoscopic approach; ultimately, the length of hospital stay tended to be shorter. Of note, there was no difference in donor renal function at discharge. In view of a growing living donor pool and its maximal benefit for patients with ESRF, minimally invasive surgery in form of robotic-assisted live donor nephrectomy may be an avenue for optimal recovery.

	Robotic-assisted (n=25)	Laparoscopic hand-assisted (n=36)	p-value
Patient demographics			
Age (years)	52.9yrs±10.4	53.3yrs±1.6	0.8907§
Gender (Male:Female, % Female)	9:16, 64%	12:24, 67%	0.5211¤
BMI (kg/m²)	25.2±4.5	25.0 ±4.2	0.8596§
Operative details			
Side of nephrectomy (Left:Right, %Left)	23L:2R, 92%	18L:18R, 50%	0.0067¤*
Duration of operation (minutes)	309±50	135±23	0.0001§*
Warm ischaemic time (seconds)	214±66	218±74	0.8291§
Postoperative outcomes			
Length of hospital stay (days)	4.8±2.3	5.6±1.3	0.0888§
Discharge within 4 days (no. of patients, %)	15, 60%	10, 28%	0.0174¤*
Donor creatinine at discharge (µmol/l)	114±21	111±24	0.6156 §
Postoperative complications Clavien I Clavien II Clavien III Clavien IV	 0 2 0 0	 0 0 2 0	/
Key *statistical significance with p-value < 0.05 § unpaired t-test ¤ Fischer's exact test			

P.1660

Ultrasonography versus CT-scanning as imaging modality for long term follow up after kidney donation

Shiromani Janki[1], Hendrikus J.A.N. Kimenai[1], Marcel Dijkshoorn[2], Khe T.C. Tran[1], Caspar W.N.Looman[3], Roy S.Dwarkasing[2], Jan N.M.IJzermans[1].
[1]Department of Surgery, Division of HPB and Transplant Surgery, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [2]Department of Radiology, Erasmus MC, University Medical Center, Rotterdam, Netherlands; [3]Department of Public Health, Erasmus MC, University Medical Center, Rotterdam, Netherlands.

Background: Live kidney donation is possible due to the fact that the remnant kidney has the capacity to compensate for renal function by hypertrophy. However, to date we include many elderly for living kidney donation and it remains to be demonstrated in larger series whether the compensating mechanism may function at older age as well as at younger age. Increase in kidney size may be an important indicator for the capacity of the remnant kidney after donation. To limit the use of CT-imaging in the follow up of living kidney donors we investigated whether ultrasound ($_{us}$) measurements for kidney size are comparable with CT ($_{ct}$) measurements.
Methods: Donors, who underwent a CT-scan during screening, were preoperatively approached for additional examination by ultrasound to determine kidney length, width and thickness. Measurements were independently performed by two ultrasonographists (US-1 and US-2). Measurements were considered to be accurate when the mean differences between both ultrasonographists were <5 mm. Ultrasound volumes were calculated with the ellipsoid formula (length x width x thickness x $\pi/6$) and an adjusted formula (correction factor 0.674), and CT volumes with the voxel count method.
Results: The mean difference of both kidneys between US-1 and US-2 for all measurements was <5 mm. The common formula underestimated the volume for US-1 with 16.9% and for US-2 with 14.8%, while the adjusted formula overestimated the volume for US-1 with 6.8% and for US-2 with 9.5%. The relation between volume$_{ct}$ and volume$_{us}$ calculated with the adjusted formula is strong for both US-1 (r 0.76, $p<0.001$) and for US-2 (r 0.80, $p<0.001$).
Conclusion: Ultrasound measurements for kidney size are comparable with CT measurements. Therefore, ultrasonography may be used during live kidney donor follow-up thus limiting costs, time and radiation exposure as compared to CT imaging.
We would like to extend our gratitude towards Wim Vermeule, radiology technician, for performing all CT volumetry measurements

P.1661

Retroperitoneal single port donor nephrectomy through lumbotomy incision - a novel approach

Ashish Sharma, Navdeep Singh, Sarbpreet Singh, Deepesh Kenwer, Sunil Kumar, Mukut Minz.
Renal Transplant Surgery, Post Graduate Institute of Medical Education and Research, Chandigarh, India.

Introduction: Laparoscopic donor nephrectomy has become the standard method of retrieval of donor kidneys from living donors. However, it has the disadvantage of breaching the peritoneal cavity with attendant risks of injury to intraperitoneal organs as well as long-term risk of adhesive intestinal obstruction. Retroperitoneal laparoscopic donor nephrectomy (RPLDN) is not popular, as it requires a muscle cutting incision for retrieval of donor kidney, thereby minimizing the benefits of laparoscopy. However, a single port RPLDN performed through lumbotomy can provide a nearly ideal approach to donor kidneys. The present study provides the first experience in the literature of performing a single port RPLDN through lumbotomy incision.
Methods: Between November 2014 to December 2015, 231 patients underwent laparoscopic donor nephrectomy at our institute. 50/231 voluntary kidney donors were offered translumbar RPLDN. 12/50 patients consented for single port translumbar RPLDN. Mean donor age was 39.6±7.7, M:F 3:9, Left: Right 10:2 and mean BMI was 21.86±2.64.
Technique of nephrectomy - Kidney was approached through 6cm curved lumbotomy incision nearly parallel to sacrospinalis muscle with donor in lateral position. Alexis® port with rubber glove was used as a single port with two 10mm and two 5mm ports placed in the finger of gloves. Renal vessels and ureter were ligated with double Hemolok® clips as a standard policy for all donors at our institute. Kidney was taken out through the Alexis® retractor after removing the glove. Visual analogue score (VAS) was used to evaluate severity of pain in the postop period
Results: RPLDN was successfully carried out in all patients. No patient required conversion to open or transperitoneal approach. Additional port for retraction was required in the two of the first three cases. No special angulated instruments were required. Only one patient had double renal artery (8.3%) and all patients had single renal vein and ureter. No intra/perioperative complications were encountered. Mean operating time was 200.7+65min (range 130-322). There was significant reduction in operative time after the initial experience. Mean WIT was 5+1.28 min (range3-8min). Average blood loss during surgery was 55.4+18 ml (range 30-100ml). Mean graft kidney weight was 129.5 +25 gm (range 85-168 gm). Mean analgesic requirement (tramadol) in postoperative period was 281.8 + 189.4 mg (range 100-800mg). Mean VAS on postop day (POD) 0 and POD1 was 5.6+1.5 and 3.9+0.9 respectively. Mean hospital stay was 2.5 days (range, 2-3 days). All recipients had good initial renal function and the mean creatinine at discharge was 1.34+0.4 mg%.
Conclusions: Single site translumbar RPLDN is a feasible alternative approach to donor surgery and can avoid inherent risks associated with the transperitoneal technique. A larger comparative study is required to know the potential benefits of this route over the transperitoneal approach.
References:
[1] Pansadoro V. The posterior lumbotomy. BJU Int. 2005 May;95(7):1121-31.
[2] Tai HC, Lin CD, Wu CC, Tsai YC, Yang SS. Homemade transumbilical port: an alternative access for laparoendoscopic single-site surgery (LESS). Surg Endosc. 2010 Mar;24(3):705-8.

P.1662

Hybrid Laparo-endoscopic single-site (LESS) donor nephrectomy with homemade port; An initial experience

Sarbpreet Singh, Ashish Sharma, Deepesh Kenwar, Sunil Kumar, Nikhil Mahajan, Navdeep Singh, Mukut Minz.
Renal Transplant Surgery, Post Graduate Institute of Medical Education & Research, Chandigarh, India.

Background: Laparoscopic method has become the gold standard for retreiving the kidney from a living donors over the last decade[1]. Laparo-endoscopic single-site donor nephrectomy(LESS-DN) is an attempt to further enhance cosmetic benefits and reduce the morbidity for potential kidney donors. Various studies show that LESS-DN is a safe although technically challenging alternative to LDN, with comparable results in terms of graft function and patient morbidity[2]. High cost of single port and articulating instruments urged us to use inexpensive alternative approach with homemade port and conventional laparoscopic instruments.
Material and Method: From March 2012 to February 2014, forty five donors underwent modified/Hybrid LESS nephrectomy. Homemade port of Alexis Abdominal wound retractor (Applied Medical,USA) with glove rolled over its ring was placed through 5cm long transumblical incision, two ten and one 5 mm ports were placed through glove fingers and secured with thread and one additional 5 mm trocar placed lateral to epigastrium. Dissection was performed as per standard laparoscopic technique with conventional laparoscopic instruments. Ureter, renal artery and vein clipped with Hemolock clips and cut. Kidney was extracted through the transumblical incision.
The lengths of vessels, ureter, retrieval incision and warm ischemia time (WIT) were noted. Post-operatively, patients were assessed for degree of pain by visual analog scale (VAS) on postoperative days (PODs) one and seven, and graft function was monitered in the recipients at the time of discharge.
Results: Mean age of donors was 42.3 ± 10.1 years, Male :Female 6:39, Mean body weight was 55.6 ± 10.1 kg, height was 155.6 ± 8.6cm. Thirty nine left and six right nephrectomies were done. Four donors had multiple arteries.
The mean operative time was 2:24 ± 0:01 hrs with WIT4.44±1.45min. Adequate length of ureter 10.9 ± 1.0 cm, renal artery 2.1 ± 0.2 cm and renal vein 2.6 ± 0.3 cm could be obtained in all patients. All kidneys had adequate diuresis intraoperatively on cutting the ureter .Intra operative complication included one small sub capsular haematoma, in one case kidney slipped while extracting so incision had to be extended. One donor required blood transfusion due to slipping of gonadal vein ligaclip. The mean incision length was 4.9 ± 0.3 cm. All donors were ambulant on the first POD. The median VAS score was 4/10 on POD 1 and 1/10 at one week. All recipients had brisk diuresis on declamping and immediate graft function. Ten recipients required biopsy for graft dysfunction. 7/45 (15.5%) had rejection whereas the rest had nonspecific changes The mean serum creatinine at the time of discharge was 1.2 ± 0.3 mg%.
Conclusion: Hybrid LESS donor nephrectomy with homemade port is safe and acceptable method to retrieve kidneys from living donors.It offers similar operative outcomes as standard laparoscopic donor nephrectomy and retains the advantages of LESS. Additional 5 mm port avoids the need of angulated instruments and makes the procedure economically and ergonomically beneficial. It can act as a bridge between conventional laparoscpic and LESS donor nephrectomy.
References:
[1] Dols LF, Kok NF, d'Ancona FC. et al. Randomized controlled trial comparing hand-assisted retroperitoneoscopic versus standard laparoscopic donor nephrectomy. *Transplantation*, 2014: 97: 161.
[2] Ganpule, A. P., Mishra, S., & Sabnis, R. (2012). Laparoendoscopic single-site donor nephrectomy. *Indian Journal of Urology : IJU : Journal of the Urological Society of India*, 28(1), 65–70. http://doi.org/10.4103/0970-1591.94960.

P.1663

To identify the risk factors for pneumocystis jiroveci pneumonia (PcP) in renal transplant recipients

Navdeep Singh, Ashish Sharma, Sarbpreet Singh, Deepesh B.Kenwer, Sunil Kumar, Mukut Minz.
Renal Transplant Surgery, PGIMER, Chandigarh, India.

Introducton: Pneumocystis jiroveci is an opportunistic pathogen causing severe pulmonary infection in renal transplant recipients(RTR). High risk of developing pneumocystis pneumonia (PcP) in the early post-transplant period; incidence 0.6 to 11.5%, mortality up to 50%1-6. Individual risk factors for the occurrence of PcP in RTRs are still poorly defined. Both recommend additional PcP prophylaxis after treatment of rejection by steroids.
Methodology: Retrospective analysis, 30 cases (10 cases, 20 controls) transplanted b/w JAN 2000 to june 2015.
Cases (n=10) were either diagnosed as PcP by identification of organism in lung tissue or respiratory secretions or were presumed as PcP because of strong clinical and radiological suspicion and rapid response to therapy.
Controls (n=20) were RTRs who were operated on the preceding and the subsequent days of the transplantation of the cases.
Parameters analysed were:
• Age,Sex Baseline S.creatinine
• Basic disease
• Maintenance immunosuppression
• Use of pulse steroids in acute rejection
• Use of ATG
• Therapy for humoral rejection
• Prior infections.
Statistical analysis was done using Chi square test and p value < 0.05 was considered significant.
Results: The mean age of the cases and controls was 36.5± 9.6 and 34.3± 12.6 years; p=0.64. All cases were males and in controls there were 17 males (85%) and 3 females (15%). The mean baseline S.creatinine in cases and controls was 1.35±0.38 and 1.19 ±0.61; p=0.46. Glomerulonephritis was the basic disease in 7/10 cases (70%) and in 12/20 controls (60%); p 0.01. Tacrolimus based maintenance immunosupression was observed in 8/10 cases (80%) and 15/20 controls (75%); p>0.05. Use of ATG, therapy for humoral rejection and prior infections were not found statistically significant when compared between cases and controls; p=1.0, p=0.15, p=0.11, respectively. Pulse steroids were used for treating acute rejection in 4/10 cases (40%) and in 1/20 controls (5%); p=0.015 and was found to be the only statistically significant parameter.
Discussion:
In review of literature, various studies have describes the following risk factors for PcP. Use of corticosteroids after rejection was found to be s ignificant risk factor by castro et al9. One retrospective case series in non-HIV immunosuppressed patients identified use of corticosteroids as risk factor in 90%11. ≥2 immunosuppressive drugs were found to be significant risk factor of PcP by Carlos et al6,10. Antilymphocyte antibodies are linked to the highest risk for PcP in first 6 months post transplant12
One retrospective study suggested a higher incidence of PCP among RTRs on tacrolimus-based regimens compared to cyclosporine based regimen 1.
CMV may be an independent risk factor for PcP13.
Conclusion: Use of pulse steroid for acute rejection in RTRs is a risk factor for PcP infection. All RTRs should receive trimethoprim-sulfamithoxazole prophylaxis for 6-12 months following use of pulse steroids while treating rejection.

P.1664

Risky organs: using organs from donors with blood-borne viruses in New South Wales (NSW), Australia, 2010-2015

Rebecca Hancock[2], Kate Wyburn[1,5], Imogen Thomson[1], Karen Waller[1], Michael O'Leary[4,6], Angela Webster[1,2,3,7].
[1]Sydney Medical School, University of Sydney, Sydney, Australia; [2]School of Public Health, University of Sydney, Sydney, Australia; [3]Centre for Kidney Research, The Children's Hospital at Westmead, Sydney, Australia; [4]NSW Organ and Tissue Donation Service, Sydney, Australia; [5]Renal Unit, Royal Prince Alfred Hospital, Sydney, Australia; [6]Intensive Care Service, Royal Prince Alfred Hospital, Sydney, Australia; [7]Centre for Transplant and Renal Research, Westmead Hospital, Sydney, Australia.

Introduction: Potential organ donors who have used drugs intravenously or engaged in other high risk behaviours pose a challenge when assessing risk of transmitting blood-borne viruses (BBVs) to recipients. Donation decisions are frequently made within potential infection windows and without knowledge of an individual's risk mitigation behaviours, and consequently may be risk averse. We sought to describe donor referrals deemed 'high risk' for hepatitis C (HCV), hepatitis B (HBV) and human immunodeficiency virus (HIV) who did and did not proceed to donation in New South Wales, Australia.
Materials and Methods: We conducted a retrospective cohort study of the NSW Organ and Tissue Donation Service referral logs, which record demographic and clinical information of all people referred for organ donation in NSW, from January 2010 to December 2015. We reviewed referrals with BBVs (hepatitis B, C and HIV) and those deemed not medically suitable due to behaviours high risk for contracting BBVs, comparing potential donors (those who did not proceed), with intended (donation abandoned before organ removal) and actual donors. Reasons for outcome variability were evaluated.
Results and Discussion: Of 3,005 total donation referrals (2,337 potential, 668 intended/actual), 220 (7.3%) were recorded as having BBVs or being high risk for BBVs due to high risk behaviours. There were 23 actual/intended donors and 197 potential donors (refer to table). None of the 5 patients with HIV became actual donors. For 44 (28%) of referrals with HCV no suitable recipient was found (38 potential, 6 intended). Among the remaining 159 potential donors, 63 were deemed not medically suitable due to high risk behaviour (n=60) and/or positive BBV infection (n=3), 66 were not medically suitable for reasons unrelated to BBVs, and for 30 consent was not granted (family or coronial refusal).
Conclusions: In NSW, referrals with BBVs are considered for and in certain circumstances proceed to organ donation. With potentially curative treatments for HCV imminent, new risk paradigms may evolve. Consideration of opportunities to increase donation rates, particularly among those identified as high risk for BBV transmission, may be warranted.

Table: Donors with blood borne viruses by donation outcome in NSW, 2015 – 2015

Blood borne virus	Total donors	Donor referral outcome	
		Potential donors (deemed not medically suitable due to risk or known BBV)	Actual & Intended donors
Hepatitis B virus (HBV)	18	15 (3)	3
Hepatitis C virus (HCV)	137	121 (22)	16
HBV + HCV	18	17 (5)	1
Unspecified hepatitis	5	3	2
Hepatitis suspected	6	6	0
High risk for BBV	31	31 (31)	0
HIV	5	4 (2)	1
Total	220	197 (63)	23

P.1665

Improving donor comfort: Perioperative dexamethasone decreases nausea, vomiting, and pain after laparoscopic donor nephrectomy

Shigeyoshi Yamanaga[1,2], Andrew M.Posselt[1], Chris E.Freise[1], Sang-Mo Kang[1].
[1]Division of Transplant Surgery, Department of Surgery, University of California, San Francisco, San Francisco, CA, United States; [2]Department of General Surgery, Japanese Red Cross Kumamoto Hospital, Kumamoto, Japan.

Introduction: Postoperative nausea and vomiting (PONV) and pain are common adverse events after laparoscopic surgery. Perioperative dexamethasone (8-10mg) has been reported to decrease PONV and pain in other laparoscopic procedures such as hysterectomy and cholecystectomy. We hypothesized that dexamethasone would also be effective for laparoscopic donor nephrectomy (LDN).
Patients and Methods: We performed a retrospective cohort study of 211 living donors who underwent LDN between February 2013 and October 2015. All nephrectomies were done using the intraperitoneal pure LDN approach by three experienced surgeons. Local anesthesia was injected into the wounds at the end of the LDN and patient-controlled analgesia was used for the first 24hrs after LDN. Total fentanyl and hydromorphone injections were converted into oral morphine equianalgesic dose. One hundred dexamethasone (DEX, 8-14mg) injected donors were compared with 111 controls with analysis of the outcomes and incidences of PONV and pain within 24hrs after LDN.
Results: DEX significantly reduced overall PONV incidence (64.9% in control and 47.0% in DEX, respectively. p=0.009) and severity (defined as PONV >3 times, 23.4% in control and 10.0% in DEX, respectively. p=0.0096). PONV was continuously suppressed in both early (<6hrs) and late phase (6-24hrs) with DEX (<6hrs; p=0.015 and 6-24hrs; p=0.03). Total intravenous opioid use in the first 24hrs was significantly lower in DEX compared to control (39.8+/-36.6 mg in control and 29.0+/-35.9 mg in DEX, respectively. p=0.009). Postoperative complication rates and postoperative hospital stays were not different between the groups. Propensity score matching on American Society of Anesthesiologists score, age, sex, body mass index and operative time slightly increased the differences between the groups. Notably, ketorolac given intraoperatively and continued for 24 hours did not appear to decrease PONV or narcotic usage.
Conclusions: A single perioperative injection of 8-10mg dose dexamethasone decreases antiemetic and narcotic requirements in the first 24hrs, with no increase in surgical complication. This translates into improved donor comfort and potentially improved safety through fewer surgical complications associated with narcotic use.

P.1666

Long period follow-up of renal function of living renal donor

Kazunari Yoshida[1,4], Daisuke Ishii[2], Yasuo Takeuchi[3], Fumino Noguchi[4], Masae Ikeda[2], Miki Tanaka[4], Rie Iriki[4], Haruki Wakai[2].
[1]Organ Transplant and Regenerative Medicine, Kitasato University, School of Medicine, Sagamihara, Japan; [2]Urology, Kitasato University, School of Medicine, Sagamihara, Japan; [3]Nephrology, Kitasato University, School of Medicine, Sagamihara, Japan; [4]Transplant Support Center, Kitasato University Hospital, Sagamihara, Japan.

Introduction: Renal transplantation basically should be done from cadaver donor. Some extent of transplantations is, however carried out from living related donor because of shortage of donor organ. Living donor should be followed up carefully for long period after kidney donation. We have observed their condition especially renal function and adverse events post transplantation for five years in our institute.
Materials and Methods: Eighty-six living renal donors who donated kidney to their relatives between Feb. 2003 and Aug 2013 in Kitasato University Hospital were involved in this observation. The average follow up period were 81.5 months after renal transplantation. All these donor nephrectomy were performed by laparoscopy. Sixty-two (72.1%) donors were female and 24 (27.9%) were male. Seventy (81.4%) were donated left kidney and 16 (18.6%) were right. Average age of these donors was 56.9 (F: 56.3, M: 58.5) year old. Pre-operative estimation of renal function was done by sCr, eGFR (Japanese scale), ERPF (evaluated by reno-scintigaraphy, MAG3), and urinalysis. Post operatively these were followed up periodically but ERPF was measured until 1 year.
Results: None of donor died nor resulted renal failure after renal transplantation in this follow up period. None of them received blood transfusion in peri-operative period. One major complication of descending colon perforation was encountered at one week after the donation, which resulted hemi-colectomy. The mean graft weight was 187.3±39.3 g. The average residual renal function estimated by pre-operative split function measured by reno-scintigraphy (MAG3) was 50.7%. The mean pre-operative eGFR (Japanese scale) were 77.88±14.0 (F: 77.5±14.2, M: 78.7±13.6) ml/min. It reduced to 44.76±9.18 (F: 45.6±10.0, M: 42.6±6.6) ml/min, which was 57.5 % of pre-operative renal function on the next day of transplantation. This reduction has recovered to 48.5±9.3 (62.1% of pre-op), 48.7±9.2 (62.4%), 48.8±9.7 (62.6%), and 48.8±10.4 (62.5%) ml/min at 1, 3, 6, 12 months post transplant respectively. Their renal function showed 49.2±10.4 (63.1%), 49.6±9.9 (63.6%), 50.2±9.1 (64.3%) ml/min at 2, 3, 5 years after donation respectively. Six (7.0%) donor noticed slight proteinuria pre-operatively, and 3 of 6 sustained slight proteinuria, but other 3 disappeared proteinuria. On the other hand 6 donors had newly onset slight proteinuria at 1 year after donation.
Conclusions: We had very few complications in donor post transplantation. The renal function of donor reduced almost half immediately after the donation, but recovered about 60% of that of pre-operative. These risks of living donation of kidney could be considered as acceptable, however, appearance of newly onset of proteinuria should be carefully followed up with appropriate treatments.

P.1667

Promotional effects of organ donation on online communities

Won-Hyun Cho[1,4], Jin-sook Yoo[4], Curie Ahn[2,4], Soon-Il Kim[3,4].
[1]Department of Surgery, Dongsan Medical Center Keimyung University, Daegu, Korea; [2]Department of Medicine, Seoul National University, Seoul, Korea; [3]Department of Surgery, Yonsei University, Seoul, Korea; [4]Vitallink, Seoul, Korea.

Objectives: Majority of teenagers tend to use SNS as their communication platforms. Therefore, we want to find out how effective a SNS promotion for securing organ donor registrants and spreading Life Sharing Culture including organ donation and transplantation to that young age group.
Methods: 1) We have selected 30 college students who were very active in running SNS on their college internet cafes, and they were given a mission as individual and team at same time. 2) Individual Mission: Each participants were asked to post 2 contents related to organ donation and transplantation on their SNS, Naver Opencast or other various social network platforms every week. 3) Team Mission: We have had them product videos for organ donation promotion. 4) We have selected best participant and team in the middle of a project and provided an opportunity to visit oversea organizations at the end of the project.
Results: 1) We have got 1,131 contents and 18 video files from individual and team activity from May 12 to August 5, 2012. 2) We have got 463,647 SNS views during a research period of 3 months and 244,436 more for 5 months after the research. The hits remain updated till now. 3) After the promotion activities by research teams, we have got a 30% increase (6,062) in organ donor registrants in the 20s compare with previous year(20,229 in 2011 vs 26,291 in 2012). 4) The rate of organ donor registrants in the 20s in 2011 was 21.3%, while 30.2% in 2012 from the annual age-specific organ donor registrants rate proportion.
Conclusion: 1) We have come to a conclusion that we could get fine effects to provide a correct understanding and education about life sharing and increase organ donor registrants from SNS promotion aims at the 20s. 2) The contents and videos made by research participants continue to be used to promote organ donation and transplantation to teenagers.

P.1668

The effects of Normal saline solution versus Hartmann's solution on the acid–base and electrolytes status and renal function after kidney transplantation

Won-Hyun Cho[1], Hyeong-Tae Kim[1], Ui-Jun Park[1], Min-Young Kim[2], Sul-Ki Hyun[1].
[1]Department of Surgery, Keimyung University, Dongsan Medical Center, Daegu, Korea; [2]Department of Nursing, Ulsan University College of Medicine, Ulsan, Korea.

Background: The purpose was to elucidate the effects of fluid on the acid–base and electrolytes status and renal function after kidney transplantation.
Methods: We retrospectively analyzed 103 patients who underwent kidney transplantation (KT). Analyses were performed separately according to the donor type (living=52, deceased=51). In the living donor KT group,
28 patients received Normal saline solution (NS) and 24 received Hartmann's solution (HS). In the deceased donor KT group, 27 patients received NS and 24 received HS. In each group, we compared the acid–base and electrolyte status, urine volume, and renal function between patients receiving NS and patients receiving HS.
Results: Regardless of donor type, there were no difference in potassium, pH, base excess, PCO2 and HCO3 between HS and NS on immediate postoperative and postoperative day 1. However, changes to neutral acid-base balance in terms of pH, HCO3, and base excess were significantly higher in HS than in NS. In living donor KT, NS increased serum potassium and chloride significantly during fluid therapy. On postoperative day 7, renal function showed no difference between two groups but urine volume was significantly larger in NS than in HS.
Conclusions: HS does not increase the incidence of hyperkalemia after KT. The use of HS resulted in less metabolic acidosis than the use of NS. Renal function was similar but polyuria was more severe in patients who received NS than in those who received HS.

Table 1. Allograft renal function and urine volume on postoperative day 7.

	NS	HS	p
Living Donor KT			
eGFR (mL/min/1.73 m²)	89.2±28.8	81.8±21.6	0.307
urine volume (mL/day)	10896.4±3889.6	8811.3±3485.2	0.049
Deceased Donor KT			
eGFR (mL/min/1.73 m²)	59.7±34.1	61.3±30.8	0.860
urine volume (mL/day)	6871.1±3579.3	4996.6±2361.1	0.031

Data are presented as mean±SD.

NS, Normal saline solution; HS, Hartmann's solution.

Table 2 Blood electrolytes and acid-base in normal saline group and Hartmann's solution in deceased donor KT

Variable	Group	Immediate postoperative (T0)	Postoperative Day 1 (T1)	Difference	p
Serum electolyte					
Sodium (mmol/L)	NS (n=27)	134,8±3,8	135,7±4,1	0,9±5,5	0,081
	HS (n=24)	132,9±2,6	134,7±2,6	1,8±2,6	
Potassium (mmol/L)	NS (n=27)	4,7±0,6	4,5±0,7	-0,2±0,7	0,121
	HS (n=24)	4,5±0	4,6±0,9	0,1±0,7	
Chloride (mmol/L)	NS (n=27)	98,8±3,8	100,1±4,7	1,3±6,0	0,243
	HS (n=24)	99,9±2,7	99,0±2,6	-0,3±3,7	
Acid-Base analysis					
pH	NS (n=27)	7,37±0,52	7,36±0,04	-0,01±0,07	0,006
	HS (n=24)	7,33±0,44	7,38±0,05	0,05±0,07	
PCO_2 (mmHg)	NS (n=27)	38,1±5,4	38,7±3,7	1,1±5,1	0,807
	HS (n=24)	37,8±5,4	39,3±4,3	1,5±5,8	
HCO_3 (mmol/L)	NS (n=27)	21,9±2,7	21,9±2,7	0,1±3,0	0,002
	HS (n=24)	19,3±2,4	23,2±4,4	3,9±4,7	
BE (mmol/L)	NS (n=27)	-1,7±4,7	-1,9±5,6	-0,1±7,5	0,009
	HS (n=24)	-5,2±2,8	-1,6±4,7	3,6±5,8	

Data are presented as mean±SD

NS, Normal saline solution; HS, Hartmann's solution

References:
[1] Magder S. Balanced versus unbalanced salt solutions: what difference does it make? Best Pract Res Clin Anaesthesiol 2014;28(3):235-47.
[2] Tellan G, Antonucci A, Marandola M, Naclerio M, Fiengo L, Molinari S, et al. Chir Ital 2008;60(1):33-40.
[3] Khajavi MR, Etezadi F, Moharari RS, Imani F, Meysamie AP, Khashayar P, et al. Effects of normal saline vs. lactated ringer's during renal transplantation. Ren Fail 2008;30(5):535-9.
[4] Prough DS, White RT. Acidosis associated with perioperative saline administration: dilution or delusion? Anesthesiology 2000;93(5):1167-9.
[5] Cittanova ML, Leblanc I, Legendre C, Mouquet C, Riou B, Coriat P. Effect of hydroxyethylstarch in brain-dead kidney donors on renal function in kidney-transplant recipients. Lancet. 1996;348(9042):1620-2.

P.1669

A report of deceased donor organ preservation with histidine-tryptophan-ketoglutarate solution (HTK) at a single, large volume U.S. center over 12 years' time

Richard Mangus[1], Jonathan A.Fridell[1], William Goggins[1], John Powelson[1], Chandrashekhar A.Kubal[1], Burcin Ekser[1], A. J.Tector[1].
[1]Surgery, Transplant, Indiana University School of Medicine, Indianapolis, IN, United States.

Introduction: There are two primary solutions utilized in the United States for preservation of solid abdominal organs, histidine-tryptophan-ketoglutarate (HTK, 35%) and University of Wisconsin (UW, 65%) solutions. Multiple studies have demonstrated clinical equivalence of these solutions in liver, pancreas, kidney and intestine transplantation. However, several large database studies have been published suggesting better outcomes with University of Wisconsin solution. This study reports on the transplant outcomes at a single, large center with primary use of HTK for liver, pancreas, kidney and intestine transplantation.
Methods: Data were extracted retrospectively from a large, single center database covering the period from 2003 to 2015. Graft and patient survival is recorded, with follow up greater than 98%. Organ preservation was performed using standard techniques including rapid exsanguination and cooling, with aortic and portal vein flush. HTK was used routinely for both living and deceased donor kidney grafts. No patients with available data were excluded from the analysis.
Results: Greater than 95% of organs at this center are preserved with HTK, including both adult and pediatric organs. HTK was utilized both for single organs, as well as combined transplants (kidney/pancreas, liver/kidney, liver/pancreas, liver/pancreas/intestine, pancreas/intestine, kidney/intestine). Current center results are available from the scientific registry of transplant recipients (release date June, 2015). Adult one-year survival at this center is 97% (kidney), 90% (liver), 94% all pancreas, and 60% (intestine). These results were all at or above the expected outcomes for all U.S. centers. Results for pediatric patients were also statistically equivalent to national outcomes. Single center, organ specific comparison of HTK and UW demonstrated clinical equivalence between the two solutions.
Conclusion: These results suggest that long-term, routine use of HTK at a high volume transplant center results in clinical outcomes equivalent to those for all centers. These findings were consistent in both adult and pediatric patients, for all abdominal organs.

References:
[1] Mangus RS. Comparison of Histidine-tryptophan-ketoglutarate solution (HTK) and University of Wisconsin solution (UW) in intestinal and multivisceral transplantation. *Transplantation.> 2008; 86(2):298-302.*
[2] Mangus RS. A comparison of histidine-tryptophan-ketoglutarate solution (HTK) and University of Wisconsin solution (UW) in extended criteria liver allografts. *Transplantation* 2008; 14:365-73.
[3] Fridell JA. Histidine-tryptophan-ketoglutarate for pancreas allograft preservation: The Indiana University experience. *American Journal of Transplantation* 2010; 10:1284-9.

P.1670

Effects of purification perfusate during subnormothermic machine perfusion for porcine liver donation after cardiac death

Hiromichi Obara[1,3], Noriyuki Morito[1], Naoto Matsuno[1,2,3], Shin Enosawa[3], Toshihiko Hirano[4], Hiroyuki Furukawa[2].
[1]Mechanical Engineering, Tokyo Metropolitan University, Tokyo, Japan; [2]Hepato-Biliary and Pancreas Surgery and Transplantation, National Asahikawa Medical Univ, Asahikawa, Japan; [3]National Center for Child Health and Development, National Research Institute for Child Health and Development, Tokyo, Japan; [4]Clinical Pharmacology, Tokyo University of Pharmacy and Life Science, Tokyo, Japan.

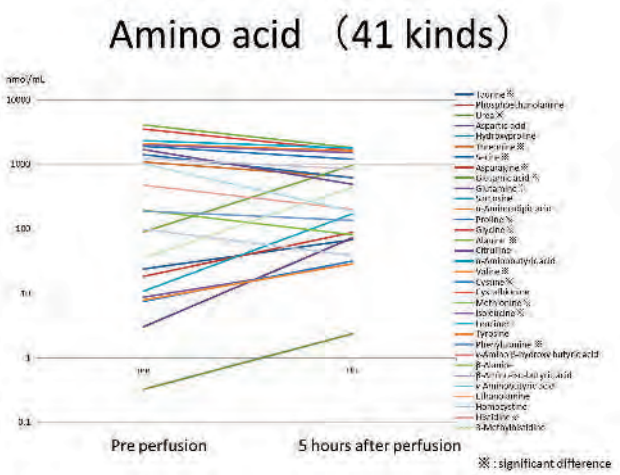
Introduction: Machine perfusion techniques for organ transplantation are particularly expected to preserve and resuscitate organs obtained from ECD or DCD grafts to expand the donated organ pool. The optimized preservation conditions for liver transplantation have been discussed and subnormothermic machine perfusion shows some advantages for the preservation of the ECD or DCD grafts. An advantage of the subnormothermic machine perfusion is to perfuse without red blood cells or an artificial oxygen carrier. Under subnormothermic condition, accumulation of various humoral factors such as cytokines and effluent enzymes might cause an ischemic reperfusion injury and dysfunctions of the organ. The elimination of the humoral factors can enhance the effect of the preservation. In this study, the effects of the purification of perfusate to eliminate the accumulate factors during the subnormothermic perfusion will investigate to consider the optimized perfusion condition.
Materials and Methods: Porcine(domestic female cross-bred with Large-Yorkshire, landrace, and Dulroc ,approximately 20 kg, 2 to 3 months old) livers were procured under warm ischemia time of 60 minutes condition. The livers were perfused under the subnormothermic condition(approximately 22℃) for 3 hours. Perfusate was used a modified University of Wisconsin solution. In this experiment, the perfusion condition was divided into two groups; regular machine perfusion and the purified condition under subnormothermic machine perfusion. In the purified condition, all perfusate was removed to substitute for purification of the perfusate after five minutes from the perfusion start. After removing the perfusate, new perfusate that has a same component and quantity was perfused to preserve the porcine liver for three hours. Effluent was obtained every 30 minutes to analyze the aspartate aminotransferase and (AST) and lactate dehydrogenase (LDH) levels.
Results and Discussion: The release rates of the effluent enzymes of AST and LDH in the perfusate as the difference from two hours after three hours were compared. The all calculated levels of the purified condition were lower than the regular condition. (delta-AST: 22.0±26.2 vs 49.0±40.8 IU/L, delta-LDH: 48.0±3.6 vs 150.7±161.2 IU/L). Furthermore, the pressure of the hepatic artery index under the purified condition shows also better than the regular condition. (0.46±0.08 vs 0.515±0.26 mmHg/ml/min). Therefore, the purified condition can propose better effects to the liver preservation compared to the regular condition. This result indicates the importance of the elimination of the accumulated humoral factors. Furthermore, this results will support to install the filtering system to remove such factors from the machine perfusion circuit under the subnormothermic condition.
Conclusion: Purification of perfusate during machine perfusion is necessary to improve the preservation and resuscitation of DCD liver grafts.
This work was supported by JSPS Kakenhi Grant Numbers 15H03922

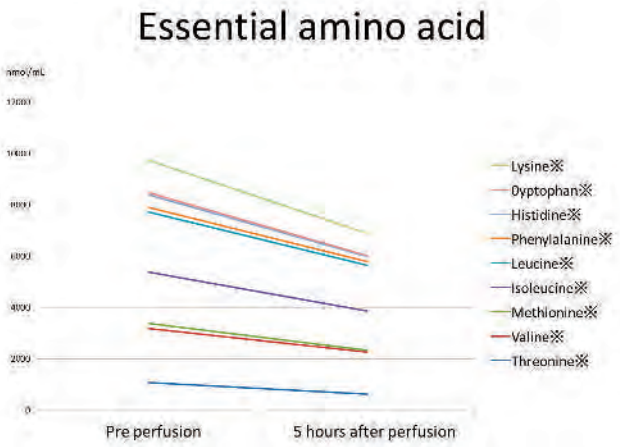
P.1671

Amino acid metabolism as viability assessment of liver grafts in a porcine model during ex vivo normothermic machine perfusion

Noriki Okada[1], Koichi Mizuta[1], Masamitsu Oshima[2], Takumi Katano[1], Yuta Hirata[1], Naoya Yamada[1], Yukihiro Sanada[1], Yoshiyuki Ihara[1], Taizen Urahashi[1], Jun Ishikawa[2], Takashi Tsuji[2], Shuji Hishikawa[3], Takumi Teratani[3], Eiji Kobayashi[3].
[1]Transplant Surgery, Jichi Medical University, Tochigi, Japan; [2]Department of Biological Science and Technology, Tokyo University of Science, Chiba, Japan; [3]Center for Development of Advanced Medical Technology, Jichi Medical University, Tochigi, Japan.

Background: Normothermic machine perfusion for the use of marginal liver graft, such as a graft from a donor after cardiac death, has been the focus of recent research to overcome the shortage of suitable liver grafts worldwide. Clinical trials using normothermic machine perfusion were reported. However, the perfusion conditions are not uniform, and the metabolic kinetics of the graft in the normothermic machine perfusion are not clearly delineated. Therefore, we measured amino acids in the perfusion solution before and after ex vivo normothermic machine perfusion and examined the amino acid metabolism of liver grafts during ex vivo normothermic machine perfusion in a porcine model.
Method: Total hepatectomy without cardiac arrest was performed for graft retrieval from crossbred miniature pigs (n=4). Liver grafts were perfused from the hepatic artery (20-30 mmHg) and portal vein (5-10 mmHg) with oxygenated perfusion solution immediately after the graft retrieval. Liver grafts were preserved at 20℃. The perfusion solution contained an electrolyte composition, glucose, amino acids and blood (10-20%) as an oxygen carrier. Amino acid concentrations in the perfusion solution were measured before perfusion and at 5 hours after the initiation of perfusion.
Results: A total of 41 amino acids were measured. Six amino acids increased significantly, and 15 amino acids decreased significantly. Eleven amino acids exhibited a relative increase, and 9 amino acids were not altered. The eight essential amino acids exhibited a significant decrease. In the amino acids involved in the urea cycle, urea and arginine increased significantly, and ornithine decreased significantly. Aspartic acid and citrulline exhibited a relative increase.
Discussion: We revealed that liver graft metabolism of amino acids during ex vivo normothermic machine perfusion decreased the eight essential amino acids. In the amino acids involved in the urea cycle, urea and arginine increased significantly, which suggests low mitochondrial activity. The measurement of amino acids in the perfusion solution reveals graft viability in ex vivo normothermic machine perfusion, and some amino acids may require to keep suitable viability of liver grafts during ex vivo normothermic machine perfusion.
Conclusion: Liver grafts metabolise amino acids in ex vivo normothermic machine perfusion. Oxygenated perfusion solution with some amino acids may provide a potential advantage for viability of liver grafts during ex vivo normothermic machine perfusion.





P.1672

Impact of machine perfusion on long-term kidney transplant outcomes

Shaifali Sandal[1], Xun Luo[2], Allan Massie[2], Marcelo Cantarovich[1], Dorry Segev[2].
[1]Medicine, McGill University Health Center, Montreal, Canada; [2]Surgery, Johns Hopkins University, Baltimore, United States.

Body: Machine perfusion (MP) has been shown to decrease DGF rates in kidney transplant (KT) recipients, particularly in high risk kidney allografts such as those from ECD. However, there is a paucity of data examining the effect of MP on graft outcomes, especially beyond 1 year.
Methods: We conducted a retrospective analysis of 78,207 adult deceased-donor KT recipients using SRTR data from 2006-2013. The cohort was stratified by donor type (SCD-DBD, SCD- DCD, ECD-DBD, ECD-DCD) and using the KDPI score (0-70, 71-80, 81-85, 86-90, 91-100). Outcomes of interest were all cause graft failure, death-censored graft failure (DCGF) and mortality. We used time-varying Cox regression to model the association between MP and graft outcomes (first year and subsequent years) adjusting for recipient, donor and transplant characteristics.
Results: When compared with SCD-DBD kidneys (26.7%), more ECD-DCD (87.6%), SCD-DCD (72.4%) and ECD-DBD (53.2%) kidneys underwent MP. MP was associated with improved graft outcomes only in ECD-DCD recipients, and only in the first year post-transplant, with no additional long-term improvement (Table 1). In several subgroups, MP was actually associated with worse outcomes, including ECD-DBD DCGF beyond 1 year, and KDPI 0-70 in the first year (Table 2). There was no association between MP use and recipient mortality.
Conclusion: Overall, MP use does not confer an improved 5-year patient or graft survival and may be associated with inferior outcomes in some donor subtypes.

Table 1: Hazard ratio for MP use and graft outcomes by donor type

	0-1 year	95%CI	1-5 year	95%CI
DCGF				
SCD-DBD	1.03	0.93-1.14	1.05	0.97-1.14
SCD-DCD	1.11	0.90-1.38	1.14	0.92-1.42
ECD-DBD	1.04	0.91-1.19	**1.15**	**1.01-1.31**
ECD-DCD	**0.56**	**0.32-0.98**	0.73	0.34-1.54
All-cause graft failure				
SCD-DBD	1.02	0.94-1.10	1	0.94-1.07
SCD-DCD	1.11	0.94-1.32	1.11	0.95-1.30
ECD-DBD	0.99	0.89-1.10	1	0.91-1.11
ECD-DCD	**0.59**	**0.38-0.91**	0.91	0.55-1.51

Table 2: Hazard ratio for MP use and graft outcomes by KDPI scores

	0-1 year	95% CI	1-5 year	95%CI
DCGF				
0-70	**1.16**	**1.05-1.27**	1.02	0.94-1.10
71-80	0.98	0.81-1.19	1.01	0.84-1.21
81-85	0.99	0.77-1.26	0.94	0.73-1.22
86-90	0.96	0.74-1.23	1.22	0.92-1.63
91-100	1.02	0.84-1.24	1.17	0.96-1.44
All-cause graft failure				
0-70	**1.10**	**1.02-1.18**	0.98	0.94-1.06
71-80	1.03	0.88-1.20	1.07	0.93-1.23
81-85	1.04	0.84-1.27	0.91	0.75-1.10
86-90	0.81	0.67-1.01	0.92	0.75-1.12
91-100	0.91	0.77-1.07	0.96	0.83-1.12

P.1673

Non-standardized preservation techniques may lead to freezing of the donor kidney during storage and may preclude transplantation

Imeshi Wijetunga, Rupaly Pande, Sonsoles Martinez-Lopez, Kathryn Brady, Muhammed Jameel, Vivek Upasani, Lutz Hostert, Niaz Ahmad.
Department of Transplantation, Leeds Teaching Hospitals NHS Trust, Leeds, United Kingdom.

Introduction: Hypothermic static storage is the standard method of kidney preservation for transplantation. Following in-situ flush, kidneys are packaged in sterile bags of cold preservation fluid which is then placed on ice at 0-4°C. Freezing of kidney during storage is associated with the formation of ice crystals, which in conjunction with subsequent thawing, destroys the integrity of the cells. An unexpected drop in temperature, inadequate volume of preservation fluid or a rise in the freezing point (mixing other solution or ice/slush to the preservation fluid) may lead to freezing of the kidney during storage. Some centres routinely add saline ice (used during back-table dissection) into the bag containing the kidney and the preservation fluid. We describe one case of a near miss where freezing was avoided due to timely intervention and two cases of frozen kidneys where kidney could not be transplanted.
Methods: Three kidneys received at our centre in the past three years met the criteria for inclusion in this review of practice.
Results: Kidney 1 was packed with ice blocks and scanty preservation fluid. Timely intervention prevented imminent freezing of this kidney. The recipient of Kidney 1 had primary function with 3month and 12month creatinine of 99, and 127µmol/L. Kidney 2 was noted to be frozen when opened immediately prior to implantation. The kidney was stored in scanty preservation fluid again with ice blocks. Kidney 3 arrived frozen with scanty perfusion fluid, again with ice blocks. Analysis of fluid biochemistry in one case confirmed contamination of preservation fluid with a sodium rich fluid.
Conclusions: Freezing renders the kidney non-transplantable. In most cases, freezing occurs due to either scanty preservation fluid or placing saline ice blocks in direct contact with the kidney. Contamination with saline may also raise the freezing point of the kidney. Appropriate packaging with preservation fluid and icing of the kidney during storage is crucial to prevent freezing. All professionals involved in organ procurement and transplantation should be aware of the detrimental effects of incorrect packaging of organs and should adhere to best practice guidelines in order to avoid these “never events”.

P.1674

Normothermic preservation of the rat hind limb with artificial oxygen-carrying hemoglobin vesicles

Jun Araki[1], Hiromi Sakai[2].
[1]Plastic surgery, University of Tokyo Hospital, Tokyo, Japan;
[2]Chemistry, Nara Medical University, Nara, Japan.

Background: For managing major limb amputation, it is important to consider ischemic time and reperfusion injury by free radicals after the blood supply is reestablished. State of preservation during transplant surgery is crucial for the survival and function of the tissue, graft, or organ. In this study, we confirmed the effect of intermittent blood flow in rat ischemic hind limb and developed a new oxygenic preservation method using artificial oxygen carrying hemoglobin vesicles (HbVs).
Methods: We first compared a continuous ischemic model and an intermittent reflow model on rat hind limb. At postoperative day 7, hind limbs were evaluated. Next, we performed total amputation, normothermic preservation by perfusion with extracellular-trehalose-Kyoto (ETK) solution or HbV, and microsurgical replantation of the left hind limb. Venous efflux was analyzed, the amputated limb evaluated after 6 hr perfusion, and the replantation outcome of each model was compared.
Results: In our early study, 24 hr continuous ischemic model necrotized, but intermittent reflow model almost survived except for partial necrosis at postoperative day 7. Scar tissue on the right limb showed myonecrosis and infiltration of inflammatory cells. Skeletal muscle on the right limb was structurally well maintained. Hemoglobin vesicleYtreated limbs appeared to have much better oxygenation than ETK-treated limbs. Aerobic respiration remained in the amputated limb, gastrocnemius muscle was well maintained, and the overall replantation was successful in the limb preserved using HbV.
Conclusion: These studies demonstrated that oxygenic preservation is effective for rat ischemic limb, suggesting that this method may be useful for other replantation and transplantation surgeries.
References:
[1] Normothermic preservation of the rat hind limb with artificial oxygen-carrying hemoglobin vesicles. Araki J, Sakai H, Takeuchi D, Kagaya Y, Tashiro K, Naito M, Mihara M, Narushima M, Iida T, Koshima I. Transplantation. 2015 Apr;99(4):687-92. doi: 10.1097/TP.0000000000000528.

P.1675

Experimental study of new vascularized composite allotransplantation: Anorectal transplantation for stoma patients

Jun Araki[1], Yuji Nishizawa[2], Naoki Fujita[3], Munekazu Naito[4], Flavio Galvao[5].
[1]Plastic surgery, University of Tokyo Hospital, Tokyo, Japan; [2]Colorectal surgery, National east cancer center, Chiba, Japan; [3]Veterinary surgery, University of Tokyo, Tokyo, Japan; [4]Anatomy, Aichi Medical University, Aichi, Japan; [5]Transplant surgery, University of Sao Paolo, Sao Paolo, Brazil.

Ostomy is an effective surgery for various anorectal dysfunctions. However, patients often suffer greatly from stress caused by their stomas. Many alternative therapies have been developed, but none have resolved this critical issue. Anal function reconstruction is a challenging issue for colorectal and reconstructive surgeons. Allotransplantation of the anorectal segment (all organs of defecation function including perineal skin, anus, rectum, and sphincter muscle) has major potential to be an innovative therapy for anal dysfunctions. We have experimented on anorectal transplantation with rats, dogs, and human cadavers. In the rat model, we succeeded in autotransplantation of the anal segment using super-microsurgery, a technique enabling anastomosis of small vessels 0.3 mm in diameter. As the next step, we examined vascularized anorectal transplantation in a canine model, as anal function is similar to that of humans. Indocyanine green angiography showed the pudendal arteries to provide more blood flow than the inferior mesenteric artery (IMA) to the anal segment. Infection and rejection were controlled with antibiotic and immunosuppressant. Anal function was suggested to be reconstructed 1 year after surgery using pudendal nerve cutting and re-anastomosis models. In addition, mock anorectal transplantation was performed on a human cadaver. One cadaver served as the deceased donor and an anorectal graft was harvested including the perineal skin, anus, rectum, and sphincter muscle. The other cadaver served as the recipient after abdominoperineal excision. The donor anorectal graft was transplanted into the recipient's defect with microanastomoses of the IMA, IMV, and pudendal nerves. Human anorectal transplantation was revealed to be technically possible. Recent progress in operative maneuvers and transplant medicine has allowed other novel transplants, such as limbs, face, larynx, and uterus, for patients. The ethics of non-life-saving organ transplants involve assessment of possible gains in the quality of life (QOL) to be achieved for the recipient relative to the risks of the procedure and the follow-up interventions necessary for maintaining organ function. Many problems remain before anorectal transplantation can be applied clinically. Thus, we physicians must continue our efforts to improve the QOL of stoma patients.

References:

[1] Anorectal autotransplantation in a canine model: the first successful report in the short term with the non-laparotomy approach. Araki J, Nishizawa Y, Nakamura T, Sato T, Naito M, Hatayama N, Hirai S, Tashiro K, Koshima I. Sci Rep. 2014 Sep 10;4:6312. doi: 10.1038/srep06312.

P.1677

Demands of overseas organ transplant recipients' family caregivers: comparing gender and Chinese family relationships

Hong-Mei Chen[1], Shoei-Shen Wang[2], Fu-Jin Shih[3].
[1]Department of Nursing, Tri-Service General Hospital, Taipei, Taiwan; [2]Department of Surgery, National Taiwan University Hospital, Taipei, Taiwan; [3]Department of Medical Research, Ditmanson Medical Foundation Chia-Yi Christian Hospital, Chia-Yi, Taiwan.

Introduction: Gender stereotypes have played an important role in ethnic-Chinese society for centuries. Overseas organ transplant recipients' family caregivers were identified the major social support resources for recipients during the pre- and post-organ transplant stages. Nevertheless, little information was available about different gender's demands of Chinese overseas organ transplant recipients' family caregivers.
Objectives: This study aimed to explore the demands of Chinese overseas organ transplant recipients' family caregivers during overseas organ transplant transitions from the perspective of gender roles and family relationships.
Methods: An exploratory qualitative method was employed using a purposive sample of overseas organ transplant recipients' family caregivers from Taiwan. Qualitative data were collected by face-to-face semi-structured interviews, and were analyzed by content analysis.
Results: The participants included 57 overseas organ transplant recipients' family caregivers (50 were spouses, 4 had father-son relationship, 2 had mother-daughter relationship, and one had sibling relationship).
Throughout the overseas organ transplant transitions, the demands shared by all participants included the access of relevant information, an adequate organ source, and collaboration and connection with international medical institutes.
During the pre-overseas organ transplant transitions, male family caregivers expected to show masculinity and help family's financial needs. Local government or charity organizations were expected to provide economical subsidies for overseas organ transplantation. Male family caregivers who had parent-child relationship or brothership required nursing assistance or regular visits from volunteers to help alleviate caring burden.
Compared to male family caregivers, female family caregivers tended to undertake the care responsibility. They accomplished more caring tasks, and spent more time with the recipient to comfort their emotional needs. Therefore, female family caregivers also demanded emotional support and affirmation from medical personnel to ease caring burdens.
Conclusions: The specific demands of Taiwanese overseas organ transplant recipients' family caregivers were compared. Health professionals would be empowered to provide better quality of care for different family relationship groups in order to promote their recovery from overseas organ transplantation.

P.1700

The use information technology to develop organ fundraising education courses in Taiwan

Ming- Hsin Hsieh[5,6], Fu-Jong Shih[7], Fu-Chi Yang[8], Shoei-Shen Wang[1,2], Fu-Jin Shih[3,4].
[1]Department of Surgery, National Taiwan University Hospital, and National Taiwan University College of Medicine, Taipei, Taiwan; [2]Department of Surgery, Fu Jen Catholic University Hospital/Fu Jen Catholic University College of Medicine, New Taipei City, Taiwan; [3]Institute of Clinical & Community Health Nursing, National Yang-Ming University, Taipei, Taiwan; [4]Department of Medical Research, Ditmanson Medical Foundation Chia-Yi Christian Hospital, Chayi, Taiwan; [5]School of Nursing, National Yang- Ming University, Taipei, Taiwan; [6]Education and Research Center, Ten Chen General Hospital, Tao- Yuan, Taiwan; [7]Department of Gerontological Care and Management, Chang Gung University of Science and Technology, Tao-Yuan, Taiwan; [8]Department of Nursing, Central Taiwan University of Science and Technology, Institute of Allied Health Sciences, College of Medicine, National Cheng Kung University, Tainan, Taiwan.

Background: Organ transplantation (OT) is a cutting edge medical breakthrough in modern surgical techniques, same in Taiwan.Organ transplantation (OT) is a cutting edge medical breakthrough in modern surgical techniques, same in Taiwan. The efficacy and usefulness of organ fundraising outcomes were argued by organ transplant health professionals (OTHP) in Taiwan. The information technology (IT) was suggested to apply for local and overseas organ transplantation (OT). Nevertheless, few of OTHP had interest in learning organ fundraising education (OFEC), they also have not learning with IT knowledge. Script theory has been already extensive research and application by scholars at home and abroad, also confirmed that the theory applied can help others and understanding their own actions, and with good training results.
Method: This project aimed to develop a friendlier OFEC from the perspectives of OTHP and IT experts. A qualitative design was employed. Both OTHP from leading medical centers and IT experts from universities were recruited to help develop the OFEC. Face- to-face in-depth interviews with the participants. Narrative data were analyzed by qualitative content analysis mode, and design IT training program based on clinical situation and script theory.
Results: Three phases of OFEC were developed. In the first phases, 15 OTHP including 10 OT nurses from ICU, and 5 OT surgeons were interviewed and reported their expectations for OFEC. In the second phases, 5 IT experts were invited to help develop a multi- discipline IT education framework to arouse OTHP's interest of OFEC and provide accurate OFEC knowledge. In the last phase, the 15 OTHP were invited again to evaluate the usefulness, friendliness, and efficacy of the OFEC. The first version of OFEC was first developed from the perspectives of OT surgeons, nurses, and IT experts in Taiwan.
Conclusion: This report identified the need of clinical education and coping strategies as perceived by organ transplant health professionals in Taiwan. It provided several suggestions. Taiwan can set up a digital learning program combined with multidiscipline professional resources, medical institutions, and government departments. This helps health professional study OFEC relevant organ fundraising team members' profession future in Taiwan. By doing so, health professionals can successfully establish and improve study effect in limited time, increase manpower of experienced OTHPs, develop the OT-expert training program for interdisciplinary members, promote cooperation between care teams to establish a singular PTOR care team with a common consensus and good communication skills, and integrate humanistic care-training program for assistance from social workers. Altogether, these strategies shall help effectively manage difficulties faced by the care team and enhance overall quality of care in clinical organ transplantation.

References:

[1] Tong A., Chapman J. R., Wong G., Josephson M. A., & Craig J. C. (2013). Public awareness and attitudes to living organ donation: Systemic review and integrative synthesis. Transplantation, 96(5), 429-437.
[2] Schiano T. D., Rhodes R. (2010). The dilemma and reality of transplant tourism: an ethical perspective for liver transplant programs. Liver Transplantation, 16: 113-117.
[3] Van Hardeveld, E. & A. Tong. (2010). Psychosocial care of living kidney donors. Nephrology, 15, S80- 87.
[4] Douville F., Godin G., Vézina- Im L-A. (2014). Organ and tissue donation in clinical settings: A systemic review of the impact of interventions aimed at health professionals. Transplantation Research, 3(8), 1-13.
[5] Johnson Robert. (2014). "Kant's Moral Philosophy", The Stanford Encyclopedia of Philosophy (Summer 2014 Edition), Edward N. Zalta (ed.), URL = .
[6] Mahanta D. & Ahmed M. (2012). E- learning objectives, methodologies, tools, and its limitation. International Journal of Innovative Technology and Exploring Engineering, 2(1), 46-51.
[7] Sewell J. P. & Thede L. Q. (2011). Nursing informatics definitions over the last 30 years. Informatics and Nursing: Opportunities and Challenges. 4th Edition.
http://dlthede.net/informatics/Informatics.html
[8] Santos D. S., Almeida, Reis R. K. (2013). Working education program in health: transforming experience of nursing teaching and practice. Revista da Escola de Enfermagem da USP. 47(6), 1427-1432.
[9] Arriola K. R., Robinson H.Z., Perryman J. P., Thompson N. J., Russell E. F. (2013). Project ACTS II: Organ donation education for African American adults. NIH Public Access, 23(2), 230-237.
[10] Taylor C. M. & Hossam Hamdy. (2013). Adult learning theories: Implications for learning and teaching in medical education: AMEE Guide No. 83. Medical Teacher, 35: e1561-e1572.
[11] Thorndike E. L. (1906). The principles of teaching- Based on psychology. Educational Psychology in Teachers College, Columbia University.
[12] Baumgartner L. M., Lee Ming- Yeh, Birden S., Flowers D. (2003). Adult Learning Theory: A primer. Information Series. ERIC Clearinghouse on Adult, Career, and Vocational Education, Columbus, Ohio.
[13] Mezirow J. (1987). How critical reflection triggers transformative learning. Fostering Critical reflection in Adulthood- A guide to transformative and emancipatory learning. 2014/9/28.
http://www.ln.edu.hk/osl/conference2011/output/breakout/4.4%20%5Bref%5DHow%20Critical%20Reflection%20triggers%20Transformative%20Learning%20-%20Mezirow.pdf
[14] Ruiz J. G., Mintzer M. J., Leipzig R. M. (2006). The impact of e- learning in medical education. Academic Medicine, 81(3), 207-212.
[15] Choules A. P. (2007). The use of e- learning in medical education: a review of the current situation. Postgraduate Medical Journal, 83(978), 212-216.
[16] Mahmud K. M. (2010). E- learning for tertiary level education in least developed countries: Implementation obstacles and way outs for Bangladesh. International Journal of Computer Theory and Engineering, 2(2), 1793-8201.
[17] McGonigle D. & Mastrian K. G. (2014). Nursing Informatics and The Foundation of Knowledge, 3rd Edition. World Headquaters Jones & Barlett Learning: Burlington.
[18] Hiltz, S.R. & Turoff, M. (2002). What makes learning networks effective? Communications of the ACM, 45:5, 56-59.
[19] Schank, R.C. & Cleary. C. (1995). Engines for education. Hillsdale. NJ: Erlbaum Assoc.
[20] Schank, R. C., Robert P. A. (1975). Scripts, plans, and knowledge. Yale University.
[21] Puto, C. P. (1985). Memory for scripts in advertisements. Advances in Consumer Research XII:404-409. Fifteenth Annual

Conference. Association for Consumer Research.
[22] Erasmus, A. C., Bishoff E., Rousseau G. G. (2010). The potential of using script theory in consumer behaviour research. Journal of Family Ecology and Consumer Sciences/Tydskrif vir Gesinsekologie en Verbruikerswetenskappe 30, no. 0 .
[23] Schank, R. C., Robert P. A. (2013). Scripts, plans, goals, and understanding: An inquiry into human knowledge structures. Psychology Press.
[24] Abelson R. P. (1981). Psychological status of the script concept. American Psychologist, 36(7), 715- 729.
[25] Charlin, Bernard, et al. (2007). Scripts and clinical reasoning. Medical education. 41, (12), 1178-1184.
[26] Ross, J. N., Nicole M. C. (2011). Gold digger or video girl: the salience of an emerging hip-hop sexual script. Culture, health & sexuality, 13(2), 157-171.
[27] McCormick, N. B. (2010). Preface to sexual scripts: Social and therapeutic implications. Sexual and Relationship Therapy, 25(1), 91-95.
[28] Alyusheva, A. R., Veronika V. N. (2012). Identity Formation and Autobiographical Memory: Two Interrelated Concepts of Development." Proceedings of World Academy of Science, Engineering and Technology. World Academy of Science, Engineering and Technology, 66
[29] Barratt, Sara. (2012). Incorporating multi-family days into parenting assessments: the Writtle Wick model. Child & Family Social Work, 17(2), 222-232.
[30] Kleinaltenkamp, Michael, J. van Stiphout, and T. Eichentopf. (2010). The customer script as a model of customer process activities in interactive value creation. Proceedings of the 11th International Research Seminar in Service Management, 25(28).
[31] Eichentopf T., Kleinaltenkamp M., and Janine Van Stiphout. (2011). Modelling customer process activities in interactive value creation. Journal of Service Management, 22(5), 650-663.
[32] Carlborg P., Daniel K., Christian K. (2013). A lean approach to service productivity improvements: Synergy or oxymoron? Managing Service Quality, 23(4), 3-3.
[33] Norman G. (2005). Research in clinical reasoning: past history and current trends. Medical Education, 39(4), 418-27.
[34] Norman G. (2006). Building on experience—the development of clinical reasoning. New England Journal of Medicine, 355(21), 2251-2
[35] Charlin B., Tardif J., Boshuizen H. P. (2000). Scripts and medical diagnostic knowledge: theory and applications for clinical reasoning instruction and research. Academic Medicine, 75(2), 182-90.
[36] Charlin B., Tardif J., Boshuizen H. P. (2000). Scripts and medical diagnostic knowledge: theory and applications for clinical reasoning instruction and research. Academic Medicine, 75(2), 182-90.
[37] Ho, Anthony M. H., Michele G., Alexander C. V. (2010). Using illness scripts to teach clinical reasoning skills to medical students. Family Medicine, 42(4), 255-61.
[38] Boshuizen, Henny P. A., Henk G. S. (2008). The development of clinical reasoning expertise. Clinical Reasoning in The Health Professions, 2008, 113-121.
[39] Dory, Valérie. (2012). How to construct and implement script concordance tests: insights from a systematic review. Medical education, 46(6), 552-563.

P.1701

How to build a liver transplant unit in India: Rajasthan's success

Giriraj Bora, Manoj Maloo, Shyam S.Mahansaria, Dilip Jain, ML Swarankar, Christopher Taylor Barry.
Liver Transplantation and Hepatobiliary Surgery, Mahatma Gandhi Hospital, Jaipur, India.

Deceased donor liver transplant in India is, to a large degree, confined to large metros in Southern India (e.g., Chennai, Coimbatore, Bangalore, Kerala, Hyderabad), with some activity in the Northwest, including Delhi and Mumbai. True progress in deceased donor transplantation in India has only recently occurred when Tamil Nadu constructed an incredibly successful deceased donor transplant program in 2008. Rajasthan is one of India's largest states, but until February 2015 did not have a deceased donor transplant program. Rajasthan's first liver transplant unit at Mahatma Gandhi Hospital in Jaipur took 6 months to conceive and realize. In that period of time, a multidisciplinary team of over 50 transplant professionals was assembled and trained using both Western and Indian protocols, policies and procedures and two successful deceased donor liver transplant were performed with excellent outcomes. The program costs, including personnel, equipment and infrastructure, were approximately 10% of American costs and the unit can offer liver transplants at less than 10% American costs ($25,000 USD). Cost savings are largely due to decreased personnel expenditures, although less expensive logistical and material costs also play a significant role. The success in Rajasthan demonstrates that deceased donor liver transplant activity can efficiently and economically grow throughout India, even outside of the corporate sector, with success comparable to international transplant standards.

P.1702

International collaborative program as a way of development of adult-to-adult living-donor liver transplantation program in National Scientific Medical Research Center

Marlen Doskali, Akhmet Seidakhmetov, Myltykbay Rysmakhanov, Aliya Taganova, Aibolat Smagulov, Aidos Kulmaganbetov, Abay Baigenzhin, Zhaksylyk Doskaliyev.
Organ and tissue transplantation, JSC National Scientific Medical Research Center, Astana, Kazakhstan.

Living donor liver transplantation (LDLT) from adult to adult has become a standard and effective treatment method and has being increasingly performed for patients with end stage liver disease. In our study we present the collaborative work experience of LDLT program development under the support of colleagues from experienced centers in National Scientific Medical Research Center during more than three years.
From January 2013 to March 2016, 34 LDLTs were performed in our center. Operation procedures were performed by standard methods in donor and recipients. In all donors right hepatectomies were performed. At a median follow up of 2 years, both the patient and graft survival were 82%. The main causes of transplantation were primary biliary cirrhosis (50%), viral hepatitis (30%) and other liver diseases. The median age of the recipients at the time of LDLT was 43.9±17.2 (19-65 years). Recipients average hospital stay was 30±5 days (23-38 days, median 30 days) found. Vascular and biliary complications were the leading cause of reoperation, graft loss and retransplantation. Postoperatively, these recipients were started on a triple therapy immunosupression. We have not seen any early or late surgical complications in donors.
In conclusion, collaborative work with centers from abroad gives opportunity to increase the development of management in liver transplantation to achieve the own strategy of LDLT program.

P.1703

First experience with donation after circulatory death transplantation program in India

Deepesh Kenwar[1], Sarbpreet Singh[1], Sunil Kumar[1], Soham Dasgupta[1], Manish Rathi[2], Ashish Sharma[1], Harbir S.Kohli[2], Vivekanand Jha[2], Krishan L.Gupta[2], Mukut Minz[1].
[1]Department of Renal Transplant Surgery, PGIMER, Chandigarh, India; [2]Department of Nephrology, PGIMER, Chandigarh, India.

Donation after circulatory death (DCD) is not prevalent in India in the absence of a legal framework and guidelines for discontinuation of life support in end of life situations. This report describes attempts at initiating a DCD program at a tertiary care centre in India. From August 2011 to December 2015, 6 donors were operated after cardiac arrest. The donor were declared dead after 5 minutes of absence of ECG signal. CPR was restarted in fourpatients till organ prefusion. Heparinization and rapid cannulation of aorta with extensive surface cooling during dissection was done in all cases. Eleven out of twelve kidneys were utilized. Mean donor age was 28±12.75 years, M: F ratio was 5:2. Mean age of recipients was 40±10.01 years, M: F ratio was 8:3. Four recipients had immediate graft function and 7 patients required dialysis in postoperative period. Mean postoperative day 0 urine output was 2.1±2.5 litres. Mean lowest creatinine was 1.43±0.37 mg/dl at an average 26.75 ±15.22 days after transplantation. Continuation of CPR efforts (n=4) resulted in better recovery of graft function (mean time to reach nadir creatinine 20.4±6.23 vs 34.3±23.7 days, mean nadir creatinine 1.38±0.31 vs 1.52±0.45 mg%). In cases when CPR was not continued, one kidney had primary non function and another had cortical necrosis. In countries such as India with a severe shortage of organs DCD donors can greatly augment the deceased donor programme. Continued CPR appears to be associated with a more rapid recovery of graft function.

P.1704

Recurrent nephrotic syndrome after renal transplantation in Syrian children: Does it compare with the international experience?

Bassam Saeed, Huda Mazloum.
Pediatric Nephrology, Kidney Hospital, Damascus, Syrian Arab Republic.

Background: Recurrent disease occurs in around 30% of children transplanted for steroid-resistant nephrotic syndrome. Its precipitating risk factors have rarely been studied in the Middle East. The aim of the study was to determine what characterize the post-transplant recurrence of nephrotic syndrome (NS) in Syrian children and how does it compare with the published experience from other parts of the world. We enrolled 12 nephrotic children who received one renal allograft at our center; the mean age at transplant was 11.3 years (8.7 - 15.3); focal and segmental glomerulosclerosis (FSGS) has been shown in 9 out of 10 patients who underwent renal biopsy; 4 patients were having affected relatives and the remainder were considered as sporadic cases; genetic screening for NPHS2, NPHS1, and WT1 mutations were done for 6 patients, one novel homozygous NPHS2 mutation has been identified in one patient; none of the patients has had one or both native kidneys removed prior to transplant; all cases were transplanted from living donors as follows: 7 from related donors and 5 from unrelated donors. 4 patients recurred the disease after transplantation, hence the overall recurrence rate was 33% (4/12); none of the recurrent patients did show an initial response to plasmapheresis therapy, however, one patient did show a complete and spontaneous remission 20 months after transplant. As expected, the patient with NPSH2 mutation didn't recur. Interestingly enough, all recurrent cases had received a kidney from related donor and were initially classified as sporadic cases. Though not statistically significant, the risk of recurrence from related donors is 6.75 times higher compared to that from unrelated donors (95%CI: 0.44-102.80; P=0.16). Sporadic cases showed a risk of recurrence that is 5 times higher compared to familial cases (95%CI: 0.33-75.11; P = 0.24). This observation may suggest that living related donor transplant is at higher risk for clinical recurrence; however, a properly designed prospective multicenter study is needed to validate this observation. After a mean post-transplant follow up period of 3.2 years, 6 patients were still having a well-functioning graft, 4 patients with CKD, and 2 patients returned to dialysis, both had lost their graft due to recurrence.
Conclusion: This data suggest that living related donor grafts should be used with constraint because of the increased incidence of recurrence.

P.1705

The impact of living-unrelated transplant on establishing deceased donor liver program in Syria

Bassam Saeed.
Pediatric Nephrology, Kidney Hospital, Damascus, Syrian Arab Republic.

Liver transplant is the criterion standard for patients with end-stage liver disease. Yet there is no liver transplant in Syria. Traveling abroad for a liver transplant is a luxury few Syrians can afford.
There is currently an on-going debate whether to start a liver transplant program using living or deceased donors. In 2003, a new law was enacted, authorizing the use of organs from volunteer strangers and deceased donors. Despite the positive aspects of this law (allowing unrelated donors to increase the number of transplants in the country); the negative aspects also were obvious. The poor used the law to sell their organs to the rich, and this model is in violation of the Istanbul Declaration.
To better document transplant communities' perceptions on organ donation, an e-mail survey was sent to a nationally representative sample of physicians (n = 115) that showed that 58% of respondents did not support the start of liver transplant from live donors, as they fear a considerable risk for the donor and the recipient. Seventy-one percent of respondents believe that unrelated kidney donation has contributed to tarnishing the reputation of transplant, and 56% believe that a deceased-donor program can run in parallel with unrelated organ donations. The interest in deceased-donor program has been affected negatively by the systematic approach of using poor persons as the source of the organ. This lack of interest has affected starting a liver program that relies on deceased donors; especially the need for kidneys is more than livers. Health authorities in Syria were inclined to initiate a liver transplant program from live donors, despite the risks of serious morbidities and mortality
Conclusion: paid kidney donation in actual effect is actually a hindrance to establishing a deceased-donor liver program.

P.1706

Novel hepatitis C treatment increases access to kidney transplantation

H.V. Patel, V.B. Kute, P.R. Shah, A.V.Vanikar, U.T. Varyani, P.S. Wakhare, S.G. Shinde, V.A. Godhela, M.M. Patel, V.B.Trivedi, G.S. Kasat, M.V. Patil, J.C. Patel, D.P. Kumar, H.L. Trivedi.
IKDRCITS, Ahmedabad, India.

Background: HCV-infected renal transplant recipients have worse patient and allograft survival after transplantation compared with non-infected renal transplant recipients. However, HCV-infected patients have a lower mortality following transplantation compared with mortality on dialysis. HCV infection is not considered a contraindication to transplantation.
The current AASLD and ISDA HCV guidelines state that Sofosbuvir - containing regimens can be considered for patients with CrCl < 30 mL/min with expert consultation, as efficacy and safety data are not yet available
Methods: treatment of HCV infection in patients with renal impairment has been limited by low cure rates and poor tolerability. The introduction of direct-acting antivirals (DAAs) has revolutionized treatment of HCV with impressive cure rates >90% and low rates of adverse events. Despite these major advancements, treatment of patients with HCV and advanced chronic kidney disease is a major challenge due to the lack of efficacy and safety data in this patient population. DAAs included simeprevir, sofosbuvir, ledipasvir, ombitasvir, paritaprevir, dasabuvir, grazoprevir, elbasvir and daclatasvir.
Results: We used in 22 ESRD patients (19 males and 3 females) on dialysis a combination of Sofosbuvir (400mg alternate day), Injection PEG-INF 2a 135 mcg subcutaneous once a week and ribavirin (200Mg once a day) for 12 weeks. Mean baseline HCV-RNA (QUANTITATIVE) was 409523.6 copies/ml. All patients has HCV genotype 1. HCVRNA by reverse transcription polymerase chain reaction (RT-PCR) was less than detected in 4, 8 and 12 weeks in 81.8 %(n=18), 90.9%(n=20) and 100% (n=22). All the patients had stable hemoglobin with optimization of iron, erythropoietin and anemia therapy. Blood transfusion was given in 3 patients. There have been no serious adverse events reported and no treatment-related discontinuations thus far. 8 Patients underwent kidney transplantation when HCV RNA was negative and post –transplant they were continued on combination of Sofosbuvir, and ribavirin (200Mg once a day) to complete therapy. There was no interaction with levels of tacrolimus and immune injury. There was no reactivation of HCV after completion of therapy after transplantation.
Conclusion: combination of Sofosbuvir, Injection PEG-INF 2a 135 and ribavirin for 12 weeks is safe and effective for treatment of HCV infection in CKD patients on dialysis however, frequent monitoring is warranted for patients with severe renal impairment. The combination of Sofosbuvir and ribavirin is safe and effective after kidney transplantation. Final results from this study will need to be confirmed before further recommendations can be made.

P.1707

Long term outcome of living and deceased donor renal transplantation in ADPKD

P.R. Shah, H.V. Patel, V.B. Kute, A.V. Vanikar, P.R. Modi, V.R. Shah, H.L. Trivedi.
IKDRC-ITS, Ahmedabad, India.

Background: Renal transplantation (RT) has become the treatment of choice for end-stage renal disease (ESRD) in autosomal dominant polycystic kidney disease (ADPKD), the most common genetic kidney disease.
Methods: We compare retrospectively RT outcomes [patient /graft survival, rejection rates] in group 1 [23 deceased donors (14 males and 9 females)] and group 2 [79 living donors (28 males and 51 females)] between 2005 and 2015.Diagnosis of ADPKD was established by family history and ultrasound. An individualized approach was applied for the need of pre-transplant nephrectomy. Mean age of patient was 47 and 42 years in group1 and 2.Mean age of donors was 46 and 46 years in group1 and 2.There were 16 male and 7 female patients in group 1. There were 69 male and 10 female in group 2.
Results: Patient survival (%) rates at 1, 5 and 10 years were 87, 58, 53 in group 1 and 97, 95, 89 in group 2.Graft survival (%) rates at 1, 5 and 10 years were 96, 91, 91 in group 1 and 99, 93, 93 in group 2.Biopsy prove acute rejection rate was 13 % and 20 % in group 1and 2 respectively. Patient loss with functioning graft due to infections were more common in group 1 than group 2 (p value <0.01).
Conclusion: In our center, in long-term patient survival is better in group 2 than group1. The graft survival is similar in the 2 groups and therefore, we believe that living donor RT should be encouraged in ADPKD compared to deceased donor RT.

P.1708

NODAT in Africans: Is it higher than in other ethnic groups?

Ihab Ahmed, Manal Omar, Nazik O.Khalifa, El-Tayeb A.Musa.
Dept. of Renal Translantation, ShargEl-Neel Hospital, Khartoum, Sudan.

Objectives: The primary aim of this study is to define the incidence of New Onset Diabetes After Transplantation (NODAT) in Sudanese renal transplant population and identify the contributing risk factors. The secondary aim is to compare the results with the literature.
Methods: Fifty eight patients who underwent living related kidney transplantation and followed-up for two years were included in this pilot study. Only patients who were not diabetic before transplantation were included. Patients who developed NODAT were compared to those who did not develop diabetes. The variables analysed were age, sex, body mass index, family history of diabetes and interval between transplantation and onset of diabetes.
Results: Five patients (5/58) developed diabetes after transplantation (8.62%). There was no association between NODAT and age, sex, BMI and family history of diabetes. The mean duration for developing NODAT was 10 months. There was no graft loss nor deterioration in the NODAT group compared to those who did not develop diabetes.
Conclusion: The prevalence of NODAT in our studied Sudanese population was found to be <10%. There was no association between NODAT and age, sex, BMI and family history of diabetes mellitus. The incidence of NODAT in the studied population was found to be equal with the figures mentioned in the literature for other ethnic groups.
Ms. Braa Dafallah

P.1709

A new renal transplant centre: Experience and challenges

Ihab Ahmed, Manal Omar, Nazik OKhalifa, Rafaa KOsman, Leena MAbdalla, El-Tayeb AMusa.
Dept. of Renal Transplantation, Sharg El-Neel Hospital, Khartoum, Sudan.

Introduction: Renal transplantation in under-developed countries can be a challenging experience due to the limited resources. The aim of this study is to review the experience of a newly established renal transplant centre in Khartoum, Sudan and to carry out a critical analysis of the results.
Methods: This is a retrospective analysis of prospectively collected data of 153 cases of living related kidney transplantation carried out at our centre during the period Nov 2011 and Nov 2015. Results are expressed as percentages.
Results: Seventy seven percent of the cohort were males. Seventy seven percent of the patients belonged to the age group 15-44 years. The percentage of delayed graft function was 3.3% and 7.8% of patients developed acute rejection during the first three months and one patient (0.6%) developed hyperacute rejection. The mortality rate for the studied group was 5.8% mostly due to infection and tuberculosis. The cause of the primary renal failure in more than 60% of those who underwent transplantation was unknown. There was a high percentage of graft failure and presumed disease recurrence.
Conclusion: Part of our results are in line with the internationally published literature. However, in other areas, our figures fall well below the standard. There is a large room for improvement if the deficiencies are addressed and rectified.

P.1710

Kidney transplantation from the United States to Sub-Saharan Africa: Establishing a kidney transplant center in a resource limited country, Ethiopia. University of Michigan - Ethiopian Federal Ministry of health collaboration

Mekdim Siyoum[1], Jeffery Punch[2], Mahteme Muleta[1], Tekleberhan Gebretsadik[1], Engida Gelan[1].
[1] Department of Surgery, Saint Paul Hospital Millenium Medical College, Addis Ababa, Ethiopia; [2]Department of Surgery Division of transplantation, University of Michigan, Ann Arbor, United States.

Background: Kidney transplantation is the standard of care for End Stage Kidney Disease (ESKD). In many resources limited countries like Ethiopia Kidneytransplantation is not available. Ethiopia found in Eastern Africa has a population of 93,815,992, the second most populous country in Africa. GDP for the year 2014 was 55.61 billion USD with GDP per Capita 315.99USD classifying Ethiopia to the resource limited countries. There is no registry of ESKD in the country and its burden is unknown. According to WHO, prevalence of ESKD for Sub-Sharan Africa is estimated to be 5%. Accordingly 4.7 people per million of the population are estimated to have ESKD in Ethiopia. The only option of renal replacement therapy provided was dialysis at private institutions which costs approximately 69 USD per session, not affordable for majority of patients. Despite its cost however the number of private institutions providing dialysis is increasing. Only few patients afford to have kidney transplantation abroad.Currently 150-200 patients who had kidney transplantation abroad are provided post-transplant care by local nephrologists in different private clinics. In response to this demand, the Federal Ministry of Health of Ethiopia (FMOH) took the initiative to establish a National Kidney Transplantation Center that will be sustainable at Saint Paul Hospital Millennium Medical College (SPHMMC), in collaboration with University of Michigan (UoM). UoM located in the United States performs up to 250 kidney transplantation per year currently, it started kidney transplantation in1965.This paper describes the collaboration between the FMOH and UoM in establishing a kidney transplant enter.
Objective: The objectives of the study is to describe the establishment of the first kidney transplant center in Ethiopia in collaboration with UoM.
Study Design: This is an observational study of the process of setting up a transplant center over a period of two years.
Methods and Materials: During the process of establishing the first kidney transplant center in Ethiopia in Collaboration UoM, preparing The Master Kidney Tansplant list was the first step. The list included Selecting a location for the center, completing the instrument, drug and reagent lists and the multidisciplinary team formation.Later Donor and Recipient Protocols were prepared and legislation of Kidney Transplantation was passed by the parliament. Finally Testing of the center's readiness with swine simulation was performed.
Results: Through three phases of collaborative effort nine kidney transplantations from live related donors was performed since September 22, 2015. Three more are planned from February 23-26, 2016.
Conclusion: Establishing a new kidney transplant center needs a huge investment. In a resource limited country like Ethiopia however it is shown that if the local government is committed, in collaboration with Institutions like UoM that has rich experience in kidney transplantation, setting kidney transplantation center with intent of making it a sustainable center can be realized benfiting ESKD patients.
Ethiopian Federal Ministry of Health; Saint Paul Hospital Millennium Medical College; Seanit Fisseha, Adjunct Professor of Obstetrics and Gynecology, University of Michigan
References:
[1] A kidney transplantation model in a low-resource country: an experience from Pakistan,Syed Adibul Hasan Rizvi et al http://www.kidney-international.org/ Meeting report,2013 International Society of Nephrology
[2] Kidney transplantation in a low-resource setting:Nigeria experience,Fatiu Abiola Arogundade et al,http://www.kidney-international.org/meeting report,2013 International Society of Nephrology
[3] Guidelines on Renal Transplantation T. Kälble, A. Alcaraz, K. Budde, U. Humke, G. Karam, M. Lucan, G. Nicita, C. Süsal. © European Association of Urology 2010
[4] University of Michigan - Ethiopia kidney transplant document

P.1711

Medical Students' Knowledge About Brain Death: A South African Contribution

Sanju Sobnach, Elmi Muller, Delawir Kahn, Rajshree Segobin.
Division of General Surgery, Solid Organ Transplant Unit, University of Cape Town, Cape Town, South Africa.

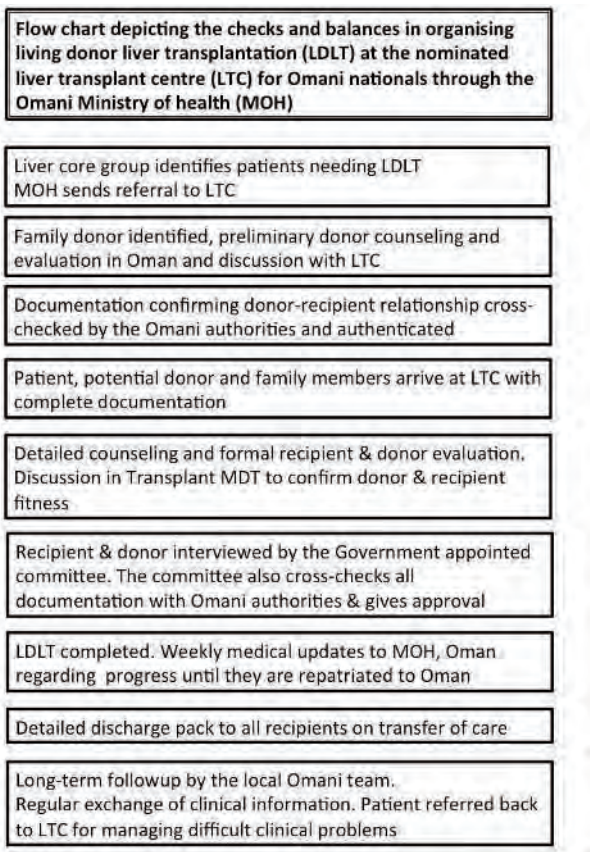
Introduction: Incorporating transplantation into undergraduate medical curricula can improve organ procurement rates.[1][2] Intricacies related to the assessment of donor suitability, the clinical diagnosis of brain death and relevant legal processes are key challenges for the newly qualified doctor.[3][4][5][6]The aim of this study was to describe and analyse the knowledge of medical students regarding the various aspects dealing with the diagnosis of brain death, with a view to designing an undergraduate training module.
Methods: The Human Research Ethics Committee of the Faculty of Health Sciences at the University of Cape Town (UCT) granted ethical approval for this study. A previously validated self-administered 26-item questionnaire was distributed to all medical students at UCT. General data included age, gender, year of study, career interests and organ donor status. Knowledge-specific questions explored the suitability of potential organ donors (eight items), the clinical diagnosis of brain death (seven items) and legal factors thereof (11 items). Statistical analysis was performed using SPSS version 15.0. Chi-square and Fisher exact tests were used to compare the data. A two-sided $P < 0.05$ was considered statistically significant.
Results: There were 346 participants; 217 (63%) were pre-clinical students and 29 (8%) carried an organ donor card. The mean and median score for the 26-items was 12 (Range: 0-25). Mean scores did not increase significantly with years of medical education: from eight in the first year of study, 10 in the second year and 17 in the final year ($P > 0.05$). Demographics, year of study, organ donor status and an interest in a surgical career did not influence knowledge levels ($P > 0.05$).
Discussion: Mean scores remained consistently low amongst all students, with no statistical difference across the six years of study. Pre-clinical students are not expected to attain high scores in this survey because of their limited exposure to clinical medicine during their first three years of undergraduate medical training.[4] On the other hand, the final year medical student should have high levels of knowledge regarding the technicalities behind the diagnosis of brain death. The mean score in the final year medical student subgroup was only 17. If the pass mark were set at 50% for this survey, only the average final year medical student would succeed.
Conclusion: Medical students have limited knowledge about brain death and identification of potential organ donors. This confirms the need for an educational intervention early in the medical curriculum.
References:
[1] Bardell, T., Hunter, D.J.W., Kent, W.D.T. et al, Do medical students have the knowledge needed to maximize organ donation rates? Can J Surg. 2003; 46:453-7.
[2] Riker, R.R., White, B.W. The effect of physician education on the rates of donation request and tissue donation. Transplantation. 1995; 59:880-4.
[3] Afonso, R. C., Buttros, D. A. B., Sakabe, D., et al. Future doctors and brain death: what is the prognosis?. Transplant Proc.2004; 36:816-7.
[4] Sobnach, S., Borkum, M., Hoffman, R. et al. Medical students' knowledge about organ transplantation: a South African perspective. Transplant Proc.2010; 42:3368-71.
[5] Dos Reis, F.P., Gomes, B.H., Pimenta, L.L., Etzel, A. Brain death and tissue and organ transplantation: the understanding of medical students. Rev Bras Ter Intensiva.2013; 25:279-83.
[6] Holling, M., Stummer, W., Friederichs, H. Teaching the concept of brain death in undergraduate medical education. J Surg Educ.2015; 72:504-8.

P.1725

A model for provision of liver transplantation services on a nation-wide basis – a novel approach by the Omani health care system

Mohamed Rela[1], Mettu Reddy[1], H.H. Khoula[2], Said Al-Busafi[2], Nadya Al Harthi[2], Ahmed Al Kindi[2], T. Tawfiq[2], Ravindranath Kancherla[1].
[1]Institute of Liver Disease & Transplantation, Global Health City, Chennai, India; [2]Liver Core group, Ministry of Health, Muscat, Oman.

Background: Many countries in Asia and Africa have limited access to liver transplantation (LT) services. Patients needing LT travel to foreign centres for treatment. This is usually unregulated exposing patients to the ill effects of transplant tourism, including unnecessary or futile transplants, poor outcomes and uncoordinated followup care. It also increases the risk of donor trafficking and unrelated transplants.
Methods: To streamline provision of LT services for its nationals, the Omani government explored the possibility of tie-up with a single foreign centre. After multiple hospital visits, an Indian liver transplant centre (LTC) with expertise in adult and pediatric LDLT was choosen as the service provider and to train local clinicians in setting up an in-house LT service. The patient care pathway included multiple in-built checks and balances to ensure transparency, accountability and donor and recipient safety (Figure 1).



Data of all patients referred from MOH to LTC from March 2010 till November 2015 was reviewed. Data regarding transplants done, reasons for not transplanting, early post-transplant outcomes, long-term followup and cost per transplant is presented.

Results: 135 patients were referred to LTC by MOH. 95 patients underwent LT in our centre. Remaining 40 patients did not undergo LT as the donor was not suitable (n=6), the recipient was too sick/ advanced HCC (n=15), too early for LT (n=10) or underwent non-transplant procedures (n=9). HCV cirrhosis was the most common indication in adults. Median MELD score at transplantation was 15. In children, PFIC with decompensated liver disease and biliary atresia were common indications. Six patients were transplanted for acute liver failure.

Mean donor age was 28.4 years and just over half were men (58%). Majority were first-degree relatives or spouses of the recipients (81%). There was no donor mortality.

No recipient had vascular complications or needed re-transplantation. 30-day survival was 97% with three deaths. Two deaths were related to graft dysfunction and sepsis, third was due to metabolic decompensation in a child transplanted for propionic academia. At last followup, survival was 94% with three late deaths (fungal endocarditis -4 months, PTLD- 8 months, metastatic cholangiocarcinoma -12 months). Three patients were referred back to LTC with anastomotic biliary stricture and underwent successful surgical reconstruction.

The average cost for the first 60 transplants was $47,000 per transplant. Training of local physicians and surgeons in liver transplantation is underway at the LTC and an in-house LT program is being developed.

Discussion and Conclusions: We present data from a novel model of provision of LT services to an entire country through a government-backed relationship with an international liver transplant centre. This system ensures excellent transplant outcomes, reliable followup and management of any post-transplant issues while ensuring transparency of donor selection and donor safety. The system also has the benefits of cost averaging for the sponsoring entity and training of local clinicians in the specialty.

P.1726

A Google Advanced Search for illegal and immoral activities related to organ transplantation in twenty-five developed and developing countries

Ruhul Kuddus[1], Robert Palais[2].
[1]Biology, Utah Valley University, Orem, UT, United States;
[2]Mathematics, Utah Valley University, Orem, UT, United States.

Introduction: Shortages of donor organs is the main problem of transplantation medicine. Despite of the recent efforts for increasing organ donation, donor organ shortage has been worsening and the waiting list for donor organs has been getting longer. Such a condition might have contributed to activities that are considered illegal and immoral as per the Declaration of Istanbul (2008). Here we compared the reports of illegal and immoral activities regarding organ transplantation in relation to the 25 countries.

Materials and Methods: We selected 25 developed and developing countries including some of the original participants of the Declaration of Istanbul (Brazil, Cambodia, Columbia, Egypt, Guatemala, India, Iran, Israel, Mexico, Nigeria, Pakistan, Philippines, Thailand, Turkey and Yemen) and some other countries (Algeria, Argentina, Bangladesh, Fiji, Greece, Indonesia, Iraq, Nepal, Ukraine and Vietnam) where citizens of the country or victors can donate organs. The 2015 population and the GDP per capita of the countries were obtained from World Bank database. We investigated Google Advanced Search (February 2016) using the following phrases “kidney trade”, “kidney sale”, “organ trafficking” and “transplant tourism” along with the name of the country. The number of hits in each category were analyzed in relation to the 2015 population and GDP per capita of the countries.

Results: The mean hits for the phrase “kidney trade” was 7880 (range: 850 for Fiji- 16,600 for Israel), that for the phrase “kidney sale” was 5073 (range: 655 for Cambodia- 11,400 for India), that for the phrase “organ trafficking” was 53,020 (range: 10,600 for Fiji- 114,000 for Turkey), and that for the phrase “transplantation tourism” was 683 (range: 42 for Fiji - 4830 for Nigeria). There was no significant correlation between the total population or GDP per capita and any of the four attributes (R= 0.01-0.58).

Conclusions: A large number of illegal and immoral activities related to organ transplantation have been reported from each of these countries including countries that participated in the Declaration of Istanbul and made significant efforts in curbing such activities. Much attention to this worsening crisis and decisive actions addressing the underlying causes of the problem are warranted.

P.1727

Interviews with patients who travelled from Macedonia, Kosovo, The Netherlands and Sweden for paid kidney transplantations

Linde J.Van Balen[1], Frederike Ambagtsheer[1], Ninoslav Ivanovski[2], Willem Weimar[1].
[1]Department of Internal Medicine, Section Transplantation and Nephrology, Erasmus MC, Rotterdam, Netherlands; [2]University Clinic of Nephrology, University of St. Cyril and Methodius, Skopje, Macedonia, The Former Yugoslav Republic of.

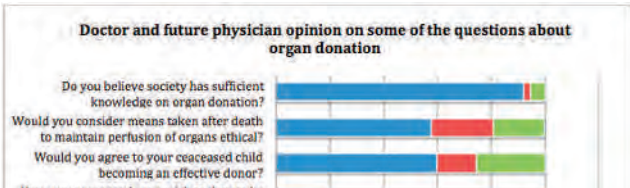
Background: Patients travel worldwide for living kidney transplants. Although travelling abroad does not directly imply an illegal transplant, it is commonly seen as an illegal and/or immoral endeavour involving risks. Lack of data makes it difficult to draw conclusions about its nature and potential illegality. We aimed to describe how, where and by whom transplants abroad were facilitated as well as to describe the motivations, experiences and characteristics of patients travelling abroad.
Methods: Between March and May 2014 interviews were performed with patients from Sweden, Macedonia and The Netherlands who travelled abroad for kidney transplantation.
Results: 22 patients (19 men; born between 1949-1985) travelled abroad from Sweden (N=5), Macedonia (N=10) and The Netherlands (N=7) for transplantation between 2000-2011. The most frequently reported countries were Pakistan (N=13), India (N=3) and Iran (N=2). 7 patients went to their country of origin. For 6 patients a facilitator organized their transplant abroad, the others received help from family or friends. 17 patients directly paid the doctor, hospital or a broker; some paid for the whole transplant service. 14 patients met their donor; 4 patients said to have paid their donor. Reported total costs varied from €280-€45.000. Almost all patients mentioned a lack of hygiene and poor hospital conditions. 11 transplantations were uncomplicated; 11 patients had severe complications (e.g. infections or kidney loss). Patients travelled because of inadequate transplant activity in their domestic countries and dialysis related complaints. However, 6 patients underwent pre-emptive transplantations. Cultural factors such as patients' affinity with destination countries, feelings of being discriminated against by the health care system and family ties help explain why patients travel abroad.
Conclusion: Despite the worldwide prohibition of organ trade, patients still purchase organs. Warning patients against the medical, ethical and legal risks and increasing the supply of organs may prevent patients from purchasing organs abroad. Furthermore, transplant professionals should implement family interventions such as education programs to increase participation in living donation. Finally, reporting mechanisms should be established for transplant professionals to anonymously disclose information about the transplant to police authorities to support the investigation and prosecution of organ trafficking networks.
We are grateful to Susanne Lundin, Martin Gunnarson and Ingela Byström of Lund University, Department of Arts and Cultural Sciences for providing us with the data from Sweden and for their valuable remarks to this manuscript

P.1728

Organ donation: viewpoint analysis of Lithuanian doctors and future physicians in accordance with opinion influencing factors

Valdone Kolaityte[1], Joana Krisciokaityte[4], Marija Krutulyte[1], Algimantas Tamelis[2], Andrius Macas[3].
[1]Medical Faculty, Lithuanian University of Health Sciences, Kaunas, Lithuania; [2]Department of Surgery, Lithuanian University of Health Sciences, Kaunas, Lithuania; [3]Department of Anaesthesiology, Lithuanian University of Health Sciences, Kaunas, Lithuania; [4]Department of Rehabilitation, Lithuanian University of Health Sciences, Kaunas, Lithuania.

Aim: Doctors play a major role in working with the families of potential donors which calls for a need to analyse doctor and future physicians' personal opinion on organ donation and factors influencing it, including analyzing physicians' working conditions and job satisfaction.
Methods: Lithuanian doctors, residents and medical students had their opinion on various organ donation issues related opinion collected by handing out anonymous 21-question questionnaire. The questionnaire data was processed and analysed by using SPSS 22.0
Results: 209 questionnaires were filled in and analyzed. 28.2% of the survey participants were male, 71,8% - female.
The major part of student respondents approve of donation after brain death (95,6 %), living-donor donation (77,1%) and non-beating-heart donation (59,5%). The respondents would agree their organs be used for donation in case of brain death (93,7%) and in the case of cardiac death (56,1%). The respondents indicated that they would donate their organs in the hope of saving another life (65%); it is a sign of good-will (39,2%)
60% of respondents would agree to give away all the organs named in the questionnaire. The majority of the respondents would agree to give away their kidney, liver and lungs, way fewer would give away skin and cornea. A major part of the student and doctor respondents (82 %) have not filled the donation card.
The majority (59,05 %) of the future doctors would agree to their underage child's organs donation in case of brain death.



	Do you support organ donation?	Have you expressed your wish to donate by filling in donation card?	Would you agree to your ceaceased child becoming an effective donor?	Would you consider means taken after death to maintain perfusion of organs ethical?	Do you believe society has sufficient knowledge on organ donation?
Yes	98%	18.10%	59,80%	57.80%	92.20%
No	2%	81,90%	14.70%	23%	2.50%
Uncertain	0	0%	25.50%	19.10%	5%

However, just one-third (32,2%) of the respondents would agree to their child being a living organ donor for them and 24,9% would disagree with such action mainly due to the damage to their child's health and moral issues.
Only 14,5% of students believe they are knowledgeable about approaching the families of potential organ donors, the majority of them acquired the information by self-study.
Respondents believe that the societal views on organ donation are negative (60,8%) and knowledge is poor (92,2%). Respondents named television, social media, doctors as the main factors influencing the

opinion about donation, whereas they expressed that doctors and national transplantation bureau together with informational websites should shape the opinion of the society.
57,8% of the respondents believed that organ preservation by performing a CPR on the deceased person in an effort to keep perfusion to their organs without having any knowledge of deceased person/relative donation opinion is ethical.
Conclusions:
1. The majority of doctors and future physicians approve of different types of donation. However, most of them have not expressed their will by signing a donor card.
2. Respondents named television, social media, doctors as main areas influencing the opinion about donation, whereas they expressed that doctors and national transplantation bureau should shape the donation opinion of the society.
3. Only minor part of students believe they are knowledgeable about approaching the families of potential organ donors, the majority of them acquired the information by self-study.

P.1729

The transplantation of organs from deceased donors due to irreversible cardiac arrest in Poland in 2013 and 2014

Edyta Skwirczyńska-Szalbierz[1], Natalia M.Serwin[2], Karol Serwin[3], Marek Ostrowski[4].
[1]Department of History of Medicine and Ethics, Pomeranian Medical University in Szczecin, Szczecin, Poland; [2]Department of Microbiology and Immunology, Pomeranian Medical University in Szczecin, Szczecin, Poland; [3]Department of Physiology, Pomeranian Medical University in Szczecin, Szczecin, Poland; [4]Department of General Surgery and Transplantation, Pomeranian Medical University in Szczecin, Szczecin, Poland.

Introduction: The demand for organ donation in Poland is three times greater compared to the number of transplant procedures performed in our country. In 2013 there were 594 donations in Poland, which accounted for 16 donors per 1 million inhabitants (pmp). In Spain this rate exceeded 30. Many patients around the world die each day waiting for available organs for transplantation. Therefore other pathways such as donation after circulatory death (DCD) should be taken under consideration. Despite one of the highest donation rates in Poland DCD donation in West Pomeranian province does not exist. There were no DCD donations in Poland to date. In this study we are trying to specify the reason for this matter.
Materials and Methods: In 2013 and 2014 a questionnaire survey was carried out among the staff in intensive care units, cardiology and cardiac surgery departments (68 respondents) and among student nurses, surgical and cardiac surgery residents (56 respondents). Questionnaire consisted of 23 questions divided into 3 sections which tested respondents for DCD donation knowledge, respondents' involvement in the process of DCD identification and asked for suggestions to increase the number of donations from deceased donors, including DCD donors.
Results and Discussion: Majority of respondents did not know the DCD definition nor the DCD classification, over half of respondents deemed that DCD donation is prohibited in Poland. The results exposed that there is no sufficient knowledge among the hospital staff involved in donation process regarding DCD donation.
Conclusions: An implementation of appropriate educational and psychological workshops regarding DCD donation is required.

P.1730

The sad story of kidney buyers on the Balkans

Ninoslav Ivanovski[1], Daniela Mladenovska[1], Galina Severova[1], Aleksandar Sikole[1], Zivko Popov[2].
[1]University Clinic of Nephrology, Medical Faculty, Skopje, Macedonia, The Former Republic of Yugoslav; [2]Macedonian Acadamey of Sciences and Arts, Department od Medical Sciences, Skopje, Macedonia, The Former Republic of Yugoslav.

The Balkan Region is dramatically changing in the last 25 years. Civil wars, new states, society destructions, introduction of democracy and market economy increased poverty of the people in the whole South-Eastern Europe. Due to the lack of organized transplant activity, approximately 120 chronic kidney disease patients according to our registry went to the third world countries (India, Pakistan, Nepal, and Egypt) to buy a kidney using mostly illegally the local and international brokers as intermediaries. Principal destinations were Bombay, New Delhi, Madras, Katmandu, Islamabad, Lahore, Rawalpindi, Karachi, Cairo, Moscow. The usual price for the kidney was between 10.000 to 22.000 Euros, excluding travel costs. Most of the kidney buyers were suffering from many health and social problems during the whole period of follow up in the last 20 years.
Thirty six of 120 patients are still alive and mostly irregularly followed at the University Clinic of Nephrology in Skopje. Most of them are from Kosovo (27) and from Macedonia (9). Among the followed kidney buyers are predominantly housewives (16), lower class trader (6), worker and farmers (4), not qualified workers (6), administrative officers (3) and pensioners (1). Most of them are unemployed and benefiters of some type of social assistance from the state which is usualy very low, up to 50 euros per month. The mean time of follow up is 94 months (range 60-240). The actual serum creatinine is 130 μ/L (range 95-300). All pts are on standard Immunosuppression including CNI, MMF and Steroids. Both countries belong to the Balkan region with weak economies, still far from EU and increased level of poverty. Due to the lack of competent nephrological team, the pts from Kosovo did not have a regular and qualitative kidney transplant patient's health care. Due to the lack of regular pharmaceutical market in Kosovo, sometimes the regular supply of the medicaments is missing and the patients should pay the expensive drugs by them-selves or simply jump the usual doses. From the other hand, the introduction of not truly verified and cheap generics (CNI and MMF from India, Turkey, Ukraine etc.) by the public health authorities and expensive and not regular lab and outpatient controls in private institutions put the pts in permanent danger for their health and life.
In conclusion, we can confirm that the illegal live donor paid transplantation in the third world countries for the Balkan patients is really a life threatening venture not only of the point of surgery and the postoperative care but also from the huge irregularity of outpatient controls, medical care and suitable immunosuppression.

P.1731

Factors affecting medication non-adherence among kidney transplant recipients

Galina Severova, Ninoslav Ivanovski, Daniela Mladenovska, Elka Masin-Spasovska, Ilina Danilovska, Lada Trajceska, Zivko Popov, Aleksandar Sikole.
University Clinic of Nephrology, Medical Faculty, Skopje, Macedonia, The Former Republic of Yugoslav.

Introduction: Adherence to therapeutic recommendations, related to drug administration, diet and healthy lifestyle, is necessary to obtain optimal medical treatment effects. Medication adherence is crucial for graft preservation and survival among renal transplant recipients. Medication non-adherence can be considered as a phenomenon that emerges from the interaction of socio-economic, psychological and health-care system related factors. Previous evidence in other medical conditions has shown that personal factors such as social support are strongly associated to adherence to therapy.
Aim: The aims of the study were to assess the level of medication adherence among our kidney transplant recipients and to identify potentially modifiable risk factors and their associations with medication non-adherence.
Materials and Methods: This cross-sectional study was performed in 50 living donor kidney recipients (male 27, mean age 39+8 years) at least 6 months after the surgery. 19 of 50 recipients were employed, 32 were married and only 10 pts were with university degree of education. In order to obtain reliable answers participation in the study was voluntary and anonymous. Medication adherence was assessed by using the 8-item self-reported Morisky Medication Adherence Scale (MMAS-8). In addition, all participants in the study were evaluated with the 12-item Multidimensional Scale of Perceived Social Support (MSPSS) and Beck Depression Inventory (BDI). Data on patients' demographic, socio-economic and living status, information about recipient's life-style and habits were collected using a non-standardized questionnaire.
Results: The majority of kidney recipients (71%) reported high medication adherence, while 23% revealed only medium level of compliance. The remaining 6% scored between 3 and 8 on the 8-point MMAS, were patients with low medication adherence. Among demographic factors potentially involved in the medication non-adherence, we confirmed detrimental effect of a lower socio-economic status ($p<0.019$) and lower educational level ($p<0.02$) of the participants. The association of non-adherence with increased depression ($p<0.01$) and active smoking ($p<0.003$) was confirmed to. Further data analysis revealed a significant correlation between medication non-adherence and lower social support ($p<0.0001$) especially among the family members and closed friends and cousins.
Conclusion: Our study confirmed than approximately 30% of the kidney recipients reported not satisfied level of medication adherence after renal transplantation. A lower socio-economic status, lower educational level, active smoking, increased depression and lack of social support are the most important factors involved in medication non-adherence. Our results suggest possible targets for future intervention that may increase medication adherence among kidney transplant recipients.

P.1732

Risk factors associated with early readmission after renal transplantation: Effects on cost and graft survival

Deanne Leonard, Thomas Gonwa, Burcin Taner, Martin Mai.
Transplantation, Mayo Clinic, Jacksonville, FL, United States.

Introduction: In the United States 90 day renal transplant re-admission rates are approximately 40%[1]. This may be associated with inferior outcomes and increase the overall cost of renal transplantation. There is a paucity of data on direct etiologies and preventable factors associated with renal transplant re-admissions.
Materials and Methods: We retrospectively analyzed 862 consecutive renal allografts from a single center performed between 1/1/2007-12/31/2014. Kidney transplant with other solid organs or after other solid organs were excluded. Data was collected on all readmissions (ReAd) within 90 days of transplant. Analysis was performed utilizing data from a prospectively maintained database between patients with and without ReAd. Statistical analysis via SPSS was conducted.
Results and Discussion: In the 862 patients mean age was 54.7 yrs (20-82) with a mean initial length of stay (LOS) of 4.7 days. 205 patients (23.8%) had at least 1 ReAd in the first 90 days with a mean time to first ReAd of 17.4 days. Reasons for the first ReAd included infection n=40 (16.4%), surgical/urologic n=40 (16.4%), GI complications n=26 (10.6%), rejection n=20 (8.2%), other n=19 (7.8%), electrolyte disorder n=17 (7%), volume related n=16 (6.6%), DVT n=9 (3.7%), CNI toxicity n=8 (3.3%) and arrythmia/CVA/TMA 11 (4.4%). The number of patients with 2, 3, and 4 ReAd were 48, 12 and 3, respectively, for a total of 271 ReAd. ReAd accounted for 1540 extra hospital days.
Statistically significant correlations with ReAd were found with pre-transplant dialysis (p 0.001), post-transplant dialysis (p 0.004), and a clinical diagnosis of diabetes (p 0.044). Expanded criteria donor (ECD) kidney had a statistically higher ReAd than non-ECD (p=0.04). Patients with allograft rejection had a ReAd rate of 31% as opposed to those without rejection who had a ReAd rate of 20% (p=0.001).
Patients with ReAd had a longer initial LOS (5.83 d vs 3.35 d, p<0.001)) and ReAd was inversely correlated with graft survival. Graft survival at 1 and 2 years without ReAd was 96% and 93.6% vs 92% and 87.6% with ReAd (p 0.004). ReAd was not correlated with patient survival. At 1 and 2 years patient survival without ReAd was 98% and 96.5% vs 96% and 95% with ReAd, (p 0.127).
Presence of a ureteral stent was not significantly associated with ReAd in all patients, but approached significance for deceased donors, (p 0.052). There was no significant association with race, gender, blood type, living donor vs deceased donor, induction type, or etiology of renal disease.
Conclusion: Readmission after renal transplant carries a substantial economic burden (1540 additional hospital days), and may be associated with decreased graft survival. In our series, 90 day re-admission rates are correlated with a diagnosis of diabetes, pre and post-transplant dialysis, and ECD donors. Further analysis is needed to identify strategies and modifiable risks to reduce re-admission rates.
References:
[1] Covert K, Fleming J, Staino C, Casale J, Boyle K, Pilch N, Meadows H, Mardis C, McGillicuddy J, Bratton C, Srinivas T, Chavin K, Baliga P, Taber D. Patient and Process-Level Factors Associated With Kidney Transplant Readmissions [abstract]. Am J Transplant. 2015; 15 (suppl 3).

P.1733

Kidney transplantation outcomes in patients with a history of renal cancer: A national perspective

Douglas Slakey, Zaid Al-Qurayshi, David Pointer, Mary Killackey, Anil Paramesh, Emad Kandil.
Surgery, Tulane University, New Orleans, LA, United States.

Introduction: Patients with a history of renal and other cancers may be candidates for kidney transplantation. In this study, we aim to examine the immediate post-transplantation outcomes in patients with a history renal malignancy using the United States National Inpatient Sample Database.
Methods: A cross-sectional analysis using the National Inpatient Sample database, 2003 – 2010. The study population of 19,697 patients included adult (≥ 18 years) inpatients who underwent kidney transplantation. Patients subsequently classified on whether they had history of renal cancer or non-renal cancer diagnosed prior to transplantation (cases) or not (controls).
Results and Discussion: A total of 228 renal cancer cases (1.2%), 975(5.0%) non-renal cancer cases, and 18,494 (93.8%) controls were included. Patients with a history of renal cancer were more likely to be >60 years old, male, and non-Hispanic (p<0.05 each), while patients with history of non-renal cancers were more likely to be <60years, female, and non-Hispanic (p<0.05 each). Renal cancer cases as compared to controls did not have a significant different risk of general postoperative complications [OR: 0.88, 95%CI: (0.64, 1.21), p=0.43], or specific complications [OR: 0.87, 95%CI: (0.67, 1.13), p=0.30]. Additionally, transplantation in patients with a history of renal cancer did not have different length of hospital stay (7.2±0.3 vs. 7.4±0.2 days, p=0.52) or a different cost of health services ($55,368.14±2,241.64 vs. $54,402.99±1,866.40, p=0.56).
Non-renal cancer cases as compared to controls showed no difference in general postoperative complications [OR: 0.98, 95%CI: (0.78, 1.23), p=0.84] or specific postoperative complications [OR: 0.92, 95%CI: (0.80, 1.06), p=0.27]. Patients with non-renal cancer had significantly shortly hospital stay after transplantation (6.9±0.2 vs. 7.4±0.2 days, p=0.009). There was no difference in cost of health service ($54,471.68±1,877.52 vs. $53,132.23±2,158.52, p=0.35).
Conclusions: This data indicates that a history of cancer does not significantly affect initial cost of transplantation. Kidney transplantation in patients with a history of primary renal cancer is not associated with different immediate clinical outcomes as compared to patients with no such history. Most likely this is due to careful selection of patients. Long-term outcomes are not included in the NIPS database, which is a limitation to determining ultimate outcomes or cost effectiveness of care using large population data sets.

P.1734

Balneotherapy - an intriguing option of rehabilitaton after kidney transplantation

Barbara Grandtnerova, Zuzana Beratsova, Miroslava Cervenova, Jan Cerven, Marian Markech.
Golden Spa, Slovak Medical Spa, Turcianske Teplice, Slovakia (Slovak Republic).

Health-related quality of life (HRQL) has emerged as an important outcome to quantify the success of transplantation. Despite proven benefit of balneotherapy (BT) in multiple medical conditions, publications concerning this topic after kidney transplantation (KTx) are surprisingly rare.
Aim: To evaluate risks versus benefits of balneotherapy after KTx (specifically its impact on HRQL) with a special emphasis on the first year after Tx.
Methods: In a retrospective study, we compared 2 groups of Ps. Group A: 53 Ps (34 F) after KTx, median age 51 years (range 24-63), median time after Tx 9 months (3-12). Group B: 60 Ps (41 F) with chronic cystitis, risky for UTI but without immunosuppression and operation, median age 62 years (30-87). All Ps were treated in bath tubes and whirpools for 15 minutes daily during 20-22 days of their stay in spa, as a part of complex physical therapy.
Results: Risks: no significant difference was found in the presence of infectious complications (group A vs B): de novo acute urinary tract infections (UTI) 2 vs 4 Ps (3.8 vs 6.7%), p=0.68, RR 0.6993 [95%CI 0.2216 to 2.207], de novo acute UTI in women only 2 vs 3 Ps (5.9 vs 7.3%), p= 1.00, RR 0.8750 [95%CI 0.2902 to 2.638], upper respiratory tract infections 4 vs 8 Ps (7.5 vs 13.3%), p=0.3728, RR 0.6871 [95%CI 0.3010 to 1.568], acute gastroenteritis 2 (3.8%) vs 0, p=0.2178. Infectious complication were a cause of early dismissal in 3 vs 1 Ps (7.3 vs 1.7%), p=0.3395, RR 1.635 [95%CI 0.8959 to 2.984].
Not any significant difference was detected in acceleration of hypertension: 4 vs 1 Ps (7.5 vs 1.7%), p=0.1845, RR 1.763 [95%CI 1.086 to 2.863], hypotensive episodes: 2 vs 1 Ps (3.8 vs 1.7%), p= 0,5696, RR 1,626 [95%CI 0.7061 to 3.745] or any medical complication during stay at SPA: 18 vs 16 (34 vs 27%), p= 0,4188, RR 1,195 [95%CI 0.7994 to 1.786]. Forty eight vs 59 Ps (91 vs 98%), p= 0,0970, RR 0,5383 [95%CI 0.3555 to 0.8152], completed full length of their stay in SPA.
Benefits: at the dismissal, all Ps after KTx who completed SPA therapy reported lesser fatigue, bodily pain, improved muscle strenght, raised well-being, vitality and energy.
Conclusion: Balneotherapy together with physical rehabilitation significantly increased HRQL after KTx. In the wulnerable period of the first year after KTx, the incidence of complications (either infectious or cardiovascular) was not significantly different in comparison to other nephrologic/ urologic patients but without immunosuppression and/or operation. Balneotherapy as a part of rehabilitation proces should be ofered to patients after KTx more frequently.

P.1735

Health-related quality of life and its influencing factors in Chinese renal transplant recipients

Hongxia Liu[1], Yabin Shang[1], Wenxin Zhang[2], Sisi Wu[1], Chunyan Du[1].
[1]School of Nursing, Beijing University of Chinese Medicine, Beijing, People's Republic of China; [2]Nursing Department, China-Japan Friendship Hospital, Beijing, People's Republic of China.

Background: Renal transplantation is considered the first-choice method of renal replacement therapy for ESRD patients. Although renal transplantation brings many benefits to recipients, it is potentially associated with a number of drawbacks, which influence their health-related quality of life (HRQOL).
Purpose: The purpose of this study was to investigate the HRQOL of renal transplant recipients and its influencing factors. The relationships among transplant-related clinical factors, cognitive appraisal of health, perceived self-efficacy, perceived social support, coping, and HRQOL were examined using a theoretically derived model from Lazarus and Folkman's (1984) Model of Stress and Coping.
Methods: Renal transplant recipients with functional kidney graft and more than 3 months after transplantation were recruited at a transplant follow-up clinic in Beijing. Standardized instruments were used to evaluate the variables. Data were analyzed using IBM SPSS Amos 21.0. Path analysis was used to examine the theoretically derived model.
Results: The mean scores of 202 renal transplant recipients for SF-36 Mental Component Summary (MCS) and Physical Component Summary (PCS) were 48.18±9.67 and 47.61±6.75 respectively. Path analysis demonstrated that perceived self-efficacy had a significant direct effect on negative primary appraisal of health and physical HRQOL, and had both direct and indirect effects on acceptance-resignation coping strategies. Perceived social support had a direct effect on negative primary appraisal, and direct and indirect effect on acceptance-resignation coping strategies. Primary appraisal of health had a significant direct effect on coping strategies, physical and mental HRQOL. Confrontation and avoidance coping strategies had no significant direct effects on physical and mental HRQOL, while acceptance-resignation coping strategies had significant direct effect on mental HRQOL. Medication side effects had significant direct effects on primary appraisal of health and mental HRQOL.
Conclusion: HRQOL of renal transplant recipients is the outcome of a complex interplay between clinical factors, psychosocial factors, and behavior factors. Information from this study may help transplant professionals develop interventions to improve HRQOL of renal transplant recipients.
This study was supported by National Natural Science Foundation of China, grant number: 81171860
References:
[1] Gabriela S, Petra S, Anja F, et al. A mixed-method study to explore patients' perspective of self-management tasks in the early phase after kidney transplant. *Progress in transplantation*, 2014; 24(1):8-18.
[2] Gentile S, Beauger D, Speyer E, et al. Factors associated with health-related quality of life in renal transplant recipients: results of a national survey in France. *Health and Quality of Life Outcomes*, 2013; 11:88.
[3] Ortiz F, Aronen P, Koskinen PK, et al. Health-related Quality of Life after kidney transplantation: who benefits the most? *Transplant International*, 2014; 27(11):1143–1151.
[4] Prihodova L, Nagyova I, Rosenberger J, et al. Health-related quality of life 3 months after kidney transplantation as a predictor of survival over 10 years: a longitudinal study. *Transplantation*, 2014; 97(11):1139-1145.
[5] White C, Gallagher P. Effect of patient coping preferences on quality of life following renal transplantation. *Journal of Advanced Nursing*, 2010; 66(11):2550–2559.

P.1736

The impact of transplant nephrectomy for patient survival over the last 15 years - a single centre study

Masaki Muramatsu[1,2], Yoji Hyodo[1,2], Atsushi Aikawa[2], Michael Sheaff[3], Magdi Yaqoob[1], Carmelo Puliatti[1].
[1]Nephrology and Transplantation, The Royal London Hospital, London, United Kingdom; [2]Nephrology, Toho University Faculty of Medicine, Tokyo, Japan; [3]Cellular Pathology, The Royal London Hospital, London, United Kingdom.

Introduction: The effect of transplant nephrectomy (TNx) on patient survival rate after returning dialysis is unclear. The aims of this study were to compare clinical management after graft loss and patient survival between TNx and non-TNx.
Materials and Methods: This study included 171 graft loss cases, who received a transplant at the Royal London Hospital between 2000 and 2015. They were divided into three groups: in situ failed graft (IS group) in 64 cases, TNx at <3 months post-transplant (ETNx group) in 51 cases, and TNx at >3 months post-transplant (LTNx group) in 56 cases. Patient survival and the risk factors for patient death were analysed. Clinical management after graft loss, such as the timing of relisting on the transplant waiting list (TWL) and immunosuppressive agent (ISA) status were compared. The indications and surgical complications of TNx were also evaluated.
Results: The incidence of acute rejection was 20.6% in the IS group, 5.9% in the ETNx group and 51.8% in the LTNx group. The duration of graft survival in the IS, ETNx and LTNx groups was 51.1, 0.06 and 21.0 months ($p<0.001$). Relisting on the TWL was possible for 46.9% of the IS group, 56.9% of the ETNx group, and 51.8% of the LTNx group ($p=0.566$). Although Only 10.9% in the IS group were ISA free, 94.1% in the ETNx group and 78.6% in the LTNx group were. Patient survival rate at 1, 3 and 5 years post-transplant in the IS, ETNx and LTNx group was 92.1%/90.5%/86.6%, 96.0%/89.7%/80.4% and 100.0%/97.9%/95.6%. The rate in the IS group did not differ significantly from those of the ETNx and LTNx group.
Risk factors for patient death were relisting on the TWL (HR 0.17, 95% CI 0.06-0.41, $p<0.001$) and the duration of the graft survival (HR 0.36, 95% CI 0.13-0.93, $p=0.036$). The common reason of TNx was insignificant blood flow for a graft in 96% of the ETNx group and suspected ongoing rejection, or graft intolerance syndrome in 58.9% of the LTNx group. The incidence of surgical complications was 39.2% in the ETNx group and 26.8% in the LTNx group ($p=0.171$).
Discussion: After graft failure, patients who did not undergo TNx had no inferior patient survival rate compared with patients who receive early and late TNx. Relisting on the TWL and graft survival time affected patient survival regardless of receiving TNx.
Conclusion: Although individual management of a failed graft is required after graft loss, the patient survival rate is not affected by TNx.

P.1737

Health-related quality of life is improved after liver transplantation and is related to disease acceptance, helplessness and perceived disease benefits

Louis Onghena[1], Wouter Develtere[1], Xavier Rogiers[2], Roberto Troisi[2], Frederik Berrevoet[2], Aude Vanlander[2], Anja Geerts[1], Hans Van Vlierberghe[1], Carine Poppe[3], Xavier Verhelst[1].
[1]Gastroenterology and Hepatology, University Hospital Ghent, Ghent, Belgium; [2]Hepatobiliary Surgery and Transplantation Medecine, University Hospital Ghent, Ghent, Belgium; [3]Transplantation Center, University Hospital Ghent, Ghent, Belgium.

Background and Aims: Liver transplantation (LT) is the only curative treatment for end-stage liver disease (ESLD) with excellent long-term outcomes. However, an important outcome parameter is health-related quality of life (QoL). Improvement of QoL has been described after liver transplantation. We wanted to confirm these findings with specific attention for psychological parameters such as acceptance, helplessness and perceived disease benefits.
Methods: We performed a cross-sectional study in a liver transplant unit. Self-report questionnaires (SF36 and ICQ) were conducted in 177 patients with ESLD: 60 patients pre-transplantation, 60 post-transplantation and control group of 57 patients without perspective of transplantation. Data were analyzed using the Mann-Whitney U test and Spearman's rank correlation coefficient.
Results: The studied groups of patients were comparable regarding age and MELD score, but the control group was significantly older ($p<0.0001$). We observed a significant increase in QoL as soon as 3 months after LT ($p=0.046$) as well regarding the mental component summary scale ($p=0.029$) as the physical component summary scale ($p=0.033$). After liver transplantation, patients report more acceptance ($p=0.001$) and disease benefits ($p<0.0001$) and a decrease in helplessness ($p<0.05$). General QoL is positively significant correlated ($p<0.001$) with acceptance ($rs=0.737$) and disease benefits ($rs=0.494$), and negatively with helplessness ($rs=-0.828$).
Conclusions: These data confirm an increase of QoL starting from 3 months after liver transplantation. We observed a better acceptance of illness and more benefits of the illness after transplantation. Patients also report to feel less helpless. We assume that these findings indicate that patients receive a lot of attention and support for their illness after transplantation but still feel slightly uncertain. These aspects could give new directions in the approach of liver patients after transplantation.

P.1750

Skin as a harbinger of rejection of underlying structures in VCA: Concordance or discordance?

LindaC. Cendales[1], Matthew Levine[2], Stephen Bartlett[3], Jennifer Cheeseman[1], Cinthia I.Drachenberg[3], Wayne W.Hancock[4], Marybeth Joshi[1], Allan D.Kirk[1], Leopardi Frank[1], Scott Levin[2], Mehmet Uluer[3], Angelica Selim[1], Mingqing Song[1], William S.Twaddell[3], Liqing Wang[4], Zhonglin Wang[2], Rolf N.Barth[3].
[1]Duke University, Durham, NC, United States; [2]University of Pennsylvania, Philadelphia, PA, United States; [3]University of Maryland, Baltimore, MD, United States; [4]The Children's Hospital of Philadelphia, Philadelphia, PA, United States.

Background: Vascularized composite allografts (VCA) are composed of multiple tissue types. It has not been determined as to whether there is an immune hierarchy or differential susceptibility of rejection amongst the included tissues. This has implications as to the utility of skin as a diagnostic sentinel of rejection and to the design of rescue strategies for rejection or graft loss. To assess the concordance or discordance of rejection of the skin and of the underlying tissues we made use of a new multicenter collaborative initiative in VCA (VCAci). We combined the preclinical results from 3 different centers evaluating all structures in animals after a VCA in mice and non-human primates
Methods: Samples were shared electronically by Aperio imaging and read by pathologists to assess the underlying structures when the skin was rejecting. Skin rejection was graded using the Banff VCA classification system. Descriptive definitions were performed for the histologic specimens of tendon, nerve, muscle, bone, artery, and vein.
Results: Pathologists reviewed a total of 104 slides of skin, tendon, nerve, muscle, bone, artery, and vein: skin (n=24), muscle (n=24), nerve (n=13), tendon (n=13), bone (n=4), artery (n=13), vein (n=13). Skin rejection by Banff grade was as follows: Grade IV (n=12), Grade III (n=5), Grade II (n=2), and Grade I (n=3), Grade 0 (n=1). When skin rejection was diagnosed, at least one other tissue in the same transplant had signs of rejection (n=22 animals). Five animals with Banff IV rejection and four animals with Banff III rejection showed signs of rejection in all underlying soft tissues. One animal with Grade 0 in the skin had simultaneous endothelialitis around the tendon. The remaining animal reached endpoint from malignancy. Necropsy demonstrated a Banff II with no signs of rejection in muscle, vessels or bone.
Summary: In the majority of cases the skin acts as a harbinger of rejection in underlying structures and thus remains an accessible and reasonably sensitive method for monitoring rejection. Rescue strategies in severe cases are unlikely to allow for the preservation of underlying structures and will likely require total graftectomy. This may have implications in planning rescue strategies for complex VCA.
This study was supported by a grant from the United States Department of Defense [W81XWH-13-2-0055]

P.1751

A clinical hand transplant program five years on: Lessons learned and future direction

Hatem Amer, Brian T.Carlsen, Thomas R.Schwab, Sheila G.Jowsey-Gregoire, Lynette A.Fix, Bridget Hannenberbger, Lori L.Ewoldt, Tammy Danielson, Keith A.Bengtson, Karen Andrews, Brooks S.Edwards, Steven L.Moran.
William J. von Liebig Center for Transplantation and Clinical Regeneration, Mayo Clinic, Rochester, MN, United States.

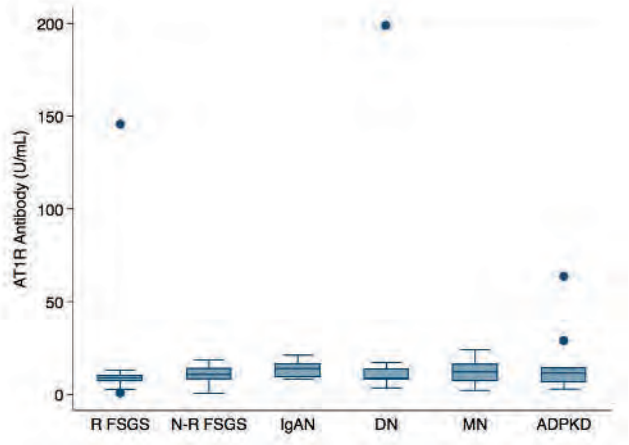
Introduction: In September 2010, as previously reported, we established the first US hand transplant program that was under clinical practice supervision and not necessarily IRB. Hand transplantation was offered in the clinical spectrum of the reconstructive algorithm for upper extremity injuries. The Procedure was deemed by the program and institution as non-experimental and in cases where patients had no insurance or insurance would not agree to cover the procedure, the institution committed to offering charity care. Subsequently the program also received a significant endowment. Here we present our experience thus far.
Methods: We reviewed the electronic medical charts, the transplant database and phone logs by our appointment coordinators. Patients who presented for clinical evaluation in the hand transplant program are offered enrollment in at least two IRB protocols. One covers serum, urine and tissue bio banking and the other, the prospective collection of clinically obtained data for future research. In this study we will focus on those patients who underwent the in-person clinical evaluation.
Results: Of 67 patients who underwent the initial phone screens for hand transplantation, 6 were invited and came for the full clinical evaluation. Age 37± 12.1 [25-58] years, 3 (50%) female, 2 (33%) were quadrimembral amputees (result of sepsis) one of whom is a recipient of a renal allograft, 3 (50%) were unilateral amputees from trauma, and 1 patient was requesting a unilateral amputation for a A-V malformation with concomitant transplantation. Three (50%) of the candidates would require above the elbow transplants (distal trans-humeral amputations). Sensitization as assessed by single antigen beads and the cPRA was 31 (0,88) [0-98]% if considering antigens with an MFI >500 as unacceptable and 0 (0,0) [0-88]% if considering MFI > 2000 as unacceptable. One patient's insurance carrier deemed the procedure as non-experimental but pending review of medical necessity. One patient is on hold due to family circumstances, two declined proceeding to transplant at the present time. The remainder was declined due to psychosocial concerns or due to the perceived lack of benefit with the proximal level of amputation.
Conclusion: Hand transplantation can be offered as part of the clinical clincial algorithm for upper extremity reconstruction. Expanding the use of this procedure in the care of amputees requires advances in the understanding of the motivation, or lack thereof, for amputees to explore this option, improvements in nerve regeneration to allow greater benefit for more proximal amputations and mitigating risks of immunosuppression.

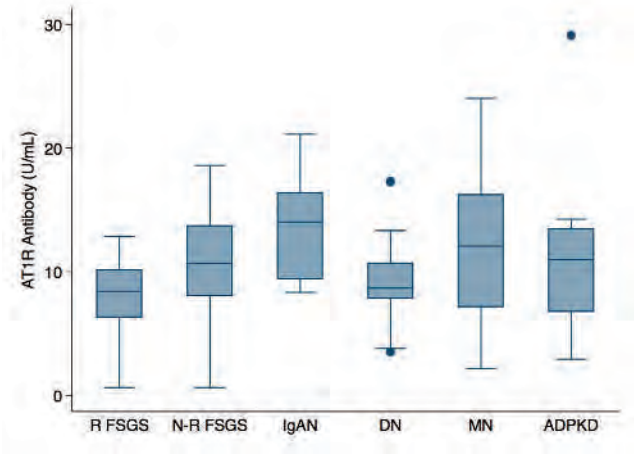
P.1752

Pre transplant at1r antibodies and the risk of recurrent focal segmental glomerulosclerosis (FSGS)

Hatem Amer[1,2], Carlos R. Franco Palacios[1,2], Nikolay Voskoboev[3], Callen D. Giesen[3], Hani M. Wadei[4], Vesna D. Garovic[1], John C. Lieske[1,3].
[1]Nephrology and Hypertension, Mayo Clinic, Rochester, United States; [2]The William J von Liebig Center for Transplantation and Clinical Regeneration, Mayo Clinic, Rochester, United States; [3]Laboratory Medicine, Mayo Clinic, Rochester, United States; [4]Transplantation, Mayo Clinic, Jacksonville, United States.

Introduction: Primary idiopathic FSGS carries a high risk of recurrence following kidney transplantation. Identifying cases at risk of recurrence is important given implications for therapy and counseling patients. An association between antibodies against AT1R and recurrent FSGS has recently been reported[1]. The objective of this study was to determine the ability of pretransplant AT1R antibodies in identifying recipients destined for FSGS recurrence.
Methods: We identified six groups of transplant recipients; ADPKD (n=15), Membranous Nephropathy (N=15), Diabetic Nephropathy (n=12), Ig A nephropathy (N=15), and 28 subjects identified pre transplant as having high risk FSGS 13 of whom recurred post transplant[2]. We measured AT1R antibodies in stored pre transplant serum using a commercially available kit.
Results: Mean (SD) age was 51.4 (13.9) years, 56 (65.9%) males, and 52 (61.2%) received a pre-emptive transplant. eGFR in the preemptive patients was 13 (5) ml/min/SA. For the entire cohort median (IQR) of AT1R antibodies was 10.5 (8.0, 14.0) U/ml. Levels where no different between groups: ADPKD
11.4 (6.7, 14.0), MN 12.1 (7.2, 16.2), DN 8.7 (7.8, 13.3), IgAN 14.0 (9.4, 16.4), NR-FSGS 10.7 (8.1, 13.7) R-FSGS 8.9 (7.2, 10.3) p=0.15. AT1R did not predict FSGS nor R-FSGS among FSGS cases OR (95%CI) 0.99 (0.98-1.01) and 1.02 (0.97-1.05) respectively





Conclusion: Pre-transplant serum AT1R antibodies did not identify subjects destined to have recurrent FSGS.
References:
[1] 1. Mujtaba MA, Sharfuddin AA, Book BL, Goggins WC, Khalil AA, Mishler DP et al. Pre-transplant angiotensin receptor II type 1 antibodies and risk of post-transplant focal segmental glomerulosclerosis recurrence. Clinical transplantation 2015;29(7):606-611.
[2] 2. Franco Palacios CR, Lieske JC, Wadei HM, Rule AD, Fervenza FC, Voskoboev N et al. Urine but not serum soluble urokinase receptor (suPAR) may identify cases of recurrent FSGS in kidney transplant candidates. Transplantation 2013;96(4):394-399.

P.1753

On-demand drug delivery system with self-assembled hydrogel in vascularized composite allotransplantation

DzhuliyaV. Dzhonova[1], Adriano Taddeo[1,2], Ashish Dhayani[3], Praveen Vemula[3], Esther Vögelin[2], Robert Rieben[1].
[1]Department of Clinical Research, Bern University, Bern, Switzerland; [2]Plastic, Reconstructive and Hand Surgery, Inselspital, Bern, Switzerland; [3]Institute for Stem Cell Biology and Regenerative Medicine, InStem, Bangalore, India.

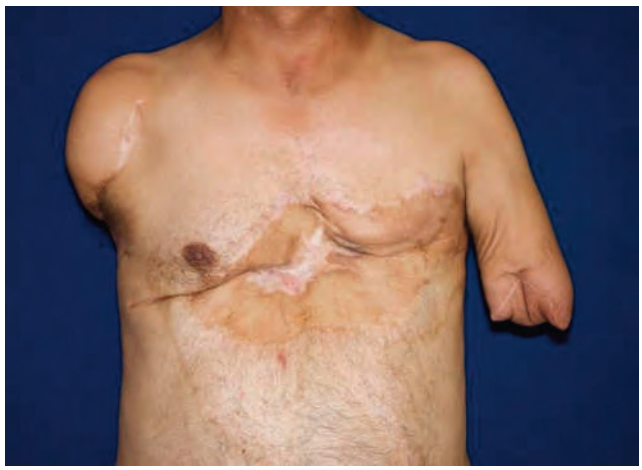
Introduction: During the last decade vascularized composite allotransplantation (VCA) has been established as clinical practice with excellent functional and aesthetic outcomes. Minimization of immunosuppression is one of the most important open questions in the field. Drug delivery systems able to release immunosuppressive drugs directly into the graft may minimize the systemic exposure, thus limiting the side effects. Recently we have shown that a single local injection of Tacrolimus-laden hydrogel significantly prolonged graft survival in a Brown Norway–to–Lewis rat hind limb transplantation model[1]. In this study we aim for a proof-of-concept for the use of this therapy in VCA by demonstrating that Tacrolimus is released in an on-demand manner and that indefinite graft survival can be achieved using this approach.
Materials and Methods: Hydrogel loaded with 7mg/ml Tacrolimus was injected in hind limb of Lewis rats. The rats were then challenged with LPS (lipopolysaccharides) to induce inflammation. Blood levels of the drug were monitored by LC-MS/MS analysis. Inflammation progression was tracked with Luminex-assay for inflammatory cytokines.
Results: To this moment our data show initial burst release in the first 48h after injection of the hydrogel, followed by a significant decrease and stabilization of drug levels for 50 days. LPS induced inflammation on day 7 after injection resulted in elevated drug release, which continuously declined. In animals, which were not challenged with LPS, no increased Tacrolimus release was observed, indicating that it indeed was related to the hydrogel induced inflammation.
Discussion: Based on our findings, we conclude that Tacrolimus loaded hydrogel system is able to release the drug on-demand, depending on the presence of an inflammatory stimulus. Currently, Brown Norway-to-Lewis hind limb transplantations are being performed to allow an in-depth investigation of the immunological mechanisms during Tacrolimus hydrogel treatment in VCA.
References:
[1] Thusitha Gajanayake et al. *Sci Transl Med*. 2014; 6: 249ra110.

P.1754

Bilateral arm allotransplantation: a case report in Mexico

Martin Iglesias Morales, Patricia Butrón, Raul Favela, Elizabeth Rodríguez, Juan S.Wong, Leonardo Abarca, Raul Granados, Miriam García, Humberto Baeza, Ricardo Villanueva, Claudio F.Reyes, María-Fernanda Ramírez, Euridice I.Lara, Fernanda Salazar, Víctor Acosta, Paulino Leal, Jorge Zamudio, Luis A.Jauregui, Carlos A.Hinojosa, Hugo La Parra.
Plastic and Reconstructive Surgery, National Institute of Medical Sciences and Nutrition "Salvador Zubirán", Mexico's city, Mexico.

Introduction: Until now, there have been eight arm allotransplants in six patients[1],[2]; their results are encouraging since they give back basic functions as a "helper arm"[3]. The main challenges to perform it involve anesthetic management, decrease in the ischemic time and distal neural regeneration[4]. Our aim is to report a bilateral arm allotransplant case, performed at the National Institute of Medical Sciences and Nutrition "Salvador Zubirán" of Mexico City, on October 16th, 2015.
Material and Methods: The patient is a 51 year old male with bilateral amputation of upper limbs due to high voltage electrical burn.

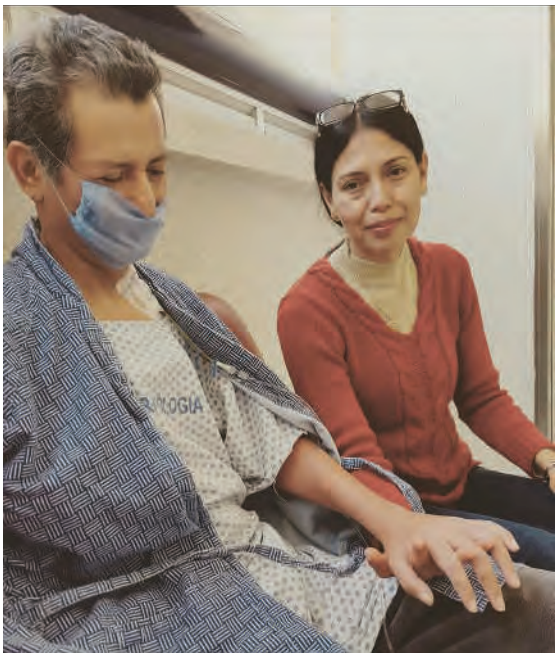


Induction was done with thymoglobuline 1.5mg/kg. The transplant in the right arm was performed at the gleno-humeral joint level and the left arm at the middle-third of the arm. The donor and recipient's characteristics were negative crossmatch, A+ blood type, with a mismatch 5/6, Cytomegalovirus -/+, Human Leukocyte Antigen Class I (2%) and Class II (7%). The total ischemia time was of 4 hours for the right arm and 6 hours for the left. In the right arm, initially the axillary artery and vein were repaired, followed by reconstruction of the glenohumeral joint. All the donor tendons of the shoulder were re-inserted incluiding the deltoid muscle. Neurorraphies of the medial, lateral and posterior cords were completed. In the left arm, a medular pin was used to bone repair, followed by anastomoses of the brachial artery and vein. Then after the biceps brachi, and triceps brachii were sutured to the muscle remnants finally the neurorraphies of the medial, radial and ulnar nerves were executed at the medial third of the upper limb. The total surgical time was of 17 hours.
Results
At twenty-four hours following surgery, the patient presented lactate levels of 4 mmol/L and CPK levels of 22.7ng/dl, both of them decreased to normality after 72 hours. There was not any important hemodynamic change. The patient was discharged home one month after the surgical procedure with tacrolimus, prednisone and mycophenolate mofetil as maintenance immunosuppression treatment. He has developed two acute rejections, Banff III and Banff II respectively, the first one was successfully treated with methylprednisolone 750 mg each 24 hours for three days and the second one with topical tacrolimus. After three

months, the left biceps brachii contraction is M2, the right deltoid muscle is M1, and the biceps brachii is M1. Tinel sign is present in the right arm at the level of the elbow and in left one at the upper third of the forearm. The first electromyography reports few motor unit potential in right deltoid, and both biceps brachii and triceps brachii muscles. The patient is with intensive rehabilitation program.

Conclusions

Proximal amputations of the upper extremity, cause severe disability, and dependence on another person. The aim of transplanting the entire upper extremity is to obtain a two-hand clip, which helps the patient to have some independence in their daily activities. The total function will be achieved after 4 years.



References:

[1] 1. Bernardon L, Gazarian A, Petruzzo P, Packham T, Guillot M, Guigal V. et al. Bilateral hand transplantation: Functional benefits assessment in five patients with a mean follow-up of 7.6 years?(range 4-13 years). J Plast Reconstr Aesth Surg. 2015;68: 1171-83.

[2] 2. Shores JT, Brandacher G, Lee AWP. Hand and upper extremity transplantation: An update of outcomes in the worldwide experience. Plast Reconstr Surg 2015; 135:351e-60e.

[3] 3. Ninkovic M, Weissenbacher A, Pratschke J, Schneeberger S: Assessing the outcome of hand and forearm allotransplantation using the action research arm test. Am J Phys Med Rehabil 2015;94:211Y 221.

[4] 4. Weissenbacher A, Hautz T, Pierer G, Ninkovic M, Zelger BG, Zelger B, et al. Hand Transplantation in Its Fourteenth Year: The Innsbruck Experience. Vascularized Composite Allotransplantation 2014; 1: 11-21

P.1755

Comic for the promotion of donation of vascularized composite allografts in Mexico

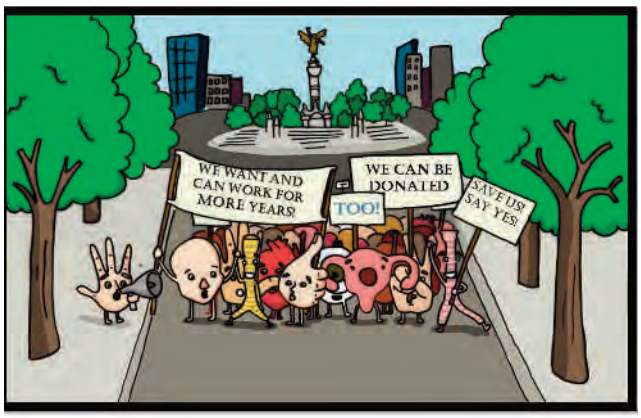
Martin Iglesias Morales, Patricia Butrón, Euridice I.Lara-Hinojosa, María-Fernanda Ramírez-Berumen, Claudio F.Reyes, María F.Salazar, Mario A.Morán, Raul Favela.

Plastic & Reconstructive Surgery, National Institute of Medical Sciences and Nutrition "Salvador Zubirán", Mexico, Mexico.

Introduction: The cadaveric donation in Mexico is made by consent of secondary disponentes[1]. Their refusal leads non-concretized donation (40.4%)[2]. One way to address ignorance about health issues is providing information through a comic[3]-[7]. We report the design of a comic that seeks to promote vascularized composite allografts (VCA) and solid organ cadaveric donation in the Mexican population.

Material and Methods: A literature search of comics directed to general population worldwide that addressed the issue of donation and transplantation was performed. A total of four were found in Argentine[8],Great Britain[9], Brazil[10] and Switzerland[11]. Significantly, there is no record of a previous comic addressing VCA donation. After asses of the main obstacles through an questionary we designed a comic to clarify the multifactorial aspects that hinder the donation process in our country, such as religious beliefs, avoidance of the topic of death among family, how to validate will to donate, traffic organ, lack of knowledge about VCA, fear of disfigurement of the body after procurement and administrative procedures.

Results: The donor is the narrator. VCA, organs and family share the limelight. The main antagonist is an aunt, which embodies myths and ignorance surrounding donation. Potential donor organs want to continue working and discover that their only chance to survive is by donation. The comic is told in "counterpoint", meaning that the two stories (realistic fiction and fanciful) are told simultaneously interrupting or resuming the narrative. Is divided into seven chapters. 1) Introduction and presentation of main characters, including the face and upper extremity along with solids organs that are currently transplanted; 2) Accident and arrival to the emergency department, which focuses in avoiding the subject of death; 3) Medical management and notification to family, 4) Brain death, both chapters clarifies religious beliefs and organ trafficking; 5) Interview and donation consent, the causes of negative for donation arises; 6) In the Public Ministry, brings out Mexican legal framework; 7) Funeral rite and allocation of organs, the myth of disfigurement and organ trafficking is broken.



Conclusion: The story highlights heroism and altruism of the donor and secondary disponentes. It's emphasized that although brain death organs remain viable. This comic is proposed as an educational tool with ability to influence the authorization of donation in the general population as well as being informative to potential recipients.

References:

[1] General Health Law, fourteenth Title; Donation, transplantation and loss of life. Chapter II.
[2] O., C. G., et al (2013). Revista Mexicana de Trasplantes, S3-S4
[3] Green et al BMJ, 2010, Vol. 340
[4] Houts PS et al. Patient EducCouns 2001;43:231-42
[5] Ingrand I. et al (2004) Eur J Public Health 14(2):147–150
[6] el-SetouhyMA, Rio F (2003) J Egypt Soc Parasitol 33(1):55–65
[7] Milleliri JM et al(2003) Sante 13(4):253–264
[8] @prender (CUCAIER).:
http://www.inerciatest.com.ar/aprender/dorganos/
[9] Chilman-Blair (2010). What's up with William? Medikidz explain organ transplants. Great Britain: Med
[10] Associação Brasileira de Transplantes de Órgãos.
http://www.abto.org.br/abtov03/Upload/file/entendad
[11] Swisstransplant. http://www.swisstransplant.org/l1/organspende-organ-transplantation-comics.php

P.1756

Rat skin morphology by reflectance confocal microscopy: a reference study for experimental vascularized composite allograft rejection studies

Huseyin Karagoz[1], Mehmet Salih Gurel[2], Asli Turgut Erdemir[2], Yildirim Karslioglu[3], Sinan Ozturk[1], Fatih Zor[4].
[1]Plastic and Reconstructive Surgery, Gulhane Military Medical Academy, Haydarpasa Training Hospital, Istanbul, Turkey; [2]Dermatology, Istanbul Training and Research Hospital, Istanbul, Turkey; [3]Pathology, Gulhane Military Medical Academy, Faculty of Medicine, Ankara, Turkey; [4]Plastic and Reconstructive Surgery, Gulhane Military Medical Academy, Faculty of Medicine, Ankara, Turkey.

Introduction: Vascularized composite allografts can undergo immune-mediated rejection, and early detection of acute rejection episodes is very important to save the allograft. Although skin biopsies are used routinely for monitoring of the transplant rejection, we introduced reflectance confocal microscopy (RCM) as a useful, non-invasive diagnostic tool for monitoring of skin containing vascularized composite allograft rejection[1],[2]. This may be a potential research area possibly with many promising results. RCM is a well-known technique that has been used very successfully to image the human skin, however, detailed rat skin morphology by RCM has not been described yet. The aim of the current study is to describe rat skin morphology by RCM and compare it with human skin.
Materials and Methods: Six male Sprague-Dawley rats were used for the study. Six different body area (abdominal region, inguinal region, medial thigh, face, back region and ear) of the each rat were marked and shaved with razor blade. The marked skin areas were assessed by RCM first, with a VivaScope® 3000 (Lucid Inc., Rochester, NY, USA). The images were obtained from superficial to deep, up to a 200-micron depth, with an interval of 2.5 microns. Following confocal microscopic assessment, skin biopsies were obtained using a four mm punch from the same area used for the RCM examination.
Results: A total of 36 concomitant RCM evaluations and skin biopsies were performed. Confocal microscopic images and histologic images of the samples were described with details and compared for each different body site. Epidermal keratinocytes, dermal papilla and dermal connective tissue structures were evaluated with RCM. Additionally, inflammatory cells, capillary blood flow and pericapillary structures were evaluated.
Discussion: The RCM primarily provides real-time in-vivo skin images. In contrast to classical histologic sections, RCM obtains horizontal sections of the skin up to 250 µm depth. We combined the horizontal images of the RCM with the vertical images of histologic assessment, and provided good orientation to the skin morphology of rats under confocal microscopic view.
Conclusion: We described detailed confocal microscopic morphology of rat skin at different body sites to be guide for potential experimental skin containing vascularized composite allograft rejection studies.
References:

[1] Zor F, Karagoz H, Erdemir AT, Karslioglu Y, Acikel CH, Kapaj R, Guzey S, Gurel MS, Isik S, Siemionow M. Reflectance confocal microscopy as a useful diagnostic tool for monitoring of skin containing vascularized composite allograft rejection: A preliminary study on rats. Microsurgery. 2015 May 11. doi: 10.1002/micr.22419.
[2] Zor F, Karagoz H. Response to comments on "Reflectance confocal microscopy as a useful diagnostic tool for monitoring of skin containing vascularized allograft rejection: A Preliminary study on rats". Microsurgery. 2015 Oct 13. doi: 10.1002/micr.22517.

P.1757

The effect of donor/recipient chimeric cells on donor-specific chimerism and composite tissue allograft survival in face transplantation model

Fatih Zor[1], Huseyin Karagoz[2], Joanna Cwykiel[3], Maria Siemionow[3].
[1]Plastic Surgery, Gulhane Military Medical Academy, Ankara, Turkey; [2]Plastic Surgery, Gulhane Military Medical Academy, Haydarpasa Training Hospital, Istanbul, Turkey; [3]Orthopedic Surgery, University of Illinois in Chicago, Chicago, United States.

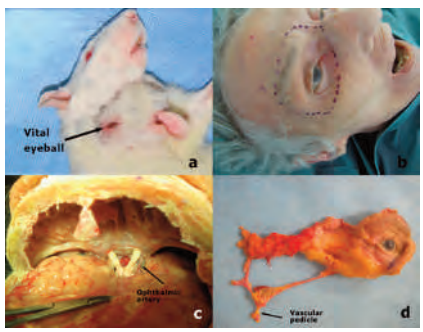
Introduction: Composite tissue allografts such face transplant require life-long immunosuppression causing significant side effects of these highly toxic immunosuppressive New less toxic therapies of tolerance induction are developing to solve this problem, The aim of this study is to determine the effect of different bone marrow based cellular therapies on donor-specific chimerism in face transplantation model.
Material and Method: Bone marrow cells (BMC) were harvested and prepared from ACI (RT1a) donors. Bone marrow stromal cells (BMSC) were obtained from ACI (RT1a) donors by culturing whole bone marrow cells in alpha-MEM medium for 5-8 passages. Chimeric animals were created by intraosseous injection of donor BMC to Lewis (RT1 l) recipients. These chimeric animals were treated with 7-day αβ-TCR/CsA and after 21 days donor/recipient chimeric cells (DRCC) were isolated from chimeric animals by MACS technique.
Twenty hemiface allograft transplantations were performed between ACI (RT1a) donors and Lewis (RT1l) recipients.
Group I was allograft rejection group. Intraosseous cellular therapy injections were delivered: In group II,: BMC (100x106 donor derived BMC) group III :BMSC (10x106 donor derived BMSC) and in Group IV: Chimeric Cell injection (10x106 donor/recipient chimeric cells). None of the groups was supported with immunosuppresion protocol.
Gene expression for proinflammatory (IL-2, TNFα, IL-6, IFNγ) and tolerogenic (IL-10, TGFβ, IL-4) cytokines were evaluated in donor and recipient face skin biopsies using Taqman® real-time PCR.
Results: In group I, composite grafts rejected on day 8-9, posttransplant. In groups II and III, composite grafts rejected on posttransplant 10-11 and 10-12 days, respectively. Extended face allograft survival was observed (13-16 days) in recipients under chimeric cellular therapy (Group IV). This correlated with lower level of chimerism (below 1 %) in groups I, II and III, and higher chimerism (2.5 %) under chimeric cell therapy.
Increased gene expression (4.7 fold) of tolerogenic IL-4 cytokine was seen chimeric therapy recipients.
Conclusion: Cellular therapy with donor/recipient chimeric cell resulted in 62 % extension of face allograft survival, without immunosuppression. This was supported by higher chimerism level and tolerogenic cytokine expression when compared with BMC and BMSC therapy groups confirming tolerogenic properties of chimeric cells.

P.1758

Composite eye and periorbital allotransplantation flap: From rat model to cadaveric study

Fatih Zor[1], Mehmet Bozkurt[2], Can Ozturk[2], Safak Uygur[2], Yalcin Kulahci[1], Risal Djohan[2], Maria Siemionow[3], Francis Papay[2].
[1]Plastic Surgery, Gulhane Military Medical Academy, Ankara, Turkey; [2]Plastic Surgery, Cleveland Clinic, Cleveland, United States; [3]Orthopedic Surgery, University of Illinois in Chicago, Chicago, United States.

Purpose: Composite tissue allotransplantation (CTA) is a novel alternative for reconstruction of severe periorbital defects with functional recovery which can offer a new hope for restoring vision to these patients.
Our group previously described the first successful eyeball allotransplantation model in rats (Figure 1a) which encouraged a cadaver study. Although there are several allotransplantation models, a cadaver model of eye along with its periorbital tissues is not described yet. The aim of this study is to describe a composite tissue allotransplantation model of eyeball and periorbital tissues on cadavers.
Methods: The study is performed on 5 fresh human cadavers. The skin island of the composite eyeball and periorbital allotransplantation flap was planned. The borders of the flap were infraorbital rim at the inferior, nasal dorsum at the medial, eyebrow at the superior and lateral orbital rim at the lateral (Figure 1b).
Following an anterior neck incision and external carotid artery was found. The facial artery and superficial temporal artery were preserved which are the arterial pedicle of the skin island of the flap. The skin and subcutaneous tissues of the periorbital region were incised according to the planned skin island and bony tissue was reached. Later, a coronal incision was performed and frontal bone was exposed. Following a frontal osteotomy, frontal lobe was retracted exposing internal jugular vein and ophthalmic artery. Ophthalmic nerve and oculomotor nerve were also dissected and included in the flap (Figure 1c). Finaly, a "box osteotomy" was performed (Figure 1d).



Results: Indocyanin was injected from the pedicle of the flap. The perfusion of the flap was confirmed with SPY Elite System to identify perfusion zones and to visualize micro-surgical arterial inflow to identifiy flap design and perfusion.
Conclusions: In this cadaver study, composite tissue allotransplantation model including eyeball and periorbital tissues was described for the first time. The perfusion of the flap was confirmed showing this transplantation is possible in the future. Although harvesting of this flap is difficult to perform, it constitutes a new alternative for reconstruction of periorbital region.
Our previous rat model showed ischemic necrosis of retinal ganglion cells (RGC), which is the main obstacle of providing a vision for blind patients. We think that dual blood supply may reduce the ischemic process of the RGC. Studies must aim to provide survival of RGC which results in optic nerve regeneration. This model may be a cornerstone for future studies about eyeball transplantation.

P.1759

Composite tissue xenopreservation: A new living tissue bank

Fatih Zor[3], Rezerta Kapaj[1], Yildirim Karslıoglu[4], Selcuk Isik[3], Vijay Gorantla[2].
[1]Plastic Surgery, Military Hospital, Tirana, Albania; [2]Plastic Surgery, UPMC, Pittsburgh, United States; [3]Plastic Surgery, Gulhane Military Medical Academy, Ankara, Turkey; [4]Pathology, Gulhane Military Medical Academy, Ankara, Turkey.

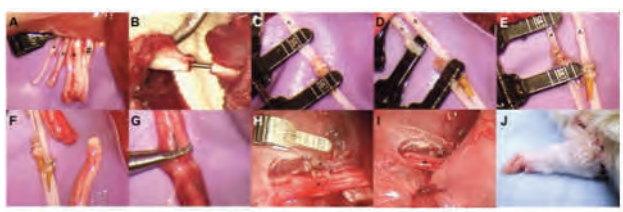
The need for preserving composite tissues and using them when needed is a clinical necessity in modern plastic surgery practice. In certain instances, amputate needs to be preserved and used at a future date, as in the amputation patient whose clinical situation will not allow a long surgical procedure. An appropriate preservation method for composite tissues has not been described up to date. In this study, preservation of composite tissue blocks via xenotransplantation (namely, xenopreservation) is described and short-term results are evaluated.
Two concordant species; Sprague Dawley Rats (n=6) and mice (n=6) were used. The groin flap of the rat was used as a xenotransplant and the vessels on the neck of the Mouse used as recipient vessels. The groin flap of the rat was transported to the neck area of the carrier mouse and microanastomoses were performed between the femoral pedicle of the flap and common carotid artery and external jugular vein of the mouse. Immunosuppression was administered in order to prevent rejection. After a 7 days period of preservation on the site, xenotransplanted groin flap is re-harvested, samples were collected from the skin and vascular structures and the flap was carried to the donor's opposite groin area. Anastomoses were performed between the flaps pedicle and the femoral artery and vein. The flap was monitored daily. Fifteen days after the second surgical procedure the rats were euthenized and samples were collected. All the samples were evaluated by Haematoxylene-Eosine Stain.
All xenopreserved groin flaps survived indefinitely. Tissue evaluation indicated prominent inflammation in carrier mouse, but these changes were reversible to some extend.
In this study a method for composite tissue preservation and a basic model for further investigation has been developed in this study. Additional studies are needed in order to find diverse strategies to modulate the tissue changes.

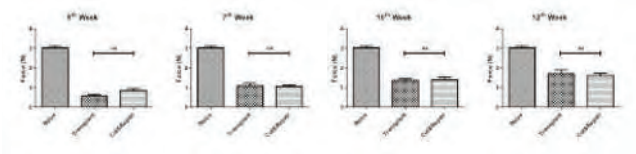
P.1760

A novel murine orthotopic forelimb transplantation model that allows for reliable assessment of functional recovery resulting from nerve regeneration

Barbara Kern[1], Joshua Budihardjo[2], Mohammed Khusheim[2], Sarah Mermulla[2], Anja Quan[2], Carol Cadmi[2], Sinan Xiang[2], Byongchol Oh[2], Georg Furtmueller[2], Joey Lopez[2], Jason Park[2], Ahmet Hoke[2], Sami Tuffaha[2], Stefan Schneeberger[1], W.P. Andrew Lee[2], Gerald Brandacher[2].
[1]Innsbruck Medical University, Operative Medicine, Innsbruck, Austria; [2]Johns Hopkins University, Plastic and Reconstructive Surgery, Baltimore, MD, United States.

Background: Improved nerve regeneration and functional outcomes would greatly enhance the utility of vascularized composite allotransplantation (VCA). However, research aimed at achieving this goal has been limited by the lack of a functional VCA animal model.
Methods: To address this problem, we developed a novel rat mid-humeral forelimb transplant model with end-to-end cuff anastomosis of the brachial artery and vein in which functional recovery is tested by scoring component forelimb motions involved with the IBB forelimb scale and by measuring progressive return of grip-strength within the transplanted forelimb at week 5, 7, 10 and 12 after transplantation. Median nerve histomorphometry and flexor digitorum myofiber cross-sectional area analysis was investigated at the end point (12 weeks).
Results: After an initial learning curve, forelimb transplantation can be performed with consistent success (operative time 180-220 minutes). Grasping skills between the transplanted group and the cut&repair group showed no statistical significance, neither on week 5, 7 or 10, nor on week 12. The transplanted group regained grip strength with a maximum of 56.4%±6.5% of baseline. Similar results were observed within the IBB scale measurements, mean score of transplanted group resulted in 2.8±0.37SEM at week 12 (1.7±0.33 at week 5), which was comparable to cut&repair group with 2.6±0.26 at week 12 (2.0±0.0). Immunohistochemistry of flexor digitorum muscle showed greater myofiber cross-sectional area in the experimental group than in the negative control group (719.5±56.9 SEM µm2 vs. 224.7±38.7 SEM µm2; P<0.0001). Myofiber cross-sectional area of naïve animals measured an average of 1325.7±19.1 SEM µm2. Histomorphometric analysis of regenerating nerve fibers in the median nerve demonstrated robust axonal regeneration in the experimental group, with greater number and density of fibers in the experimental group than in naïve median nerves (fiber count 5324±266 vs. 2997±231 SEM; p=0.004; fiber density 9500±191 vs. 25269±2664 fibers/mm2; p=0.017).
Conclusion: Rat forelimb transplantation may represent the first VCA model that allows for reliable and reproducible measurement of functional recovery. Statistical analysis of grip strength data will elucidate the degree of variability at each time-point and the degree of improvement from week to week as compared to non-innervated controls.



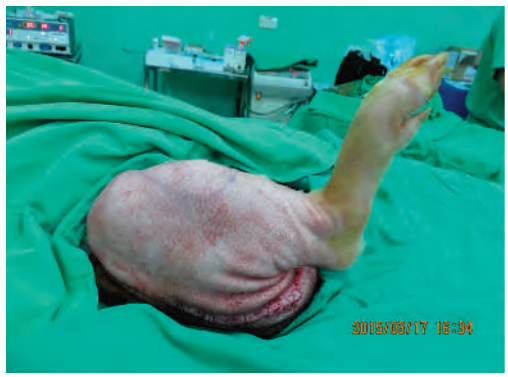


P.1761

Vascularized composite allotransplantation in orthotopic porcine hind limb model

Chen Hsiang Kuan, Sung-chuan Chao, Nai-Chen Cheng, Jung-Hsien Hsieh, Hao-chih Tai, Hong-Shiee Lai.
Department of Surgery, National Taiwan University Hospital, Taipei, Taiwan.

Purpose: Vascularized composite allotrasplantation(VCA) for aesthetic and functional purposes in face and upper limb reconstruction has been developing in decades in Western. Beyond the aspect of immunomodulation, ethical issues and procurement difficulties are obstacles to VCA in Eastern Asia. To simulate, we present a porcine model of orthotopic hind limb allotransplantation.
Materials and Methods: Three small-ear Lee-Sung strain miniature pigs were assigned as the group of replantation, following transfemoral amputation and bone shortening before osteosynthesis. Other twenty pigs, ten donors and ten recipients, underwent ipsilateral hind limb allotransplantation according to the protocol of replantation group except bone trimming and ischemia time saving. Cyclosporine-A based immunosuppression regimen was given for VCA group. Temperature records, blood exams, Cyclosporine-A level, soft tissue sonography, and pathological follow-up reports were collected.
Results: All the grafts in the replantation group survived more than one week and the one pig still had its replantated hind limb for 7 months with partial weight bearing function. In the allotransplantation group, one graft survived for 2 weeks and it failed because of fulminant infection over the graft. The second one has survived for 7 weeks and 3 days. However, the recipient died in sepsis and pneumonia and invasive line infection were blamed for. The other eight allotransplantations have survived through 4-week immunosuppressive regimen without evidence of acute rejection.
Conclusion: Our porcine model of orthotopic hind limb allotransplantation is not only technically feasible, but a good simulation of immunomodulation and functional evaluation. The data would facilitate further clinical trials.



P.1762

Our experience with immunosuppression protocols for composite tissue transplant (bilateral hand transplantation)

George Kurian[1], Zachariah Paul Polachirakal[1], Subramanyam Iyer[2], Mohit Sharma[2], Anil Mathew[1], Rajesh R.[1], Sandeep S.[1], Jimmy Mathew[2], Kishore P.[2], Malini Eapan[3], Ravi S.[4], Karthik Ganesh[1].
[1]Department of Nephrology, Amrita Institute of Medical Sciences, Ernakulam, India; [2]Department of Plastic and Reconstructive Surgery, Amrita Institute of Medical Sciences, Ernakulam, India; [3]Department of Pathology, Amrita Institute of Medical Sciences, Ernakulam, India; [4]Department of Physical Rehabiltation, Amrita Institute of Medical Sciences, Ernakulam, India.

Introduction: Hand transplant is the only alternative for patients that have lost both hands. But unlike a solid organ transplant involves multiple tissues called composite tissue allotransplantation (CTA)[1],[2].
On 13 January 2015, surgeons at the Amrita Institute of Medical Sciences and Research Centre in India successfully did the country's first hand transplant. In this article we will describe the immunosuppression protocols, clinical and histological assessment for rejection and graft survival of our two hand transplant recipients.
Materials: Our index patient has is 12 months and second patient 9 months post bilateral hand transplantation. We have analyzed their clinical course, rejection episodes, adverse events and graft function. Patients underwent weekly punch biopsies to assess for skin rejection.
Induction -Thymoglobulin (ATG) @ 2 mg/kg and methylprednisolone (MP) 500 mg; Thymogobulin was continued for total of 5 days. Tacrolimus 0.2 mg/kg/d and Mycophenolate Mofetil (35 mg/kg/d).
Maintenance - Prednisolone, tacrolimus and mycophenolate mofetil.
Results and Discussion: First patient was a pottery artist, asian male aged 31 yrs. Lymphocyte cross match was negative. There were 3 episodes of rejection on postop days 14 (Antibody mediated rejection), 118 (Grade II Acute cellular rejection) and 238 (Grade III Acute Cellular rejection). Antibody mediated rejection was treated with intravenous immunoglobulin (100 mg/Kg/d) and rituximab @ 375 mg/m2. For acute cellular rejections MP (15 mg/kg/day for 5 days) were given.
Second patient was a soldier, caucasian male aged 30 yrs. Lymphocyte cross match was negative. He had 1 episode of acute cellular rejection on postop day 38, for which MP (10 mg/kg/day for 3 days) was given.
Induction: Kidney transplantation induction is with Basiliximab or ATG. However in CTA, ATG induction is required and in a higher dose (2 mg/kg/d against 1 mg/kg/d). Tacrolimus trough level in kidney transplantation is maintained at 5 – 10 ng/ml as against 10 – 15 ng/ml in CTA.
Immunosuppression: Standard triple immunosuppression protocol. Tacrolimus is preferred over cyclosporine (trough level 10-15 ng/ml). No infection, renal failure or other adverse events have occurred as yet.
Rejection: Rejection episodes manifested as petechial skin lesions. Episodes were highest in the initial four months, with acute cellular rejection being dominant. Rejection episodes responded to standard treatment.
Graft survival: Both patients have remarkable allograft survival with progressive improvements in intrinsic muscle activity, return of functional grip, pinch strength and sensation.
Conclusion: Both patients of bilateral hand transplants have demonstrated good functional recovery. Our protocol of immunosuppression for CTA was adapted from our kidney transplant protocol. Overall the burden of rejection is higher in CTA (high skin immunogenicity), hence immunosuppresion regime more intense.
References:
[1] S. Schneeberger, V.S. Gorantla, T. Hautz, B. Pulikkottil, R. Margreiter, and W.P.A. Lee. Immunosuppression and Rejection in Human Hand Transplantation. *Transplantation Proceedings* 2009; 41: 472–475.
[2] P. Petruzzo,, J. Kanitakis, L. Badeta, et al. Long-Term Follow-Up in Composite Tissue Allotransplantation: In-Depth Study of Five (Hand and Face) Recipients. *Am J Transplant* 2011; 11: 808–816.

P.1763

AMD3100 (plerixafor) as a clinically relevant stem cell mobilizing agent in vascularized composite tissue allograft (VCA) transplantation

David Mathes[1,2], Bruce Swearingen[1,2], Scott Graves[2], Rainer Storb[2].
[1]Division of Plastic Surgery, Dept. of Surgery, University of Colorado, Aurora, CO, United States; [2]Transplantation Biology Research Laboratory, Fred Hutchinson Cancer Research Center, Seattle, WA, United States.

Introduction: Vascularized Composite Allograft (VCA) transplantation is a clinical reality but limited by toxicities of chronic immunosuppression and rejection. Current clinical tolerance protocols rely on recipient conditioning and donor cell mobilization limiting there use to living donor transplants. We sought to design a clinically relevant protocol applicable to cadaveric organs. We modified our existing non-myeloablative stem cell canine VCA transplant model to use AMD3100 (Plerixafor) for stem cell mobilization.
Methods: 5 DLA-haploidentical, related canine recipients received conditioning with 350cGy TBI, AMD3100-mobilized donor stem cells and VCA transplantation with a short course of immunosuppression (MMF: 84 days/CSP: 133 days; including taper). CD34+ hematopoietic progenitor cells were quantified via flow cytometry. Peripheral blood chimerism was evaluated by PCR techniques weekly. VCA graft survival was followed clinically and histologically.
Results: All 5 canines tolerated the conditioning regimen. 4 were followed long-term. Stem cell engraftment and donor chimerism were seen in all dogs. Median COBE apheresis cell counts of 6.12x10^8 cells/kg and CD34+ cell count of 5.27x10^7 cells/kg were obtained. No acute rejection nor evidence of GVHD was seen. An unexpected finding of persistent thrombocytopenia resolved on loss of donor cell chimerism.
Conclusion: This study demonstrates proof of principle for AMD3100 as a single-dose stem cell mobilizing agent for a clinically relevant tolerance protocol. Use of AMD3100 led to stem cell engraftment in all animals transplanted with no evidence of acute rejection in the VCA. Current application of AMD3100 is limited by thrombocytopenia but we are currently modifying the protocol to address this.

P.1775

The natural course of renal function with everolimus after heart transplantation

Tsao Chuan-I[1], Chou Nai-Kuan[2], Wang Shoei-Shen[2].
[1]Nursing, National Taiwan University Hospital, Taipei, Taiwan;
[2]Surgery, National Taiwan University Hospital, Taipei, Taiwan.

Background: Renal insufficiency after heart transplantation is a frequently observed comorbidity. Most studies have shown that an initial steep decay with 1-2 years after HTx is followed by less pronounced decline thereafter. The mTOR inhibitors has the potential to reduce CNI nephrotoxicity by minimizing or eliminating the need for their use. The natural course of immunosuppressive strategies combining Calcineurin inhibitors (CNI) with everolimus (EVR) and steroids after heart transplantation (HTX) are rarely published. This study was aimed to describe the change over time of renal function in two-year after HTx, in patients treated with CNI-EVR.
Methods: Two-year follow-up data of patients who received a first HTx at National Taiwan University Hospital(NTUH) between2004 and 2014 were analyzed. As a study purpose, the patients were received everolimus with CNI. All patients were aged 18 years or older. The The renal function was obtained from chart review and was collected before HTx(T0), every months for up to 1 year, and then every 3 months for up to 2 years. We also compared in HTx two immunosuppressive regimens: EVR plus tacrolimus (FK) and EVR plus cyclosporine (CsA).
Result: The study included 117 patients. At 24 months, patient survival and ESRD were 82.8% and 4.9%. The mean follow time was 21.4±6.5 months. Estimated median glomerular filtration rate (eGFR) by the four-variable Modification of Diet in Renal Disease formula was 76.5ml/min/1.73m2 before HTx. After HTx, the GFR was 64 ml/min/1.73m2at Month 3,and 64 ml/min/1.73m2at Year 1. The difference was significant between pre-HTx and post-HTx GFR (P=0.000) during the first year. The study was also completed by 42 patients inEVR plus CsA group and 75 patients in EVR plus FK group. Renal function was not significantly different in the two groups despite a trend towards a poor impairment of renal function in the EVR plus FK group between three and nine(p = NS) months.
Conclusion: The result had shown that an initial steep decay within 3 months after transplant is followed by a less pronounced decline thereafter. Our short-term result is different than before report. The EVR-based strategy may provide a more benefit in renal insufficiency. The post-transplant renal insufficiency is a complex and multifactorial condition. Further follow up could evidence the benefits in the middle-term follow-up related to the anti-proliferative effects of EVR.

P.1776

Life's a beach - spending holidays after LVAD implantation

Christian Heim, Nattipong Mekkhala, Markus Kondruweit, Michael Weyand, Rene Tandler.
Cardiac Surgery, University of Erlangen, Erlangen, Germany.

Objectives: Improving quality of life has become an important goal in LVAD therapy, especially in destination therapy patients. Going on holiday is a main factor in re-establishing quality of life. The aim of this study was to evaluate the attitude towards spending holidays in patients provided with a continuous flow left ventricular assist device.
Methods: Destination therapy patients who underwent continuous flow LVAD implantation (HeartWare HVAD or BerlinHeart Incor) between January 2010 and December 2014 and who were followed up in our outpatient care unit were provided with a standardized questionnaire regarding their holidays (destination, duration, reason of denial). The data were prospectively collected, anonymised and analysed for the purpose of this study.
Results: Out of 62 ambulatory LVAD patients during the survey period, 16 completed the questionnaire (response rate 26%). 56% of them have gone on holidays after successful LVAD implantation. Most of the holidays were spent within Germany (67%), 75% of the patients spending holidays quoted their holiday destination abroad including Austria, Croatia, Turkey, Thailand, and Dubai. 3 of them declared medical problems including collapse with cerebral haemorrhage, aggravation of a driveline infection due to tropic climate, and admission to a LVAD site abroad due to VAD alarms on the basis of dehydration. 56% of all surveyed LVAD patients had plans for future holidays. Reasons of not spending holidays included personal factors (money, job) and fear of medical issues or support during travelling.
Conclusion: A substantial proportion of LVAD patients has spent holidays or has plans to do so, some of them even abroad. Analysing the reasons of patients not going on holidays accentuates the need for improved patients´ consulting with regard to medical support during travelling and especially discussing cooperating LVAD sites abroad..

P.1777

Cardiac allograft vasculopathy involving the artery to the atrioventricular node: an under-recognized important pathological finding in the autopsy

E. Rene Rodriguez, Carmela D.Tan.
Anatomic Pathology, Cleveland Clinic, Cleveland, OH, United States.

Introduction: The development of cardiac allograft vasculopathy (CAV) is the main limiting factor in the long-term survival of heart transplant recipients. Pathological examination of the conduction system is rarely performed in determining the extent of CAV in the autopsy setting. Here we describe a series of 4 patients with CAV involving the artery to the atrioventricular (AV) node.
Materials and Methods: The anatomic pathology database was queried for autopsy cases with findings of CAV from 2006 to 2015. There were 4 cases where involvement of the AV node artery was identified.
Results: All 4 patients were male with age ranging from 42-72 years at the time of death. Posttransplant survival ranged from 7 to 94 months. Two patients were symptomatic with shortness of breath and near syncopal attacks, while the 2 other patients died suddenly. The electrocardiographic findings range from sinus tachycardia, first degree AV block and second degree AV block. Two patients with second degree AV block underwent implantation of pacemaker devices. Coronary angiography performed within 6 months from the time of death revealed normal coronary arteries in 1 patient, mild non-obstructive stenosis in 2 patients and severe stenosis in medium and small sized coronary arteries in 1 patient. Autopsy findings reveal more severe stenosis and predominant involvement of the small epicardial and intramural coronary arteries compared to the large epicardial coronary arteries. The pathology of the AV node artery includes marked intimal proliferation, abundant inflammatory infiltrates in the intima and media and occlusive thrombosis.
Patient demographics and clinical data

Patient	Age/Gender	Survival (in months)	ECG findings	Pacemaker	Coronary angiogram
1	43/M	70	second degree AV block	Yes	CAV3
2	64/M	7	sinus tachycardia	No	CAV0
3	72/M	94	second degree AV block	Yes	CAV1
4	42/M	32	first degree AV block	No	CAV1

Discussion: In this subgroup of patients, CAV manifested predominantly in the small epicardial and intramural coronary arteries. The antemortem recognition of small vessel disease is limited by nonspecific signs and symptoms and the lack of sensitivity of coronary angiography in detecting small vessel involvement. In these cases, the autopsy provides important data in understanding the causes of death, particularly in sudden death cases.
Conclusion: Involvement of the AV node artery in CAV is infrequent but explains the development of atrioventricular block and sudden arrhythmic deaths in a subset of heart transplant recipients. Investigation of sudden unexpected deaths in cardiac transplant recipients should include examination of the conduction system.

P.1778

Endocan and renalase: are they related in heart transplant recipients?

Piotr Przybylowski[1], Jacek Malyszko[2], Ewa Koc-Zorawska[3], Jolanta Malyszko[3].
[1]Cardiac SUrgery and Transplantation, Collegium Medicum, Cracow, Poland; [2]1st Nephrology, Medical University, Bialystok, Poland; [3]2nd Nephrology, Medical University, Bialystok, Poland.

Endocan (endocan- ESM-1) is a novel soluble dermatan sulfate proteoglycan derived from endothelium. It has the capacity of binding to different biologically active molecules associated with cellular signaling, adhesion and regulating proliferation, differentiation, migration, and adhesion of different cell types in health and pathology. Its elevated level is connected with endothelial activation, neovascularisation and inflammation or carcinogenesis. Endothelial dysfunction had been associated with hypertension. Renalase, a recently identified flavin adenine dinucleotide– dependent amine oxidase secreted into the blood by the kidney, metabolizes circulating catecholamines. Renalase is preferentially expressed in proximal tubules, but it is also present in glomeruli and distal tubules, as well as in cardiomyocytes, liver, skeletal muscle and even adipose tissue. It was reported that renalase, secreted by the kidney and circulating in the blood, degrade catecholamines and might play a role in the regulation of sympathetic tone and blood pressure. The aim of the study was to assess possible correlations between endocan, renalase, blood pressure, and kidney function among heart transplant recipients
Materials and Methods: The level of serum endocan and renalase among 131 heart transplant recipients on three-drugs immunosuppression (CNI, MMF/MPA, steroids) in correlation with other markers of endothelial damage was estimated. Additionally 22 healthy volunteers were studied as well. In cross-sectional study the markers of endothelial damage like endocan, vWF, markers of inflammation: hsCRP, IL-6 and kidney function: cystatin C using commercially available assays were measured.
Results: The endocan, renalase, vWF, IL-6, hsCRP, cystatin C level were significantly higher in heart transplant recipients comparing to healthy volunteers. In our cohort endocan level was correlated weakly ($p<0.05$) to renal function (eGFR by MDRD; creatinine, cholesterol; LDL; NYHA class) and moderately ($p<0.01$) to ; hsCRP; IL-6, vWF, erythrocyte count, hemoglobin, NT-proBNP. ;In multifactorial analysis the predictors of endocan levels were cholesterol level, cystatin C, IL-6 predicting 54% of variability. Among heart transplant recipients renalase correlated weakly ($P < .05$) with time after transplantation; moderately ($P < .01$), with ejection fraction and age; and strongly, with kidney function, IL-6, vWF, and New York Heart Association class ($P < .05$). Multiple regression analysis revealed renalase values to be 70% predicted by kidney function (either eGFR by MDRD or serum creatinine) measurements. There was no correlation between renalase and endocan.
Conclusion: Endocan concentration among heart transplant recipients is potentially connected with endothelial damage as long as subclinical inflammation resulting from hyperlipidemia. Impaired kidney function was strongly associated with endothelial damage and inflammation. Renalase, which was highly elevated among heart transplant recipients, was predominantly dependent on renal function, which deteriorated with time after transplantation and in correlation with age.

P.1779

VItamin D status in heart and kidney transplant recipients

Jolanta Malyszko[3], Jacek Malyszko[2], Ewa Koc-Zorawska[3], Piotr Przybylowski[1].
[1]Cardiac SUrgery and Transplantation, Jagiellonian University, Cracow, Poland; [2]1st Nephrology, Medical University, Bialystok, Poland; [3]2nd Nephrology, Medical University, Bialystok, Poland.

Background: One of the main action of vitamin D is the bone mineralization regulation. Vitamin D is linked also to hypertension, diabetes, cardiovascular disease. Vitamin D deficiency may result in osteomalatia, while its excess in bone calcium mobilization. Kidney transplant recipients are also at risk of hypovitaminosis D due to impaired graft function. The aim of the study was to asses vitamin D concentration in patients on after heart and kidney transplantation
Material and Methods: 98 stable heart transplant recipients were enrolled to the study, 80 kidney transplant recipients and 22 healthy volunteers served as controls. The laboratory tests, including parameters of 25(OH) vitamin D –calcidiol were assayed using commercially available kits.
Results: Calcidiol deficiency (level below 10 ng/ml) was observed in 10% of Tx group and 55 % of OHT There was the positive correlation between calcidiol concentration, hemoglobin, kidney function and serum glucose in kidney transplant recipients. In OHT vit D correlated with age, kidney function, hemoglobin, cholesterol, LDL cholesterol, and glucose. Both groups have similar kidney function. In both groups of patients with eGFR above 60 ml/min/1.72m2 vit D was significantly higher. In OHT vit D was higher in non-diabetic patients. In OHT in multivariate analysis vit D was predicted in 24% by kidney function (beta=-0,30, p=0.02) and hemoglobin concentration (beta =0,25, p=0.03).
Conclusions: Patients after heart transplantation have more common vit D deficiency that kidney allograft recipients despite similar kidney function. The possible associations between cardiovascular system and vit D merit further studies.

P.1780

Underrecognition and underestimation of disturbances In calcium-phosphate balance in kidney transplant recipients

Jolanta Malyszko[2], Jacek Malyszko[1].
[1]Nephrology and Transplantation, Medical University, Bialystok, Poland; [2]2nd Nephrology, Medical University, Bialystok, Poland.

Introduction: Together with impairment of renal function, disturbances in mineral metabolism, namely chronic kidney disease-metabolic bone disease became more profound. In dialysis patients assessment of serum calcium, phosphate, alkaline phosphatase and PTH is a standard procedure. However, in outpatient transplant clinic standard protocol varied in different centres.
Aim: The aim of the study how often calcium, phosphate, alkaline phosphatase and PTH are measured in kidney transplant recipients relative to hemodialyzed patients. In addition, prevalence of hypercalcemia defined as calcium concentration over 10.5 mg/dL was assessed.
Patients and Methods: We studied 200 kidney allograft recipients (at least 3 months after transplantation) and 100 hemodialyzed patients. Data were obtained retrospectively on the basis of the outpatients charts. Calcium, phosphate, alkaline phosphatase, 25(OH) vitamin D and PTH were studied using standard laboratory methods.
Results: Kidney transplant recipients were significantly younger than hemodialyzed patients. All the studied parameters were available in 100% of the hemodialyzed patients, while only in 80% of kidney allograft recipients, calcium and phosphate levels were available, alkaline phosphatase activity was available in 40% of patients, while PTH in less than 10%. 25 (OH) vitamin D was available only in a few cases of kidney allograft recipients (1%), while in 100% of dialyzed patients. Hypercalcemia was present in 10% hemodialyzed patients, while in 5% of the kidney allograft recipients. Vitamin D analogs was administered to 98% of hemodialyzed patients, whereas vitamin D was administered to 28% of kidney allograft recipients, in particular with impaired kidney function. Cinacalcet was administered only to 12% hemodialyzed patients, not to kidney allograft recipients (lack of reimbursement and therapeutic programme). None of the patients were administered with vitamin D receptors activators (paricalcitol).
Conclusion: Calcium and phosphate are assessed to seldom on the outpatients basis in kidney allograft recipients making difficult the diagnosis and treatment of secondary hyperparathyroidism in this population. Care of kidney transplant recipients could be substantially improved, in particular in regard to chronic kidney disease- metabolic bone disease (CKD-MBD), when regular check-ups for calcium-phosphate balance is implemented and proper treatment could be introduced to prevent further CKD-MBD.

P.1781

Clinical implication of non-complement-binding de novo donor-specific anti-HLA antibodies in heart transplant recipients

Takuma Sato, Osamu Seguchi, Seiko Nakajima, Sachi Matsuda, Yoshiaki Kanaumi, Kyoichi Wada, Masanobu Yanase, Hatsue Ueda, Norihide Fukushima, Takeshi Nakatani.
Transplantation, National Cerebral and Cardiovascular Center, Osaka, Japan.

Background: The development of de novo human leukocyte antigen (HLA) donor specific antibodies (DSA), detected by both cytotoxic or solid phase assays, was considered the major risk factor for cardiac graft failure in heart transplantation. However, it was shown that not all patients with persistent production of DSA suffered loss of their allografts. The ability to activate complement may be an important factor differentiating clinically relevant DSA from non- relevant DSA. Recently, a C1q-binding assay (C1qScreen; One Lambda, Inc. Canoga Park, CA) has been developed to identify complement-fixing HLA antibodies with high sensitivity and specificity.The aim of this study was to investigate the association between C1q-binding ability of HLA-DSA and the clinical outcomes post-transplant to identify clinically significant de novo DSA in heart transplantation.
Methods: We enrolled 64 consecutive patients who received heart transplant between May 1999 and January 2015 in our institute. Sixty of 64 patients (93.7%) were screened for the presence of circulating DSA using Luminex Single Antigen Flow Bead assays between June 2014 and August 2015, and patients with post-transplant DSA with mean fluorescence intensity (MFI) >500 were selected to assess C1q fixation by C1q-binding assays. The clinical outcomes were compared with the results.
Results: Of 60 patients, twelve patients were considered as DSA positive (MFI>500, range 698-5952, class 1: 75% class 2: 17%, class1+2:8%). All of these patients were identified as C1q negative. As the results, we divided all patients into two groups; group DSA+, C1q-(n=12) and group DSA- (n=48). Rejection episodes, development of cardiac graft vasculopathy, cardiac function (EF, BNP), cardiac event-free survival and overall mortality were not statistically different between the two groups.
Conclusion: Patients producing C1q-negative DSA had good graft survival, which was comparable to that of DSA negative patients. Adding the assessment of the complement-binding capacity of DSA might redefine the traditional risk stratification of de novo DSA positive patients.

P.1782

Influence of CYP3A5 polymorphism on pharmacokinetic interaction of fluconazole and tacrolimus, and effect of fluconazole on everolimus pharmacokinetics in heart transplant recipient

Yuka Terada[1,2,3], Kyoichi Wada[1], Sachi Matsuda[1], Takeshi Kuwahara[1], Atsufumi Kawabata[2], Mitsutaka Takada[3], Eriko Hisamatsu[4], Haruki Sunami[4], Seiko Nakajima[4], Takuma Sato[4], Osamu Seguchi[4], Masanobu Yanase[4], Norihide Fukushima[4], Takeshi Nakatani[4].
[1]Department of Pharmacy, National Cerebral and Cardiovascular Center, Suita, Japan; [2]Division of Pharmacology and Pathophysiology, Kinki University School of Pharmacy, Higashi-Osaka, Japan; [3]Division of Cardiovascular Drugs and Therapy, Kinki University Graduate School of Pharmacy, Higashi-Osaka, Japan; [4]Department of Transplantation, National Cerebral and Cardiovascular Center, Suita, Japan.

Introduction: Tacrolimus (TAC) and everolimus (EVL), immunosuppressant drugs commonly given to heart transplant recipients, are metabolized by cytochrome P450 (CYP) 3A4/5. Agents that affect their metabolism by inhibiting CYP3A enzymes, such as azole antifungals, may change the blood concentration levels of these drugs, resulting in side effects or rejection. Previous reports have suggested that a reduced TAC dose is required when fluconazole (FLCZ) is concomitantly used. Moreover, it has been shown that the CYP3A5 genotype markedly influences TAC pharmacokinetics. Interestingly, itraconazole co-administration did not alter the dose-adjusted C_0 value of TAC in a patient possessing the CYP3A5*1/*1 allele, whereas it changed that in patients with the CYP3A5*3 allele. On the other hand, it was reported that EVL C_{max} and the area under the blood concentration-time curve (AUC) were increased, and elimination half-life time was extended when used in combination with ketoconazole. However, changes in the pharmacokinetics of EVL when given in combination with FLCZ have not been clarified. We report findings in a heart transplant patient who discontinued FLCZ after starting combination treatment with TAC and EVL, probably due to an interaction between FLCZ and TAC caused by the CYP3A5 gene polymorphism.
Case Summary: A 50-year-old male with a CYP3A5*1*3 gene polymorphism underwent heart transplantation for idiopathic dilated cardiomyopathy and was treated with triple immunosuppressive therapy consisting of TAC, mycophenolate mofetil (MMF), and prednisolone. At 154 days after transplantation, FLCZ was initiated to treat fungal pyogenic spondylitis. At 368 days, MMF was switched to EVL for progression of transplant coronary vasculopathy and renal impairment, then at 612 days successful subsidence of the fungal infection was shown and he finished FLCZ therapy.
Results and Discussion: FLCZ C_0 was lower than the effective concentration at 2 days after FLCZ withdrawal, then decreased to about 1% after 24 days, prior to withdrawal. Dose-adjusted C_0 values for TAC and EVL rapidly decreased from 2 days after FLCZ withdrawal, and their target blood concentration was reached by a dose increase of 2.9 and 3.3 times, respectively (Fig. 1, 2). Those dose-adjusted C_0 values then stabilized at 15 and 11 days, respectively, after FLCZ withdrawal. At 34 days after withdrawal, weight-adjusted dose and CL/F/kg were increased, and dose-adjusted C_0, C_{max}, and $AUC_{0\text{-}12}$ were decreased.
Conclusion: When TAC and EVL are used concomitantly with FLCZ, TAC and EVL blood levels should be closely monitored, with dosage carefully and frequently adjusted during and after FLCZ therapy. In addition, CYP3A5 genotype testing is useful to predict pharmacokinetics changes of TAC when used in combination therapy with CYP3A inhibitors in heart transplant recipients, as previously reported for other organ transplant recipients.

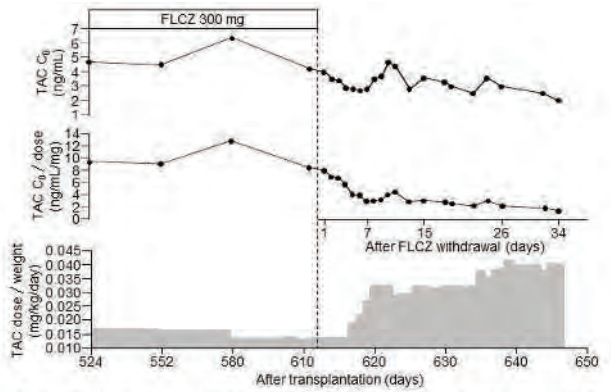


Fig. 1 Effects of fluconazole (FLCZ) withdrawal on trough blood level (C_0) of tacrolimus (TAC).

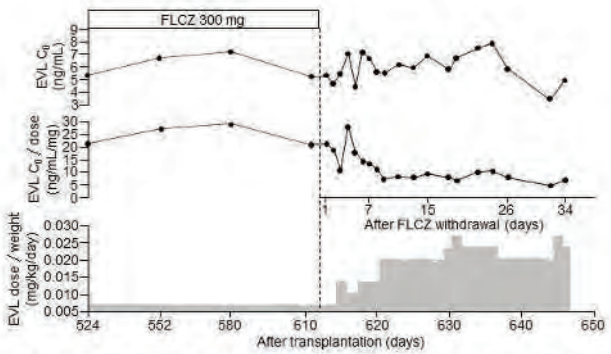


Fig. 2 Effects of fluconazole (FLCZ) withdrawal on trough blood level (C_0) of everolimus (EVL)

P.1783

Initial experience of lung transplantation with a national organ procurement organization effort to use lungs from brain and cardiac death donors or cardiac death donors in a single Chinese center

Wenjun Mao, Xiaowei Nie, Wei Xia, Mingfeng Zheng, Jingyu Chen. Department of Lung transplant center, Key Laboratory of Human Organ Transplant in Jiangsu Province, Affiliated Wuxi people's hospital,Nanjing Medical University, Wuxi, People's Republic of China.

Background: Lung transplantation remains the only therapeutic option for various end-stage pulmonary diseases. Ethical issues limited the development of organ transplantation in china.However,voluntary organ donation after death of citizens become the sole source in organ transplant from January 1, 2015,which stopped major organ source from executed prisoners.The purpose of the study was to estimate the initial experience in lung transplantation using lungs from donation after brain and cardiac death(DBCD) or donation after cardiac death(DCD) under Chinese organ procurement organization(OPO) practices.
Methods: Retrospective data from Chinese network registry for lung transplantation performed in our center were evaluated between January and September,2015, and reported to the registry as of October 2015. The donors were DBCDs or DCDs allocated by OPO nationally. All the transplants were approved by the Ethics Committee of our hospital.
Results: There were 58 lung transplantation performed using lungs from brain and cardiac death or cardiac death donors during the study period.The mean age of recipients was (52.78±12.66) years old.Forty-three patients were males and 15 were females,43 patients received bilateral lung transplantation,10 underwent right single lung transplantation,and the remaining received left single lung transplantation.The main indications of the cohort includes 24 cases of idiopathic pulmonary fibrosis,13 chronic obstructive pulmonary disease,7 silicosis,5 bronchiectasis,3 lymphangioleiomyomatosis,3 bronchiolitis obliterans,and 3 pulmonary hypertension.Extracorporeal membrane oxygenation(ECMO) was utilized in 44.8% of the patients(26 cases),with 20 recipients weaned from ECMO immediately after transplants.The remaining 6 patients required prolonged ECMO support due to poor oxygenation or instable hemodynamics.Postoperative complications includes 30 infections(51.7%), 38 primary graft dysfunction(PGD,65.5%),15 hemorrhage(25.9%),11 renal injury(20.0%),8 heart failure(13.8%),and 3 acute rejection(5.2%).Four patients died during hospitalization for sudden cardiac death, PGD,sepsis,multiple organ failure in one patient,respectively.Thirty-day survival was 94.8%.
Conclusion: This small restrospective study shows that lung transplant program under current national OPO practices may expand donors after brain and cardiac death or cardiac death in China.Further research is warranted to improve donor lung quality and decrease postoperative complications.
None

P.1784

The early outcome of extracorporeal membrane oxygenation for primary graft dysfunction after lung transplantation: Experience in Korean single center

Jin Gu Lee[1], Kyung Sik Nam[1], Seokkee Lee[1], Hee Suk Jung[1], Jee Won Suh[1], Chang Young Lee[1], Seok Jin Haam[2], Hyo Chae Paik[1].
[1]Thoracic and Cardiovascular Surgery, Yonsei University College of Medicine, Seoul, Korea; [2]Thoracic and Cardiovascular Surgery, Aju University College of Medicine, Suwon, Korea.

Background: Primary graft dysfunction(PGD) is the leading cause of early morbidity and mortality following lung transplantation (LTx). However, recent advances in extracorporal membrane oxygenation (ECMO) device may allow sufficient time for the graft to recover from severe PGD. Our current practice of LTx program have extensively used ECMO support since 2010 and reviewed our experience of ECMO during the peri- and post-operative period.
Methods: Between 2010 and 2014, 82 patients received LTx in our institution (one heart lung transplantation was excluded). Among them, 28 (34.1%) patients required ECMO support immediately after LTx and additional 3 (3.6%) patients needed ECMO support during the course of postoperative period. Five (6.1%) patients necessitated re-insertion of ECMO after the successful weaning from the initial ECMO support. We retrospectively reviewed 31 (37.8%) patients who needed an ECMO to recover from graft dysfunction.
Results: Mean age of the recipient was 49.2 ± 13.9 and 18 (58.1%) patients were male. The disease entities for LTx were 24 pulmonary fibrosis, 3 bronchiolitis obliterance after bone marrow transplantation, 3 acute respiratory distress syndrome, and 1 bronchiectasis. Sixteen patients (51.6%) were on a ventilator support prior to LTx. Two patients received single lung and 29 patients received double lung transplantation. The ECMO usage immediately following LTx was veno-venous type in 19, veno-arterial in 8, and veno-veno-arteral in 1 patient. Three patients who required ECMO during the late course of postoperative period all underwent veno-venous ECMO, and patients who had re-insertion of ECMO was veno-venous type in 2 and veno-arterial in 3. Among them, 26 (83.9%) patients could be weaned from ECMO with a mean duration of ECMO support of 4.5 ± 5.2 days (range, 1 to 27). Early postoperative period with a ventilator support (33.5 days ± 49.2) and ICU stay (34.6 days ± 48.5) were longer compared to the group without ECMO. Thirty-day and 1- and 3-year survival were 78.1%, 50.0% and 45.5%, respectively.
Conclusions: The patients who required ECMO support during the postoperative period seems to have longer time to recover. But given the high risk of severe PGD patients, the early outcome seems acceptable, and weaning rate from ECMO was relatively high. We need further study regarding their long-term outcome.

P.1785

Optimum serum level of mycophenolate mofetil in chronic phase after lung transplantation

Yasushi Matsuda[1,2], Hideki Mitomo[1], Ken Onodera[1], Hiroshi Yabuki[1], Masafumi Noda[1], Fumihiko Hoshi[1], Akira Sakurada[1], Yasushi Hoshikawa[1], Miki Akiba[2], Masafumi Kikuchi[3], Nariyasu Mano[3], Yoshinori Okada[1].
[1]Department of Thoracic Surgery, Tohoku University, Sendai, Japan; [2]Division of organ transplantation, Tohoku University Hospital, Sendai, Japan; [3]Pharmaceutical Sciences, Tohoku University Hospital, Sendai, Japan.

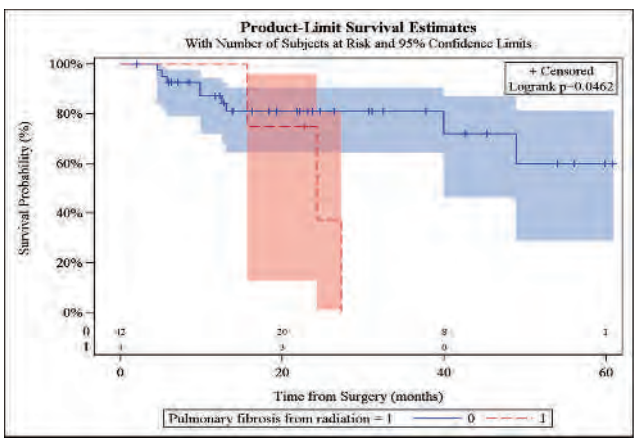
Introduction: We commonly use three immunosuppressive drugs for lung transplant recipients in order to suppress acute and chronic rejection. These are calcineurin inhibitor, steroid and mycophenolate mofetil (MMF). We often take care of the trough level of calcineurin inhibitor, however the optimum serum level of MMF in chronic phase of lung transplantation is unclear. Our objective is that we determine the optimum serum level of MMF to avoid rejection and infection in chronic phase of lung transplantation.
Material and Methods: Our program has performed 90 lung transplantations since 2000. We investigated serum level of MMF in 54 adult recipients of all who underwent single or double lung transplantation, survived more than one year after the operation and are taking MMF for immunosuppressant. We investigated the level of MMF by AUC0-12 calculation and examined complications with severe graft dysfunction or infection such as fungus or mycobacterium. Statistics was done by student t-test.
Results: As for control group, we selected forty recipients who did not have severe graft dysfunction and severe infection. Their average serum level of MMF was 34.8ug h/ml. However, severe graft dysfunction occurred in 5 recipients who needed listing for re-transplantation or steroid pulse therapy due to low respiratory function. Their average serum level of MMF as graft dysfunction group was 24.6ug h/ml. The serum level of MMF in graft dysfunction group was significantly lower than that in control group ($p<0.05$). In contrast, infectious disease by aspergillus or mycobacterium occurred in 9 recipients who needed to treat the infection. Their average serum level of MMF as infection group was 50.2ug h/ml. The serum level of MMF in infection group was significantly higher than that in control group ($p<0.05$).
Discussion: We have started to investigate the serum level of MMF in lung transplant recipients since 2014. According to some report about MMF in the field of kidney transplantation, optimal serum level of MMF in acute phase was 30-60ug h/ml in order to avoid acute rejection and side effect of MMF such as decrease of WBC count or digestive symptoms. In the chronic phase of lung transplantation, we need to consider chronic rejection and infection because they are the major causes of death after lung transplantation. We finally decided the optimum level of MMF in chronic phase of lung transplantation as 25-50ug h/ml to keep away from major complications.
Conclusion: For the purpose of management for the severe complications as graft dysfunction and infections in chronic phase of lung transplantation, we would like to determine that the optimum serum level of MMF was 25-50ug h/ml.
References:
[1] Kiberd BA, Lawen J, Fraser AD, Keough-Ryan T, Belitsky P. Early adequate mycophenolic acid exposure is associated with less rejection in kidney transplantation. *Am J Transplant*. 2004 Jul;4(7):1079-1083
[2] Mourad M, Malaise J, Eddour CD, De Meyer M, Konig J, Schepers R, Squifflet JP, Wallemacq P. Parmacokinetic basis for the efficient and safe use of low-dose mycophenolate mofetil in combination with tacrolimus in kidney transplantation. *Clin Chem*. 2001 47:7,1241-1248.

P.1786

Pediatric lung transplantation in patients with chemo-radiation associated pulmonary fibrosis: limited medium term survival compared to age-matched controls

David Horne[1,4], Brandi BScully[1,4], Ernestina Melicoff[2,5], Kathleen Hosek[3], Wei Zhang[3], E. DeanMcKenzie[1,4], George B.Mallory[2,5], Jeffrey S.Heinle[1,4].
[1]Congenital Heart Surgery, Texas Children's Hospital, Houston, TX, United States; [2]Pulmonology, Texas Children's Hospital, Houston, TX, United States; [3]Outcomes and Impact Services, Texas Children's Hospital, Houston, TX, United States; [4]Surgery, Baylor College of Medicine, Houston, TX, United States; [5]Pediatrics-Pulmonary, Baylor College of Medicine, Houston, TX, United States.

Background: In the current era, median pediatric lung transplant survival approaches 5-6 years. Pulmonary fibrosis patients present unique operative and post-operative challenges. Outcome reporting in pediatric lung transplantation for chemo-radiation associated pulmonary fibrosis (CRAPF) is limited.
Methods: Single center retrospective review of all pediatric patients that underwent bilateral lung transplantation for CRAPF between 2010-2015, compared to age-matched controls (any etiology for lung transplantation). Baseline characteristics collected were compared with univariate analysis. Overall survival outcome was compared with Kaplan-Meier logrank method. Secondary outcomes of primary graft dysfunction (PGD) ≥ grade 3, acute cellular rejection (ACR) ≥ grade 2 and bronchiolitis obliterans (BOS) were compared with univariate analysis.
Results: Four patients had CRAPF compared to 42 age matched controls. CRAPF mean age was 12.9 (SD 3.34) compared to 14.6 (SD 2.95, p=0.3). All CRAPF patients had restrictive lung disease before, as well as after transplantation (median 21.3 months post-transplantation). There were no statistical differences in any baseline characteristics while CRAPF patients trended to have longer cardio-pulmonary bypass times (CRAPF 378 ± 71 min. vs. controls 313 ± 61 min., p=0.07). Mortality rate was significantly higher in CRAPF patients compared to age-matched controls (3/4 (75%) vs. 9/42 (21%), p=0.049) – see Kaplan-Meier survival curve in figure 1. There was no statistical difference in PGD (CRAPF 26% vs. control 29%), ACR (CRAPF 0% vs. 14%) or BOS (CRAPF 25% vs. control 20%).



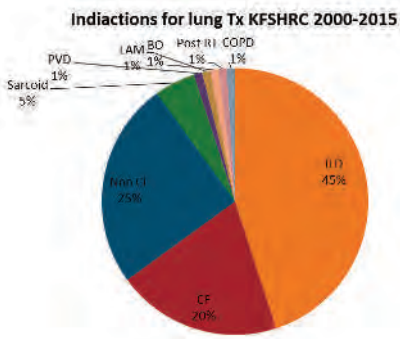
Conclusion: Lung transplantation in patients with end stage lung disease due to CRAPF has a significantly higher mortality rate and needs further investigation to determine causality and viability as treatment option.

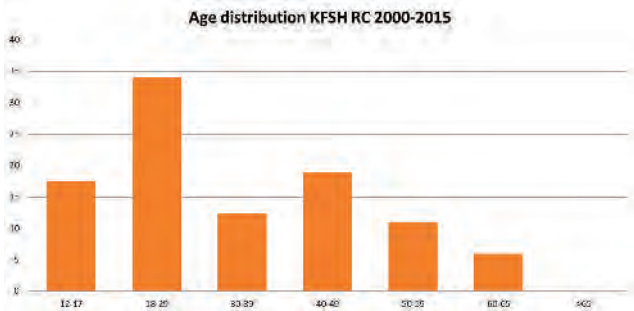
P.1787

Journey of lung transplant program in Saudi Arabia

Imran Nizami, Mohamed A.S. Aabdeen.
Lung Transplant Section/Organ Transplant Center, King Faisal Specialist Hospital and research center, Riyadh, Saudi Arabia.

Purpose: To highlight the diferences in lung transplant (LTx) recipient and donor population in Saudi Arabia (SA).
Methods: Retrospective chart review of 80 patients transplanted between January 2010 and December 2015.
Summary of Results: The most common indication for (LTx) was pulmonary fibrosis including UIP 36 (45%), followed by non CF bronchiectasis 20(25%), CF 16 (20%), sarcoidosis 3(4%), COPD 1 (1%), others 4 (5%).The lung transplant patients were younger with 82% being youger than 50 years of age. Most donors were colonized with bacteria (70%) including multiple drug resistant bacteria (50%). The 1,2 and 5 years survival was comparable to the ISHLT registry.
Conclusion: During the study period the common indications for lung transplant in SA were different from ISHLT registry, The patient population was younger, Majority of donors were colonized with bacteria including multiple drug resistant bacteria. The patient survival however is comparable to ISHLT registry.





P.1788

Mycobacterium tuberculosis infection after lung transplantation, Saudi Arabia experience

Imran Nizami, Saeed Akram, Mohamed A.S. Aabdeen.
Lung Transplant Section/Organ Transplant Center, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia.

Purpose: To study the incidence, time of onset and outcome of patients diagnsed with MTBI after lung transplant in Saudi Arabia.
Methods: Retrospective chart review of patients transplanted at the king Faisal Specialist Hospital and Reseasrch center, Riyadh, Saudi Arabia.
Summary of results: A total of 80 patients underwent lung transplantation during the study period. 5 (6%) developed MTBI between 1-102 days after the transplanatation. 4 patients were negative for latent MTB at the time of transplant. one patient had positive IGRA (Quanteferon) at the time of transplant and recieved INH prophylaxis. Unfortunatelly developed INH resistant MTBI while on prophylaxis. All patients are alive at the time of this data submission.
Conclusion: MTBI is not rare after lung transplanatation in Saudi Arabia. All patients developed MTBI within 4 months of the transplant. MTBI and its treatment did not cause excess episodes of acute rejection or mortality.

Age	sex	Diagnosis	Pre-Tx Quanteferon	Diagnosis of TB post Tx (days)	# of acute rejection
19	F	Bronchiectais	Negative	1	1
26	F	CF	Negative	102	0
50	F	ILD	Negative	97	0
49	M	ILD	Negative	93	0
35	M	Sarcoidosis	positive	95	0

P.1789

The prevalence, possible risk factors and the outcome of swallowing abnormalities in post lung transplant patients.

Imran Nizami[1], Amber Hassan[2], Hatem Elabd[1].
[1]Lung Transplant Section/Organ Transplant Center, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia;
[2]Speech Language Pathology/Otolaryngology, King Faisal Specialist Hospital and Research center, Riyadh, Saudi Arabia.

Purpose: To study the prevalence, possible risk factors and outcome of swallowing abnormalities in post lung transplant patients.
Methods: retrospective chart review of patients that underwent lung transplant at our center between 2010 and 2013. The prevalence of swallowing abnormalities was judges by bedside examination and modified barium swallow. A correlation between swallowing abnormalities (SA) and the duration of mechanical ventilation (DMV) and need for tracheostomy (Tr) was calculated. Those with SA were compared with patients without SA to assess the effect of SA on Length of hospital stay (LOS), episodes of acute rejection (AR) and episodes of pneumonia requiring hospitalization (PRH) in the first year post transplant.
Summary of Results: Total of 50 lung transplants were performed during the study period. The data was available in 42.The prevalence of SA was 75.68%. All patients recovered from SA. The average duration of mechanical ventilation in pts with and without SA was 19 and 8.1days (p=0.16). The number of pts requiring tracheostomy in SA group was 9 (32%) vs 1 (11%) without SA (p= 0.21). The pts with SA stayed in hospital for an average of 46.5 days vs pts without SA 32.1 days (p=0.21). The ICU length of stay was 23.5 days in SA group vs 12.7 in non SA group (p= 0.16). The number of acute rejection were 11 (39%) and 1(11%) and pneumonia requiring hospitalization 8(88%) in SA group and 19(67%) in no SA group.
Conclusion: SA was common in post lung transplant patients. With speech therapy intervention the SA recovered in all patients. In our small study the DMV and Tr. did not significantly affect the prevalence of SA. Similarly there was no difference in the LOS, AR and PRH in patients with SA.

P.1800

Pre- transplant immunological risk assessment: CDC cross-match supplemented by Luminex antibody Screen (Qualitative) - more sensitive approach to prevent early antibody mediated rejection

Feroz Aziz[1], Sajith Narayanan[1], Ismail Na[1], Benil Hafeeq[1], Jyotish Gopinathan [1,2], Renjith Narayanan[2], Elyas K.K. [3].
[1]Nephrology and Renal Transplantation, Malabar Institute of Medical Sciences, Calicut, India; [2]Nephrology, Malabar Institute of Medical Sciences, Kottakkal Campus, India; [3]Department of Biotechnology, Calicut University, Calicut, India.

Introduction: Antibody-mediated rejection(ABMR) remains the Achilles heel of successful renal transplantation(R-Tx). Though antibody profiling has evolved from less sensitive complement-dependent-cytotoxic(CDCXM) methods to more sensitive luminex-based tests, clinician finds it difficult to utilize these tools cost-effectively. The aim of our study is to outline a cost-effective strategy to profile patients utilizing both cytotoxic and luminex-based tests.
Methods: We retrospectively studied 98 consecutive R-Tx at our institute from Nov-2012 to July-2014. All underwent R-Tx if they had negative pre-transplant CDCXM. Fourty three of 98 patients randomly underwent additional pre-transplant qualitative screen(Screen-group) for anti-HLA antibodies(Class-I& II) by luminex based multiple antigen bead method(Lab-Screen), whereas 55 patients had only pre-transplant CDCXM (CDC-group). We compared the incidence of acute rejections (AR)- both Cellular(ACR) and Antibody medicated (ABMR), graft loss (GL) and patient loss (PL) between the two groups.
Results: Of the 43 patients of screen-group, 29 were negative for both class-I and II antibodies. Of 14 who had positive 'Lab-Screen', 7 had only class I, 1 had only Class-II and 6 had both class-I and II antibodies. There were 4 AR in each group, 9.3% in Screen-group and 7.2% in CDC-group (ns). Screen-group had 3 ACR and 1 ABMR whereas CDC-group had 3 ABMR and 1 ACR. None of the 29 patients with negative Lab-Screen developed ABMR .There were 2 GL due to non-immunological reasons in screen-group; 5 GL in CDC-group of which 3 were due to ABMR. There were no patient loss in either group.
Conclusion: CDCXM supplemented by qualitative HLA-antibody screening is associated with decreased early ABMR. Sending patient sera to a lab with luminex facility and in-house CDCXM is a cost-effective option for upcoming centers.

Table 1

Outcomes	Lab screen+ CDCXM (n = 43) Screen group		Only CDCXM (n = 55) CDC group
	Lab Screen positive for anti - HLA antibodies (n = 14)	Lab Screen negative for anti - HLA antibodies (n = 29)	
Acute rejections	4 3 ACR & 1 ABMR	1 1 ACR & 0 ABMR	4 1 ACR & 3 ABMR
Graft loss	One graft loss (Recurrent FSGS)	One graft loss (Recurrent HUS)	5 graft loss 3 ABMR 2 – Technical reasons
Patient loss	None	None	None

CDCXM – Complement dependent cytotoxic crossmatch, ACR – acute cellular rejection
ABMR- acute antibody mediated rejection, HUS – hemolytic uremic syndrome, FSGS – focal segmental glomerulosclerosis.

Sameer N M

P.1801

HLA-antibody testing with LabScreen/Luminex single antigen beads - the relation to CDC results

Reinhard Kelsch[1], Thomas M. C.Binder[3], Katharina Schütte-Nütgen[2], Barbara Suwelack[2].
[1]Institute of Transfusion Medicine and Transplantation Immunology, University Clinics of Münster, Münster, Germany; [2]Dept of Medicine D, General Internal Medicine, Nephrology, Rheumatology, University Clinics of Münster, Münster, Germany; [3]Department of Transfusion Medicine, University Medical Center Hamburg Eppendorf, Hamburg, Germany.

Patients on the waiting list for kidney transplantation in Germany are screened four times a year for HLA-antibodies in order to define the inacceptable HLA antigens, against which the patients are immunized. This is performed using different laboratory techniques including complement dependent cytotoxicity technique (CDC), ELISA or the Luminex single antigen bead assay (SAB). CDC is supposed to detect HLA-antibodies, which are most relevant for transplantation. The SAB technique is more sensitive and usually detects more HLA-antibody specificities in a given serum and thus a different result than CDC. The results and signals are disturbed by prozone effects, where the binding of the fluorochrome detector antibody is blocked by complement C1q binding from the serum or an HLA-antibody overload of the system. Prozone effects can be diminished by adding EDTA to the serum, by heat inactivation, DTT-treatment or dilution of the serum. In order to investigate, how the results of CDC and SAB are comparable, we compared sera positive in CDC with T- or B-lymphocytes with SAB technique with and without the addition of EDTA to the serum.
Materials and Methods: Sera from 434 patients from the kidney waiting list were tested for HLA-class I/II antibodies by ELISA (AbScreen HLA Class I/Class II, Bio-Rad). HLA Class I antibody positive sera were further tested in T-CDC (SeraScreen FCT 60, BAG). Sera solely positive for HLA-Class II antibodies were tested in B-CDC (LymphoscreenDR30, Bio-Rad). All ELISA positive sera were tested in the LABScreen Single Antigen HLA-Class I (SAB-1) and HLA-Class II (SAB-2) assays (One Lambda) in two different modes: 1. Untreated serum (SAB-1/2-IgG), 2. Serum supplemented with 8 mmol/L EDTA (SAB-1/-IgG/EDTA).
Results and Discussion: 22 of the 62 ELISA Class I positive sera were reactive in T-CDC. 24 out of 68 ELISA Class II positive sera, were negative in ELISA Class I. 19 of these 24 sera were reactive in B-CDC-testing. The SAB-IgG testing of the untreated sera showed that the antibody specificities found in CDC assays only rarely led to the highest signal intensities in the SAB-IgG assays. However, the addition of EDTA led to a shift of T- or B-CDC reactive antibody specificities to the highest ranks of MFI-values in the SAB assays and thus reflected much better the CDC results.
Conclusion: The addition of EDTA to serum for Luminex SAB assays opens the possibility, to use the signal intensities or HLA-specificities not only in a qualitative, but in a quantitative manner. It should be used generally in these tests, since the signal intensities reflect the CDC results and thus the clinical significance of antibody specificities and their relation to the test cutoff much better than in SAB tests performed without EDTA. Clinically relevant CDC positive antibody specificities and most likely clinical relevant CDC-negative specificities too move to highest mean fluorescence intensity values of the SAB profile according to their strength.

P.1802

The frequency and the reactivity patterns of HLA class I donor-specific epitope antibodies identified in Korean patients

Soo-Kyung Kim, Chang Ahn Seol, Heung-Bum Oh.
Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea.

Introduction: This study aimed to analyze PRA results at the epitope level utilizing the concept of HLA eplets which are considered to represent B-cell receptor epitopes on HLA[1]. In addition, immunogenicity and antigenicity of eplets frequently identified in Koreans were investigated to see if the eplets are clinically relevant.
Materials and Methods: A total of 96 patients were enrolled who had undergone kidney transplantation. They were tested with class I Luminex single-antigen bead assay (One Lambda, Canoga Park, CA). Total 250 class I PRA assay results were obtained. A computer program was developed, which uses a tail analysis algorithm to identify HLA-A and -B donor-specific epitope antibodies (DSEA). The immunogenicity of various eplets was investigated by analyzing the correlation between the frequencies of eplets in Korean and the frequencies of DSEAs. The antigenicity of some eplets was tested with T-cell flow cytometric crossmatching (T-flow XM). T-flow XM serum were from 10 patients who were DSA negative and DSEA positive. T-flow XM lymphocytes were from healthy volunteers. Each patient serum was crossmatched with 2 or 3 volunteer lymphocytes.
Results: Among 250 PRA test results, 76 specimens were DSA+/DSEA+, 57 were DSA-/DSEA+, 116 were DSA-/DSEA-, and 1 was DSA+/DSEA-. The total eplet frequency and DSEA frequency were positively correlated (R = 0.4783) for an eplet frequency less than 50%, and negatively correlated (R = -0.2859) for an eplet frequency greater than or equal to 50%. T-flow XM results are as follows. Two tests with 82LR+s145R/149A eplet pair DSEA crossmatched with HLA-B*57:01 lymphocytes were negative. One test with 24T DSEA which were crossmatched with HLA-B*40:01 lymphocytes was negative. The other results were all positive. The 82LR eplet is located at α1 chain and s145R/149A is located at α2 chain, which suggests that eplet pair override the peptide binding groove. The 24T eplet was unexposed.
Discussion: The correlation of eplet frequency and DSEA frequency was relatively low when eplet frequency was greater than 50%. This was because some eplets had much higher frequency of DSEA than average frequency observed in the eplets with similar frequencies. This is considered to be indicating that immunogenicity of each eplet varies. Structural analysis of eplets in the test samples that were negative in T-flow XM revealed that eplets overriding peptide cleft or hidden by surrounding amino acids could be crossmatch negative in spite of high MFI in PRA analysis.
Conclusion: We developed a computer program that could conveniently count eplet mismatches of HLA-A and -B antigens and interpret the PRA results at the epitope level. Eplet analysis, if it entails structural analysis, was useful for the interpretation of discrepancy between results of HLA antibody tests. However, it is thought that studies revealing immunogenicity and antigenicity of each eplet should be continued to fully understand the clinical relevanceof DSEA identified.
References:
[1] Duquesnoy RJ. *Transplant Proc*. 2001;33:493-7.

P.1803

Positive rates of preliminary T and B cell flow cytometry crossmatches among transplant candidates in the Korean Network for Organ Sharing

Myoung Hee Park, Sohyun Kim, Hyewon Hwang, Heeseo Park, Jiye Kwak, Eun Kyung Kwon, Hye Young Sung, Boknyun Han.
Laboratory, Korea Organ Donation Agency, Seoul, Korea.

Introduction: For deceased donor organ transplantations, virtual crossmatches (XMs) have not yet been adapted in Korea. Preliminary T cell XMs are routinely performed using stored frozen sera and negative T cell XM results are mandatory for organ allocation in the Korean Network for Organ Sharing (KONOS) organ allocation system. Submission of serum to the KONOS with periodic renewal is mandatory for kidney and pancreas transplant candidates and optional for other organ transplant candidates. Sensitization of the transplant recipients reacting with donor-specific HLA class II antigens is also known to adversely impact the transplant outcomes. Our laboratory is performing about 40% of the KONOS XMs and we have investigated positive rates of T and B cell flow cytometry XMs among transplant candidates waitlisted for different organs.
Materials and Methods: For KONOS T cell XMs, we are routinely performing both complement-dependent cytotoxicity (CDC) and flow XMs. In recent one year period (August 2014-August 2015), a total of 239 deceased donors were crossmatched in our laboratory. For randomly selected 53 donors, 1,387 candidates (26.2 candidates per one donor) were retrospectively tested for B-flow XMs and positive rates of T- and B-flow XMs were analyzed for transplant candidates waitlisted for different organs. Cells were treated with pronase (0.5 mg/mL final concentration) for B-flow XMx, and untreated cells were used for T-flow XMs.
Results and Discussion: T-flow XM positive rates (overall 15.3%) varied among transplant candidates waitlisted for different organs (Table 1). Positive rate was high for kidney (16.8%; 204/1,216), intermediate for heart (9.5%), and low for pancreas & kidney/pancreas (0%) and lung (5.9%). Nearly 4% of the candidates (3.9%, 54/1,387) showed T-flow negative and B-flow positive results. Thus, T- and/or B-flow positive rate in the candidates was increased to 19.2%, compared to T-flow positive rate of 15.3%. Although pancreas & kidney/pancreas recipients showed very low T-flow positive rate (0%), their B-flow positive rate was relatively high (6.3%). Among B-flow positive cases, 13.0% (7/54) showed strong positive reactions: median fluorescence intensity (MFI) ratio ≥6.0; or median channel shift (MCS) ≥200.

Table 1. Positive results in KONOS preliminary T and B cell flow crossmatches for transplant candidates

Organ (No. of crossmatches)	T (+) No. (%)	T (−) / B (+) No. (%)	T &/or B (+) No. (%)
Kidney (n=1216)	204 (16.8%)	46 (3.8%)	250 (20.6%)
Panc, Kid/Panc (n=80)	0 (0%)	5 (6.3%)	5 (6.3%)
Lung (n=17)	1 (5.9%)	0 (0%)	1 (5.9%)
Heart (n=74)	7 (9.5%)	3 (4.1%)	10 (13.5%)
Total (n=1387)	212 (15.3%)	54 (3.9%)	266 (19.2%)

Conclusion: Nearly 4% of deceased donor transplant candidates show T-flow negative and B-flow positive results in the preliminary KONOS XMs. Some of the candidates show strong positive reactions for B-flow XMs, which might impact transplant outcomes. Prospective B-flow testing in KONOS XMs would be of help for pre- and post-transplant clinical management of the recipients in solid organ transplantations, especially for those centers not routinely performing appropriate HLA antibody monitoring and pre-transplant final XMs.

P.1804

An inventory of the presence of pretransplant donor-specific HLA antibodies as tested with luminex single antigen tests of lifecodes and one lambda and their complement variants

Simone Brand-Schaaf, Janneke Langerak-Langerak, Sophia Stein, Frans Claas, Dave Roelen.
Dept of Immunohaematology and Blood Transfusion, LUMC, Leiden, Netherlands.

Introduction: Pretransplant donor-specific HLA-antibodies (DSA) have been associated with acute humoral rejection and inferior kidney graft survival. We investigated the incidence of DSAs with two different Luminex SA platforms in 77 patients, who have been transplanted.
Methods: 77 kidney transplant recipients of living or postmortal donors with intermediate and high immunological risk (PRA >60%) were included in this observational, retrospective study. The last available pretransplant sera of these patients were analysed by single antigen bead (SAB)-analysis, for the presence of HLA class-I and -II antibodies of 2 providers Lifecodes (LC) and One Lambda (OL) (positivity was defined according to manufacturer's instructions). Furthermore, in case of positivity the analysis was followed by C3d and C1q assays.
Results: Out of the 77 patients, transplanted between 1995 and 2004 in the presence of a negative CDC crossmatch, we found donor-specific HLA class I antibodies with both SA tests in 43 patients while 27 patients were negative in both tests. OL showed 5 times a DSA not detected by LC and 2 times the opposite was found. For HLA class II, 12 samples had DSA in both tests and 63 were negative in both tests while OL showed 2 times a DSA not detected by LC. A significant positive correlation was found between the outcome of OL and LC tests, both for class I and class II DSA. The C3d SA luminex test of LC was positive in 11 of the 45 positive samples, whereas the C1q test was positive in 12 out of the 43 tested positive samples. The neat MFI samples of both the LC and OL tests could to a certain extent predict the positivity of the C3d and C1q assays.
Conclusion: Measurement of the presence of pretransplant HLA specific antibodies with SA luminex tests of the 2 providers shows results that were very similar. The complement variants of these tests, C3d and C1q, were positive in about 25% of the positive neat tests.

P.1805

Effect of recipient age (<3 years) during liver transplantation on the prevalence of post-transplant class ii anti-human leukocyte antigen antibody

Kazuaki Tokodai, Satoru Yoshida, Koji Miyazawa, Kai Maida, Toshiaki Kashiwadate, Yasuyuki Hara, Chikashi Nakanishi, Shigehito Miyagi, Naoki Kawagishi, Noriaki Ohuchi, Susumu Satomi.
Department of Transplantation, Upper Digestive and Vascular Surgery, Tohoku University Hospital, Sendai, Japan.

Introduction: Post-transplant donor-specific anti-human leukocyte antigen (HLA) antibodies (DSA) reportedly have detrimental effects on organ transplantation outcomes. We previously reported that liver transplant recipients who tested positive for post-transplant class II DSA were younger at the time of living donor liver transplantation (LDLT) than those who did not test positive, although this difference was not statistically significant. Therefore, this study aimed to assess the effect of recipient age (<3 years) at the time of pediatric LDLT on the prevalence of post-transplant class II DSA during long-term follow-up.
Materials and Methods: We identified 84 pediatric LDLT recipients who underwent follow-up checks at the outpatient department of our institution. Of these, 40 patients with HLA typing data available were recruited for this study; informed consent was obtained for DSA evaluations from recruited patients, and the local institutional internal review board approved the study. Patients were divided into 2 groups based on their age at the time of LDLT: younger (Y) group, age < 3 years; older (O) group, age ≥ 3 years. To evaluate DSA prevalence, Luminex single-antigen bead assays were performed; the cut-off for a positive result was set at mean fluorescence intensity (MFI) ≥1000.
Results: The mean recipient age at the time of LDLT in groups Y and O was 12.4 months (range, 6–35 months) and 109 months (range, 40–203 months), respectively (p<0.0001). Donors in group Y were younger than those in group O (30.7 vs. 39.8 years; p<0.0001). There were no differences regarding recipient sex, primary disease, ABO incompatibility, calcineurin inhibitor, acute rejection, and periods from LDLT between the groups. Basiliximab was only administered to 4 patients in group O (p<0.005). No class I DSA was detected in any of the 40 patients. Significantly more patients tested positive for class II DSAs in group Y (38.5%) than in group O (7.1%; p=0.02). A high MFI value (≥5000) was detected only in 3 patients in group Y compared to none in group O, although the difference did not reach statistical significance (p=0.10).
Discussion: Here, we found that the prevalence of class II DSA was higher in patients who underwent LDLT at ages < 3 years. The mechanism underlying this higher prevalence is unclear, but the use of basiliximab may have a role in this finding because class II DSA was not detected among the 4 group O patients to whom basiliximab was administered.
Conclusion: Recipients aged less than 3 years might be at a higher risk of testing positive for class II DSA during long-term follow-up after pediatric LDLT. These findings may help in cost-effective screening of post-transplant DSA levels.
This study was supported by JSPS KAKENHI Grant Number 26861036 for the analysis and writing of the manuscript

P.1806

Transplantation of highly sensitized patients with circulating preformed donor specific antibodies: how safe is it?

Angeliki Vittoraki[1], Maria N.Darema[2], Pavlina V.Markaki [1], Ilias K.Makropoulos[2], Sofia I.Ioannou[1], Emmanouil G.Tzamparlis [2], Aliki G.Iniotaki [1], John N.Boletis [2].
[1]National Tissue and Typing Center, General Hospital of Athens ''G.Gennimatas'', Athens, Greece; [2]Transplantation Unit, General Hospital of Athens "Laikon Hospital", Athens, Greece.

Introduction: Preformed anti HLA donor specific antibodies (DSA) of IgG subtype are associated with increased graft loss in kidney transplants related to an increased risk of antibody mediated rejection (AMR). Although, the introduction of solid phase assays radically changed the antibody monitoring and the stratification of the immunological risk in kidney allocation, there are still questions regarding their clinical significance especially in highly sensitized patients (HS).
Materials and Methods: In this study, we present the follow up results from 56 HS patients (% PRAs>70) who received a graft between 2009-2015 in our center. The patients were divided into two groups: group A-37 patients transplanted with no preformed DSA (MFI<1000) and group B- 19 patients transplanted with preformed DSA (mean MFI 5213±6249). A control group of 37 non-HS patients (%PRAs 0-58), transplanted the same period with no preformed DSA was also used (CG). All patients (n=93) were transplanted from deceased donors, with negative CDC and T/B FCM. As induction treatment Basiliximab was given to all patients with addition of rituximab in 15 HS and 2 CG patients. Maintenance immunosuppression of MPA or mTORi/FK506/MP was administered in HS patients and of MPA or mTORi/CNI/MP in CG. The mean follow up period was 39±21 months post transplantation for AMR episodes, de novo DSA development, infections, baseline and current sCr levels and graft loss.
Results and Discussion: No significant difference was found between the three groups regarding patients' or donors' age at transplantation, AMR episodes, de novo development of DSA, graft loss or infection rates. Biopsy proven acute rejection episodes (n=9) were developed during the follow up with no significant difference between the groups: 4/37 group A, 3/19 group B and 2/37 from CG. The episodes were defined as AMR (n=3) or T cell mediated rejection-TCMR (n=6). AMR episodes were developed only in group A (n=2) and group B (n= 1) patients. The median baseline sCr levels at first month post-Tx was 1.3, 1.2 and 1.4 mg/dL for group A, B and CR respectively (p=NS).
During the follow up, six patients lost the graft: two from group A (one patient with preformed IgM DSA before Tx and the second from surgical complications), two from group B (surgical complications) and two from CG (chronic rejection with de novo DSA in one case). Finally, the current sCr levels (median value) were 1.15, 1.2 and 1.18 mg/dL for group A, B and CR, respectively with no difference between the groups (p=NS).
Conclusion: We conclude that HS renal transplant candidates with preformed DSA and negative CDC and T/B Flow crossmatch may proceed to safe transplantation with low immunological risk and long term good stable graft function.

P.1807

Antibodies against Angiotensin II type 1 receptor and HLA donor specific antibodies in renal allograft rejection

AngelikiG. Vittoraki[1], Christina A.Melexopoulou [2], Ioanna G.Glynou[1], Pavlina V.Markaki [1], George C.H.Liapis[3], John N.Boletis [2], Aliki G.Iniotaki [1].
[1]National Tissue and Typing Center, General Hospital of Athens ''G.Gennimatas'', Athens, Greece; [2]Transplantation Unit, General Hospital of Athens "Laikon Hospital", Athens, Greece; [3]1st Department of Pathology, Medical School, University of Athens and General Hospital of Athens "Laikon Hospital", Athens, Greece.

Introduction: Antibodies against Angiotensin II type 1 receptor (anti-AT1R abs) are autoantibodies that may induce a wide variety of allograft injuries reflecting the complexity of their action and a possible synergistic effect with HLA donor specific antibodies (DSA). The aim of this study was to investigate the influence of anti-AT1R abs on graft outcome in renal allograft recipients who experienced biopsy proven graft rejection.
Materials and Methods: Thirty one renal allograft recipients, 39±12 years old, 20 males and 11 females, who experienced antibody mediated rejection-AMR (n=19), T cell mediated rejection-TCMR (n=10) or mixed AMR and TCMR rejection (n=2) were studied. Anti-AT1R abs were measured retrospectively in all sera at the time of rejection (EIA-AT1R kit, One Lambda Inc). Anti-AT1R antibody levels were classified as low <10U/mL, at risk 10-17U/mL and high>17U/mL. DSA were defined at the time of rejection by Single Antigen Bead Assay (Luminex, One Lambda Inc). For DSA an MFI>1000 was considered positive.
Results and Discussion: Anti-AT1R abs in at risk or high levels were present in 14/31 cases (45.2%): 9/19 AMR, 4/10 TCMR and 1/2 mixed rejection type cases. DSA were detected in 17/31 cases (54.8%) and in 9/17 cases anti-AT1R abs were also present. The parallel detection of anti-AT1R abs and DSA was significantly associated with AMR (p=0.02). Anti-AT1R ab detection was not associated with class I or class II DSA. Twelve out of 31 patients (38.7%) lost the graft: 10 with AMR or mixed type rejection and two with TCMR. Graft loss was associated with the presence of both anti-AT1R abs and DSA in patients serum even not significantly so (p=0.057). Nineteen patients had functioning graft 89±77 months (mean time) post transplantation. Current median serum creatinine levels were 2.55, 1.63 and 1.67 mg/dl in patients with high, at risk and low levels of anti-AT1R respectively.
Conclusion: We conclude that anti- AT1R abs may be detected in the serum of renal transplant recipients with different types of rejection. The association of AMR and graft loss with the presence of both anti-AT1R abs and HLA DSA indicate a possible synergistic effect of allo and auto reactivity in graft injury. Monitoring for anti-AT1R and DSA could help to define patients with particular risk for irreversible graft injury and may improve graft outcome.

P.1808

Presence of day-14 post transplantation donor specific IgM antibody predicts poor graft survival in HLA-incompatible renal transplantation

Adarsh Babu[1,3,4], Avgi Andreou[1,4], Mihaela Porumb[2], Torgyn Shaikhina[2], David Briggs[4], Nithya Krishnan[1], Tom Barber[1,3], Dan Mitchell[3], Rob Higgins[1], Natasha Khovanova[2], Sunil Daga[1,3,4].
[1]Renal medicine, University Hospitals Coventry and Warwickshire, Coventry, United Kingdom; [2]School of Engineering, University of Warwick, Coventry, United Kingdom; [3]University of Warwick, Coventry, United Kingdom; [4]Histocompatibilty and Immunogenetics, NHS Blood and Transplant, Birmingham, United Kingdom.

Introduction: IgG antibodies against donor HLA are monitored in the post-transplant period due to their association with rejection and allograft survival. IgG can initiate both classical complement pathway activation and antibody-dependant-cell cytotoxicity (ADCC).
IgM: In primary responses precedes IgG appearance.
Most potent activator of the classical complement pathway but cannot initiate ADCC. The role of IgM donor HLA specific antibodies (DSA) is not fully understood, especially in highly sensitised patients undergoing direct transplantation. Previous studies of conventional renal transplantation have shown contradictory effects of HLA-specific IgM[1],[2], and one study suggests IgM antibody incompatible transplantation may be refractory to C5 blockade[3]. We designed this study to determine if additional post-transplant monitoring of IgM DSA predicts rejection episodes or graft failure.
Methods: Serum samples from 92 patients who had undergone HLA-antibody incompatible transplants were tested at post-transplantation days 7, 14 and 30.
All samples screened by microbead assay with EDTA containing wash buffer (LAB screen SAB, One Lambda, CA, USA). Screen IgM positive samples were further characterised for specific class I and class II HLA using single antigen bead assay (One Lambda, CA, USA). IgM antibody was defined positive if the MFI>2000.
Presence of post-transplant IgM was correlated with early antibody mediated rejection (within day 30 post transplantation) episodes and graft failure.
Statistical analyses were performed using SPSS IBM software (Fischer exact 2 tailed test and Kaplan Meier survival analysis).
Results: IgM DSA was positive in 32 patients (35%) of which 17 patients had an episode of antibody mediated rejection. IgM DSA was negative in 29 patients who had an episode of antibody mediated rejection. Post-transplant IgM was associated with graft survival (p=0.037), particularly d14 post-transplantation (p=0.008) (Figure 1). Day 30 IgM did not predict graft survival (p=0.46). Post-transplant IgM MFI trend was not similar to pan IgG (Figure 2). IgM MFI peak at day 14 correlates with IgG3 peak at day 30 (data not shown). Presence of IgM DSA day 7, 14 or 30 was not associated with predicting rejection (P=0.83).
Discussion: In this study, we have shown additional value of post-transplantation IgM DSA measurement, predominantly day 14 IgM over and above IgG in prediction of death censored graft survival. IgM resynthesis did not predict early AMR. In this particular cohort we have previously shown an association of IgG3 DSA response at day 30 with inferior graft survival[4]. Hence, our findings suggest that a sequential anti-donor humoral response, starting with IgM, contributes to poor graft outcome in antibody incompatible transplantation. Interference of this process may provide a strategy to improve outcome.

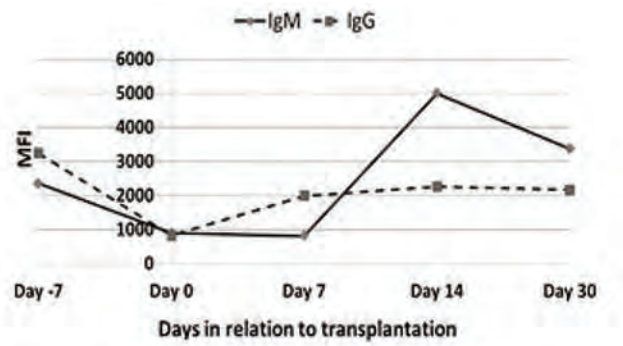


Figure 2 IgM DSA and pan IgG DSA trend

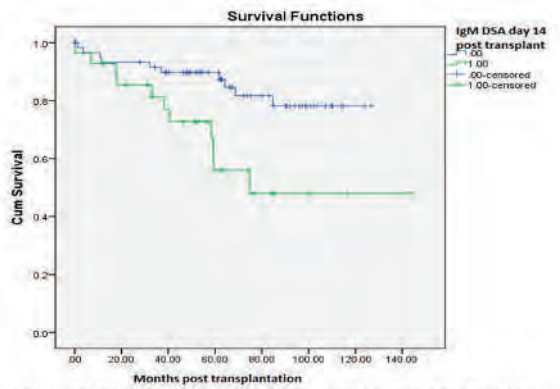


Figure 1 Kaplan Meier survival analysis: Day 14 IgM DSA predicted renal allograft survival (p=0.008)

References:

[1] Worthington JE, Thomas AA, Dyer PA, Martin S. Detection of HLA specific antibodies by PRA STAT and their association with transplant outcome. Transplantation 65:12,1998.
[2] Tardif GN, McCalmon RT. Successful Renal Transplantation in the presence of Donor specific HLA IgM antibodies. Transplant Proc. 1995;27(1):664-5.
[3] Bentall, Andrew, et al. "Antibody-mediated rejection despite inhibition of terminal complement." Transplant International 27.12 (2014): 1235-1243
[4] Khovanova N, Daga S, Shaikhina T, Krishnan N, et al. Subclass analysis of donor specific IgG in antibody incompatible renal transplantation reveals a significant association of IgG4 with rejection and graft failure. Transpl Int. 2015 Dec;28(12):1405-15. doi: 10.1111/tri.12648. Epub 2015 Sep 1.

P.1809

Clinical relevance of pre-formed IgM HLA-donor specific antibodies (DSA) in HLA-incompatible kidney transplantation

Adarsh Babu[1,3,4], Avgi Andreou[1,4], Mihaela Porumb[2], Torgyn Shaikhina[2], David Briggs[4], Nithya Krishnan[1], Tom Barber[1,3], Dan Mitchell[3], Rob Higgins[1], Natasha Khovanova[2], Sunil Daga[1,3,4].
[1]Renal medicine, University Hospitals Coventry and Warwickshire, Coventry, United Kingdom; [2]School of Engineering, University of Warwick, Coventry, United Kingdom; [3]University of Warwick, Coventry, United Kingdom; [4]Histocompatibilty and Immunogenetics, NHS Blood and Transplant, Birmingham, United Kingdom.

Introduction: Pre transplant IgG antibodies against donor human leucocyte antigens (HLA) are screened due to its established role in predicting rejection and renal allograft survival.
• IgG can initiate both classical complement pathway activation and antibody-dependant-cell cytotoxicity (ADCC).
IgM
• In primary responses precedes IgG appearance.
• Most potent activator of the classical complement pathway but cannot initiate ADCC.
The role of IgM donor HLA specific antibodies (DSA) is not fully understood, especially in highly sensitised patients undergoing direct transplantation. Previous studies of conventional renal transplantation have shown contradictory effects of HLA-specific IgM[1]-[3], and one study suggests IgM antibody incompatible transplantation may be refractory to C5 blockade[4]. We designed this study to determine if additional post-transplant monitoring of IgM DSA predicts rejection episodes or graft failure. We designed this study to determine if additional testing IgM DSA prior to transplantation predicts rejection episodes or graft failure.
Methods: Samples from 92 patients who had undergone HLA-antibody incompatible transplants were tested at pre-conditioning or pre-transplant for cases that did not require antibody removal therapy. All samples were screened by Luminex microbead assay with EDTA containing wash buffer (LAB screen SAB, One Lambda, CA, USA). Screen IgM positive samples were further characterised for specific class I and class II HLA using single antigen bead assay (One Lambda, CA, USA). IgM was defined positive if the MFI values> 2000. Presence of pre-formed IgM DSA was correlated with early antibody mediated rejection (within day 30 post transplantation) episodes and graft failure. Statistical analyses were performed using SPSS IBM software (Fischer exact 2 tailed test and Kaplan Meier survival analysis).
Results:
• IgM HLA DSA was present in 35 of the 92 patient samples studied.
• Early antibody mediated rejection (within 30 days) was seen in 47 of the 92 cases studied. IgM DSA was positive in 17 cases (36%).
• There was no significant association of episodes of early rejection with pre-formed IgM HLA-specific DSA, P = 0.83 (Table 1).
• Similarly, there was no significant association of pre-formed IgM HLA-specific DSA and death censored graft survival, P=0.44 (Figure 1).
Discussion: In this single centre study we have shown that additional testing for pre-formed IgM HLA DSA is not useful for prediction of rejection or graft survival in a cohort of HLA-incompatible kidney transplantation. Findings of pre-formed IgM DSA is in line with other published literature, where renal transplants have been performed successfully in the presence of IgM DSA. There has been no published cases of hyperacute rejection solely due to IgM antibodies. It is interesting to note that although IgM antibodies are strongly complement activating donor HLA specific IgM are relatively harmless. One possible explanation for this finding is relatively low affinity of IgM antibodies or importantly lack of T-cell activation. This finding needs to be validated in multi-variate analysis accounting for other baseline characteristics and for larger cohort in a multi-centre study.

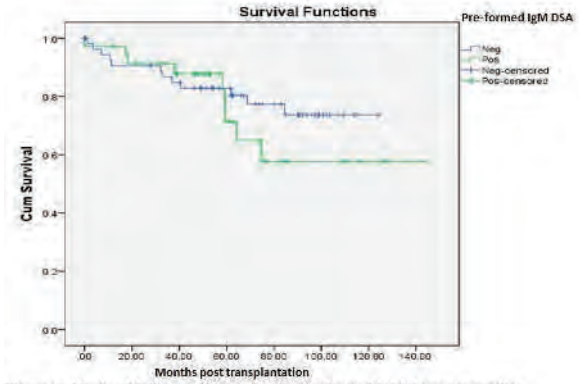


Figure 1: Kaplan Meier survival analysis: Preformed IgM does not predict allograft survival (p=0.44)

		Rejection	
		Yes	No
IgM DSA	Yes (35)	17	18
	No (57)	30	27
Total no (92)		47	45

Table 1: IgM DSA association with rejection was not statistically significant (P=0.83)

References:
[1] Worthington JE, Thomas AA, Dyer PA, Martin S. Detection of HLA specific antibodies by PRA STAT and their association with transplant outcome. Transplantation 65:12,1998.
[2] Tardif GN, McCalmon RT. Successful Renal Transplantation in the presence of Donor specific HLA IgM antibodies. Transplant Proc. 1995;27(1):664-5.
[3] Suzuki M, Ishida H, Komatsu T, Kennoki T, Ishizuka T, Tanabe K. Kidney transplantation in a recipient with anti-HLA antibody IgM positive. Transplant Immunology. 2009;21(3):150-4.
[4] Bentall, Andrew, et al. "Antibody-mediated rejection despite inhibition of terminal complement." Transplant International 27.12 (2014): 1235-1243.

P.1810

Removal of unacceptable antigens to increase the likelihood of a patient receiving a renal transplant: A cross-sectional study

Gemma Napaul[1], Claire Collins[2], David Briggs[2], Nithya Krishnan[1].
[1]Renal Unit, University Hospitals Coventry & Warwickshire NHS Trust, Coventry, United Kingdom; [2]Histocompatibility & Immunogenetics, National Health Services Blood & Transplant, Birmingham, United Kingdom.

Background: There are limited options for highly sensitised patients requiring a renal transplant with many remaining on the waiting list longer than average. The aim of this study was to determine if patients on the renal waiting list could have antigens listed as unacceptable, removed from their transplant profile to improve the chance of compatibility with a donor.
Methods: This cross-sectional study identified thirty-nine patients on our transplant list who had been waiting longer than 1096 days. Human leukocyte antigen-antibody reports generated using the Luminex® platform for twenty males and eighteen females aged 18-72 were analysed on an individual basis. A median fluorescence intensity cut-off of greater than 5000 was employed and HLA-A, -B, -C, -DR antigen-antibody reactions below this were determined as removable. An adjusted calculated reaction frequency percentage demonstrating how each patient would react to the donor organ pool was generated and compared with the previous value.
Results: Of the thirty-eight participants included in the study twenty-eight saw an improvement in their calculated reaction frequency percentage. Of these twenty-eight, eighteen saw an improvement between 16-99% with five of these being reduced to 0%. Four participants remained unchanged with a calculated reaction frequency of 100% and six remained unchanged at 0%. On average there was a 23% improvement in the calculated reaction frequency as the adjusted percentage reduced to 55% from the original 78%.
Conclusion: Increasing the median fluorescence intensity cut off led to an improvement in the calculated reaction frequency. This should hopefully result in an increased chance of a kidney offer for these patients. However, a multitude of factors are considered for kidney allocation and the increasing age of the population studied may affect their chances. If successful kidney transplants occur, these will be followed up and reported to see whether this can be utilised on a larger scale to reduce waiting list times and ultimately improve patient quality of life.

P.1811

Comparison of baseline proteinuria and outcomes and baseline comorbidities and new onset proteinuria in live kidney donors - UK cohort study

Nithya Krishnan[1], Lisa Bradbury[2], Neil Raymond[1].
[1]Renal Unit, University Hospitals Coventry & Warwickshire NHS Trust, Coventry, United Kingdom; [2]Statistics, National Health Services Blood & Transplant, Bristol, United Kingdom.

Living kidney donation has significantly improved recipient and graft survival world wide. With a move to increase these numbers further, it becomes mandatory to have a better understanding of the long term outcomes and risks of kidney donation.
Analysed the effect of baseline proteinuria and outcomes and the effect of baseline comorbidities and new onset proteinuria in live kidney donors. From January 1, 2001 until December 31, 2013 inclusive, all live kidney donors in the U.K were included in the study. No formal sample size estimate was produced for the study; all eligible patients records were used. December 31, 2014 was considered the study end, meaning that all patients had at least one year of follow-up. Datasets, based on regular returns from individual transplant centres across the UK, were obtained from NHSBT.
There were 9750 donor records available. Out of which 9500 donors were tested for proteinuria at baseline. 8858 had no evidence. 437 had trace, 182 had 1+, 18 had 2+ and 5 had 3+ proteinuria. There were no significant relationships between baseline proteinuria and 1, 5 and 10 year mortality and morbidity outcomes. Multiple logistic regression models adjusted for age and sex was used to analyse baseline comorbidities and proteinuria at 1 year. The baseline comorbidities analysed were gender, ethnicity, GFR risk groups (1. meeting the recommended levels. 2. up to 5 mls/min/m2 less than the recommended levels and 3. more than 5 mls/min/m2 less than the recommended levels), different BMI bands, BMI <18.5, 18.5 -<25, 25-<30, 30-<35, 35-<40, 40+, cardio vascular disease, kidney stones or renal mention, microscopic haematuria, hypertension. diabetes, depression, asthma, hypercholesterolemia and any morbidity.
Proteinuria was more prevalent and statistically significant in male donors (P=0.012). Ethnicity was of borderline significance, with white ethnicity having less frequent proteinuria (P=0.052). BMI bands suggested a trend towards an increase in frequency of proteinuria as BMI increased (P=0.037); Higher proportion of kidney mention patients had proteinuria at 1 year and this was statistically significant (P=0,031). Patients with any baseline morbidity were more likely to have proteinuria (P=0.009). Baseline proteinuria did not have significant association with morbidity or mortality at 1 year. However, certain baseline comorbidities were significantly associated with proteinuria at 1 year.

P.1812

Glass cliff effect in transplantation – myth or reality?

Nithya Krishnan[1], Deborah Biggerstaff[2], Neil Raymond[1], Monica Dolton[3], Hillary Braun[4], Sondra Livingston[5], John Hattersley[1], Josette Eris[6], Nancy Ascher[4], Robert Higgins[1], Kathryn Wood[3], Ala Szczepura[7].
[1]Renal Unit, University Hospitals Coventry & Warwickshire NHS Trust, Coventry, United Kingdom; [2]Psychology, University of Warwick, Coventry, United Kingdom; [3]Transplantation Research Immunology Group, University of Oxford, Oxford, United Kingdom; [4]Transplantation, University College San Francisco, San Francisco, CA, United States; [5]The Transplantation Society, Montreal, QC, Canada; [6]Transplantation, University of Sydney, Sydney, Australia; [7]Health Technology Assessment, University of Coventry, Coventry, United Kingdom.

It is well known that representation of women in many fields of medicine is much lower than men[1] and historically, gender has precluded females from becoming leaders in some fields. This 'glass-ceiling' effect is recognised to be more prevalent in male-dominated specialities with fewer women in senior positions. Transplantation is one such area. Moreover, studies in other professions show that women who reach a senior position can easily fall from a position of leadership because of a single mistake[2]. To date, there have been no studies in transplantation looking at this 'glass cliff' effect.
Aim: To investigate if there is any difference in perception regarding wrong decisions made by senior medical staff in transplant leadership roles, based on gender.
Methodology: Ethical approval was obtained from West Midlands Research Ethics Committee, U.K and Biomedical and Scientific Research Ethics Committee, University of Warwick, U.K.
The study was a prospective web-based survey involving five clinical scenarios, with either a male or female protagonist (Set1 and Set2). Each scenario was followed by two questions (i) rating of clinical performance (score from 1 to 10, where 1= unacceptable and 10 = exemplary) and (ii) action to be taken based on clinical performance (no action; informal action; written report to department; written report to national regulatory body).
To ensure elimination of any inherent biases in responses, the survey was advertised as a study exploring whether adequate actions are taken following adverse clinical incidents in transplantation. Participants were recruited by advertising through the websites of various professional bodies. Individuals were randomly assigned to either Set1 or Set2 questionnaires, with each Set receiving the same scenarios in the same order, differing only in whether a male or female protagonist was mentioned.
Results: 189 invitees responded; 55 did not complete any questions and were excluded from the main analysis, leaving 134 (64 Set1 and 70 Set2) completed questionnaires. Comparison of respondent characteristics showed no significant differences between the two randomly allocated Sets with respect to age group, gender, ethnicity, country and respondent status.
Mean clinical performance scores in each of the Scenarios 1 to 5 between Set1 versus Set2 were not statistically significant. Analysing grouped scores using chi-squared tests similarly found no significant differences between Sets. There were no significant differences between Sets with regards to actions or grouped actions.
Analysis of the Sets versus clinical performance groups and action within gender defined groups also showed no significant difference between the Sets.
Conclusions: Overall there was little evidence to suggest any differences in responses between Set1 and Set2. Thus, this study did not find any quantitative evidence of gender bias in the assessment of either clinical performance scores or recommended actions. However, since 29% of responses were incomplete, this raises the question of the degree to which this result can be generalized and whether responses are truly representative. Qualitative analysis of participants' comments will be forthcoming.
The Transplantation Society, The British Transplantation Society, National Health Service Blood & Transplant, UK, Royal College of Paediatrics and Child Health, UK, National Kidney Federation, UK and Warwick Medical School, UK
References:
[1] 1. AAMC. Distribution of residents by specialty, 1996 to 2006. http://www.aamc.org/members/wim/statistics/stats07/table02.pdf.
[2] 2. Victoria L. Brescoll, Erica Dawson and Eric Luis Uhlmann. Hard Won and Easily Lost: The Fragile Status of Leaders in Gender-Stereotype-Incongruent Occupation. Psychological Science. 21(11) 1640–1642, 2010.

P.1813

Non-HLA and HLA antibodies in lung transplantation (LTx): Results from a prospective study

Duncan Walton[1], Steven Hiho[1], Linda Cantwell[1], Mary Diviney[1], Monika Loskot[2], Bronwyn Levvey[2], Greg Snell[2], Miranda Paraskeva[2], Glen Westall[2].
[1]Victorian Transplantation and Immunogenetics Service, Australian Red Cross Blood Service, Melbourne, Australia; [2]Lung Transplant Service, Alfred Hospital, Melbourne, Australia.

Introduction: The aims of this prospective study were 1)investigate a cohort of LTx recipients for the presence and clinical associations of anti-Angiotensin II receptor antibody type 1 (AT1R), anti-Endothelin Receptor A antibody (ETAR) and anti-HLA antibodies 2)identify potential impact these antibodies may have on chronic LTx allograft dysfunction (CLAD).
Materials and Method: From Jul 2014-Aug 2015, 47 patients were enrolled at time of listing for LTx, and blood samples obtained pre-transplant and at 3 and 12 months post-LTx. Samples were HLA typed (SSO, One Lambda Inc.) then screened for antibodies by single antigen beads for HLA (One Lambda Inc.), and ELISAs for AT1R (One Lambda Inc.) and ETAR (Celltrend GmbH) by the VTIS laboratory. HLA compatibility was assessed using HLAMatchmaker (version 2.1) and de-novo donor specific antibodies (DSA) were determined using a MFI cut off>500. AT1R and ETAR antibody status was determined by kit specifications as either negative (<10U/ml), at risk (≥10 and <17) or positive (≥17). HLA and non-HLA antibodies measurements were correlated with patient demographics and clinical LTx outcomes including acute rejection & development of CLAD at 3 and 12 months.
Results and Discussion: HLA antibodies were detected pre-transplant in 19/47 patients (40%); 4 with Class I, 3 with Class II and 12 with both Class I&II, and four patients having pre-transplant DSA. 13/47 (28%) developed de novo HLA DSAs by 3 months post LTx; 1 with Class I, 8 with Class II, 4 with Class I&II. When DSA development was correlated with eplet mismatch no significance was observed at class I, however DRB1* eplet mismatches correlated to HLA Class II DSA formation ($p<0.05$).
Pre-transplant AT1R screening showed 17/47 patients (36%) were classed as "at risk" and 6 were AT1R antibody positive (13%). Of these 23 patients, at 3 months post LTx 6 were negative for AT1R antibodies and 17 recorded similar levels to pre-transplant. Of 24 patients negative for AT1R antibodies pre-transplant, 4 were "at risk" at 3 months-post LTx.
Pre-transplant ETAR screening showed that 20/47 patients (43%) were classed as "at risk" and 8 were positive (17%). Of these 28 patients, at 3 months post-LTx 8 were negative for ETAR antibodies while 20 had similar levels. There was no correlation between the presence of HLA antibodies and AT1R/ETAR antibodies, however when AT1R antibodies were detected, ETAR antibodies were usually also detected. There was no association with HLA and non-HLA antibody levels and the early measures of LTx outcomes, ICU and hospital days or acute rejection rates at 3 months post LTx.
Conclusion: HLA DRB1*eplet mismatch was associated with de-novo HLA Class II antibodies at 3 months post-transplant. The prevalence of AT1R and ETAR antibodies are in line with previous literature. This analysis will be expanded to include 12 month antibody results and eplet mismatch to ascertain if there is a relationship between CLAD and non-HLA and or HLA antibodies post LTx.

P.1825

Perspectives of the development of renal transplantation in children in Kazakhstan

Venera Altynova[1], Dinara Galiyeva[2].
[1]Kidney Centre, National Research Centre for Maternal and Child Health, Astana, Kazakhstan; [2]Usher Institute of Population Health Sciences and Informatics, The University of Edinburgh, Edinburgh, United Kingdom.

Background and Aim: In Kazakhstan the prevalence of renal replacement therapy in children with end stage renal disease (ESRD) was 6 per million of the age-related population in 2013. Kidney transplantation is the best way to reach the medical and social rehabilitation of children with ESRD. Paediatric kidney transplant program in Kazakhstan was launched in 2012 in Astana. The aim of this study is to evaluate the results of the renal transplantation in children in Kazakhstan in the period from 2012 to 2015.
Methods: We have conducted a retrospective analysis of the 29 transplanted children under the age of 18 who received kidney transplants in Astana during 2012-2015 years within paediatric kidney transplant program.
Results: Mean age of the transplanted children was 11.2 years (Standard Deviation (SD) 3.9). Girls accounted for 69% of all recipients (n=20). The main primary renal diseases which caused ESRD were congenital anomalies of the kidney and urinary tract (44%) and glomerulonephritis (39%). 21 patients (72%) received a kidney transplant of the living donor from their relatives, while 8 children (28%) received a renal transplant from a deceased donor. Median waiting time was 1 year (Interquartile Range 0.5-2.5). Majority of children (47 %) received peritoneal dialysis (PD) before getting transplantation, 28% - haemodialysis (HD), 10% - PD and HD and 15% received pre-emptive transplantation. All children underwent immunosuppressive therapy consisted of induction therapy with monoclonal antibodies (Basiliximab (Simulect)) и Anti-thymocyte Anti-thymocyte immunoglobulin (Thymoglobuline) and primary therapy with Calcineurin inhibitors (Tacrolimus or Cyclosporine), Mycophenolic acid (Cell-Cept) and glucocorticoids (Prednisolone or Methylprednisolone). Surgery complications after kidney transplantation were in 6 children (21%). Out of them 4 patients experienced chylorrhea, 1 child had the failure of the ureter (necrosis) and another child had bleeding. Mean follow-up time was 1.4 years (SD 0.9) resulting in total of 43 patient-years. Two patients died (7%). Mortality rate was 4.7 per 100 patient-years. Graft loss occurred in one child. Eight patients had graft dysfunction, the causes of which in most cases was infection (4 children). One-year patient and graft survival was 94% and 97% respectively. Three-year patient and graft survival was 95% и 85% respectively.
Conclusion: assessing the experience of the first 29 transplanted children in Astana from 2012 to 2015, we can conclude that all the efforts that have been made for the implementation of this program have been successful. However, there are number of issues that require further consideration, such as allowing nephrologists to use protocols with minimal immunosuppressive support (early cancellation of steroids), carrying out a kidney transplantation in children with incompatible ABO system, increasing the number of cadaveric transplantation and the introduction of post-mortem donation for children.

P.1826

A ten-year review on the surgical outcomes of paediatric renal transplantation: a Hong Kong experience

Alan Lo, T.Y. Chu, C.K. Chan, Y.C. Lam, K.L. Ho, Y Chiu, F.K. Cheung.
Division of Urology, Department of Surgery, Princess Margaret Hospital, Hong Kong, Hong Kong.

Introduction: To review the surgical outcomes for paediatric patients who underwent renal transplantation from 2005 to 2015 in Hong Kong.
Materials and Method: We retrospectively reviewed all paediatric patients who received renal transplantation in Hong Kong from 2005 to 2015. Patient's demographic characteristics, surgical and medical outcomes were studied.
Results: A total of 55 paediatric patients (female =28, male =27) with a mean age of 15 year-old were included. Mean follow up time was 68 months. Mean waiting time from the start of renal replacement therapy to renal transplantation was 3 years. 10 (18.2%) patients had living related renal transplant and 45 (81.8%) patients had cadaveric renal transplantation. Mean operative time was 3 hours and 16 minutes, mean cold ischemic time was 9 hours and mean second warm ischemic time was 40 minutes. Surgical complications were noted in 32(58%) patients: 9 (16.4%) had vascular complications, 18 (32.7%)had infection, 6 (10.9%) had delayed graft function, 2 (3.6%) had lymphocele requiring drainage, and no mortality was recorded. Graft failure rate was in 5 (11.1%) in the cadaveric renal transplantation group, and 0 (0%) in the living related renal transplantation group. All graft failure occurred within first year post-operation: 2 due to arterial/venous thrombosis; 2 due to acute rejection; and 1 due to recurrence of primary disease. The 1-year, 3-year and 5-year graft survival rate was 88.9% for cadaveric renal transplantation and 100% for living related renal transplantation.
Conclusion: Renal transplantation remains a safe and effective mode of renal replacement therapy for paediatric patients with end-staged renal disease.

P.1827

Predictive value of early period doppler ultrasonography on early and long-term allograft function

Engin Melek[1], Esra Baskin[1], Kaan Gulleroglu[1], Nihal Uslu[2], Gokhan Moray[3], Mehmet Haberal[3].
[1]Pediatric Nephrology, Baskent University, Ankara, Turkey; [2]Radiology, Baskent University, Ankara, Turkey; [3]General Surgery, Baskent University, Ankara, Turkey.

Introduction: Doppler ultrasonography is a useful, noninvasive diagnostic tool for the management and follow-up of the transplanted kidney. The measurement of intrarenal arterial resistance index (RI) by Doppler ultrasonography (DUSG) has been proven to reliably predict short-term allograft function. We evaluated the value of DUSG performed during the early post-transplant period to predict short and long-term renal allograft function in pediatric transplant patients.
Materials and Methods: We retrospectively analyzed clinical data, laboratory data and DUSG parameters of 70 (35 male, 35 female) renal transplant recipients. DUSG was performed at 3rd and 7th days after transplantation. A RI value <0.7 was considered as normal. Patients were grouped as normal graft function (NGF) and abnormal graft function (AGF). Abnormal graft function was defined as 30% increase of the creatinine level from the baseline value or need for dialysis.
Results: The mean age of patients was 13.6±4.0 years. The mean follow-up time of patients was 41.7±30.4 months. At 3rd day, 58.8% of patients with AGF had RI value ≥0.7, only 25% of patients with NGF had RI value ≥0.7. Also at 7th day while 74.7% of patients with AGF had RI value ≥0.7, only 26.9% of patients with NGF had RI value ≥0.7. BUN and creatinine level of patients with RI ≥0.7 were higher than that of patients with RI <0.7 at 3rd and 7th days. The RI values were correlated with the graft function at early post-transplantation period ($p<0.05$). RI values obtained by DUSG at 3rd and 7th days were not correlated with allograft function at 1 year and at last visit. The graft function at early post-transplantation period was correlated with creatinine level at 1st year and with glomerular filtration rate at 1st year and last visit. The patients with AGF at early period after transplantation has higher creatinine level at first year and lower GFR at both 1st year and last visit than that of patients with NGF.
Conclusion: We demonstrated that RI is correlated with graft function at early period after transplantation. In addition, the graft function at early post-transplantation period has predictive value for long-term graft function. Patients with higher RI values at the early period after transplantation should be followed carefully for the development of chronic allograft dysfunction.

P.1828

Effects of renal transplant age on the graft functions in pediatric transplant patients

Begum Avci[1], Esra Baskin[1], Kaan Gulleroglu[1], Mahir Kirnap[2], Gokhan Moray[2], Mehmet Haberal[2].
[1]Pediatric Nephrology, Baskent University, Ankara, Turkey; [2]General Surgery, Baskent University, Ankara, Turkey.

Introduction: Renal transplant is the best renal replacement therapy choice for children. It provides a long-term survival. Graft outcome can be affected by many factors. Transplant age is one of these factors. In this study, we aimed to evaluate the relationship between graft loss and age at renal transplant.
Materials and Methods: We retrospectively evaluated the data files from 141 pediatric renal transplant patients (74 boys, 67 girls). Patients were divided into 2 groups according to the age of renal transplant. Patients younger than 12 years-old are considered as children group (47 patients) and older than 12 years-old are considered as adolescent group (94 patients). Demographics of the patients, etiology of chronic renal failure, donor type, acute rejection episodes and graft loss were recorded.
Results: 110 patients received a living-related donor allograft and the remaining 31 patients received the allograft from a deceased donor. Gender (22 boys/25 girls for children group and 52 boys/42 girls for adolescent group) and mean follow-up time (63.70±44.88 months for children group, 64.29±45.23 months for adolescent group, p=0.94) of two groups were similar. There was any significant difference for donor type and acute rejection episodes between two groups. 17 patients (12.1%) were lost their graft during follow-up. 15 (16%) of these patients were in adolescent group and the remaining 2 (4.3%) patients in children group. Graft loss was significantly higher in adolescent group ($p<0.05$).
Conclusion: We demonstrated that adolescents has poorer graft outcome. It may be related to different risk factors such as primary disease, immunosuppressive non-adherence. These risk factors must be evaluated for each patient, especially at adolescence ages.

P.1829

Waiting time of children in list for renal transplantation: one center experience

Kaan Gulleroglu[1], Esra Baskin[1], Asli Kantar[1], Begum Avci[1], Gokhan Moray[2], Mehmet Haberal[2].
[1]Pediatric Nephrology, Baskent University, Ankara, Turkey; [2]General Surgery, Baskent University, Ankara, Turkey.

Introduction: Renal transplantation is accepted the best treatment choice of end stage renal failure in children. Successful renal transplantation normalizes metabolic and endocrine abnormalities and it allows a most near-normal life. The goal is to apply transplant within 6 months for children 0-5 years old, within 12 months for children 6-10 years old and within 18 months for children 11-17 years old. General In this study we evaluated time of waiting of our patients for renal transplant.
Methods: We retrospectively evaluated the data files from 96 pediatric renal transplant patients over the last past 5 years. Demographics of the patients, cause of chronic renal failure, time of waiting for transplant, donor type were recorded.
Results: The mean age of patients was 9.66±5.75 years. The mean time of waiting was 28.86±24.28 months. 70 patients received a living-related donor allograft and the remaining 26 patients received the allograft from a deceased donor. The mean time of waiting is significantly long for patients who received the allograft from a deceased donor when compared with patient who received a living-related donor allograft (38.16±22.97 vs. 25.23±23.98, p=0.02). 12 patients were included to the waiting list before 5 years old, 35 patients were included between 6 to 10 years old and 49 patients were included after 10 years old. The mean time of waiting was 17.70±11.12 months for patients between 0-5 years old. The mean waiting time was 2 times longer for patients between 5-10 years old (37.35±29.37 months). The mean time of waiting was 25.70±21.11 months older than 10 years old. 9 patients received a living-related donor allograft and the remaining 3 patients received the allograft from a deceased donor for patients who were included to the waiting list before 5 years old. 27 patients received a living-related donor allograft and the remaining 9 patients received the allograft from a deceased donor for patients who were included to the waiting list between 5 to 10 years old. 35 patients received a living-related donor allograft and the remaining 14 patients received the allograft from a deceased donor for patients who were included to the waiting list after 10 years old. Although it is not statistically significant, waiting time is longer for patients who received the allograft from a deceased donor when compared with patients who received a living-related donor allograft for each age group (26.33±12.74 months vs. 14.00±8.85 months, for 0-5 years old; 50.37±33.01 months vs. 32.82±27.32 months for 5-10 years old; 33.71±14.73 months vs. 22.41±22.60 months for >10 years old).
Conclusion: Short waiting time provide minimum complication related dialysis and end stage renal failure. Although living-related organ donation provides patients with an opportunity to apply an early renal transplant, we are so far of the goal of the waiting time for children. Further efforts are needed for reduce the waiting time for renal transplant.

P.1830

Mortality on first linting for kidney transplant in pediatric patients in Argentina: Period 1998-2015

Liliana Bisigniano[1], Viviana Tagliafichi[1], Daniela Hansen Krogh[2], Marta Monteverde[4], Maria del Carmen Bacque[3].
[1]Scientific and Technical Direction, INCUCAI, Buenos Aires, Argentina; [2]Informatic Direction, INCUCAI, Buenos Aires, Argentina; [3]Presidence, INCUCAI, Buenos Aires, Argentina; [4]Pediatric Nephrology, Hospital de Pediatraia J. P. Garrahan, Buenos Aires, Argentina.

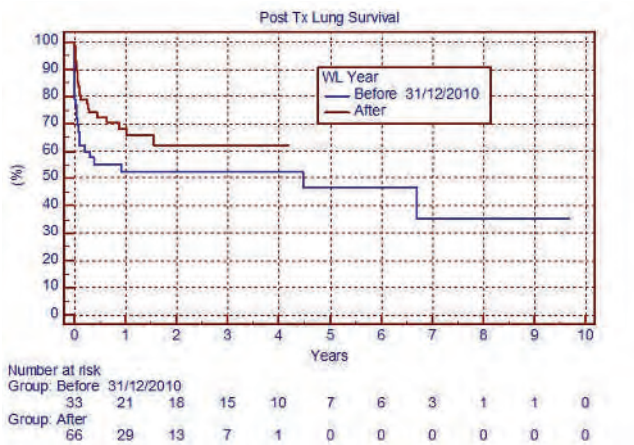
Objective: to describe the characteristics, access to transplant and mortality in patients under the age of 18 on first listing for kidney transplant in Argentina in the last 17 years.
Materials and Methods: 1760 patients listed between January 1, 1998 and December 10, 2015 were assessed. The information was obtained from SINTRA. Results are reported as a percentage of categorical variables and the median value of numerical variables. Confidence intervals (CI) were estimated for 95%. A multivariate model was designed, proportional Cox Analysis, to analyze the effect of age, gender, time on dialysis at the time of listing and etiology for listing on mortality. Mortality was estimated as incidence density.
Results: 54% of the 1760 patients listed were male. The mean age was 12.8 (CI95% 12.6-13.1). The median time on dialysis at the time of listing was 1.1 (CI95% 1.04-1.2). The etiologies on listing were: glomerulonephritis 23%, obstructive nephropathy 19%, unknown 12%, hemolytic uremic syndrome 8%, polycystic kidney disease 3% and other 35%. Eighty two percent of the patients listed underwent transplantation (74% from a cadaveric donor), 7% is still on waiting list, 6% were withdrawn from the list for other reasons, and 4% died on waiting list. Etiologies such as glomerulonephritis and other exhibited a 5% cumulative mortality rate. The incidence density of death on waiting list was 3.1% per year. Mean age of the patients who died on waiting list was 16, (CI 95% 13.28-18.00). Mean years on waiting list at the time of death was 1.97 (CI 95% 1.23-2.63) and at the time of transplant 0.44 (CI95% 0.40-0.48). Patient´s age was statistically significant to account for mortality in children over and under 12. Patients under the age of 12 had a HR of 2.31 (1.37 and 3.92), even when adjusting for etiology and gender.
Conclusions: Patient´s age adjusted for etiology and gender was statistically significant to explain mortality; patients under the age of 12 had a higher risk. The cumulative mortality rate was 4% in the study period. Although it was not statistically significant, etiologies such as glomerulonephritis and other exhibited a higher cumulative mortality than the rest of the etiologies.

P.1831

Lung transplantation in Argentina: Results after changes in the clinical emergency setting

Liliana Bisigniano[1], Viviana Tagliafichi[1], Alejandro Bertolotti[3], Daniela Hansen Krogh[2], Maria del Carmen Bacque[1].
[1]Scientific and Technical Direction, INCUCAI, Buenos Aires, Argentina; [2]Informatic Direction, INCUCAI, Buenos Aires, Argentina; [3]Lung Advisory Comitte, Sociedad Argentina de Trasplante, Buenos Aires, Argentina.

Objective: to analyze differences in mortality in terms of the waiting list and survival rate after transplant in patients listed for lung transplant with clinical emergency criteria before and after the modification of the definition of this clinical setting.
Materials and Methods: 251 patients were listed between January 1, 1995 and December 31, 2014, 117 of these patients underwent lung transplant. The legal framework modifying the definition of the emergency setting was implemented as of January 1st, 2011, so two time periods were studied: 01/01/1995 to 31/12/2010 and 01/01/2011 to 31/12/2014. A global population study was conducted for each period according to both donor and recipients´ age and gender, etiology, mortality on waiting list, post-transplant survival and percentage of patients listed undergoing transplant (transplantability).
A Kaplan-Meir curve was used to estimate the survival rate. The median times on waiting list were compared using the Mann Whitney test (independent samples) and the percentages were analyzed using the c2 test.
Results: 122 of the 251 patients were listed before 31/12/2010 (period 1) and 129 after that date (period 2). One hundred and twenty one patients (48%) died on waiting list during period 1, 61% (75/122) and 36% (46/129) in the second period (p=0.7775). The median age of both donor and recipient were 39.3 (CI 95% 35.9-51.2) and 28.2 (CI 95% 22.7-36.6) respectively in period 1, and 35.6 (CI 95% 29.0-43.3) and 24.9 (CI 95% 20.5-30.8) both p=n/s; 63/66% of the donors and 57/58% of the recipients were males, in the respective periods.
Pulmonary fibrosis accounted for 37% in both periods; and cystic fibrosis increased from 24% to 35%, p=0.1051. The median time in days on waiting list to transplant was 148 days, and decreased from 466 in period 1 to 111 in period 2 (p=0.0002).
Transplantability in period 1 was 35%, and 57% in period 2. Post transplant mortality in both periods decreased from 51% in period 1 to 30% in period 2 (p=0.1010).
Conclusions: For patients in the clinical emergency setting, the mortality rate on waiting list and in the post transplant period was lower in the second period; although the differences between both variables were not statistically significant. Increased transplantability and shorter time on waiting list were observed in the second period. A larger number of patients should be listed before drawing final conclusions.



P.1832

Postoperative mortality in infants following liver transplantation; impact of large for size graft and preoperative portal vein size

Chan Woo Cho, Sanghoon Lee, Kyeong Sik Kim, Jonghwan Lee, Seounghyun Kim, Ji Soo Lee, Jong-Man Kim, Gyu-Seong Choi, Choon Hyuck D Kwon, Jae-Won Joh, Suk-Koo Lee .
Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea.

Background: Liver transplantation (LT) is an effective treatment but challenging procedure for infants (defined as children< 12 months of age at the time of LT) with end-stage live disease. Moreover, risk factors of postoperative mortality in infants after LT has not been well established yet.
Methods: We performed a retrospective study of 101 infants who were performed LT from May 1997 to March 2015 at a single institute. We assigned patients to the dead infants (n=9) or alive infants (n=92) according to the presence of mortality within 30 days after LT. We compared baseline characteristics, operative factors, and donors' factor between two groups, and assessed the risk factors for postoperative mortality in infants following LT.
Results: Patients' characteristics such as height, preoperative total bilirubin, creatinine clearance (CrCl), primary diagnosis, and graft to recipient weight ratio(GRWR) were significantly different between the two groups. In binary logistic regression model for multivariate analysis, independent factors associated with postoperative mortality in infants after LT were primary diagnosis of genetic disorder with co-morbidity (P = 0.029; HR, 26.304; 95% CI, 1.392-496.959), and higher GRWR (≥4) (P = 0.018, HR:19.872; 95% CI, 1.669-236.674). Out of 9 dead infants, 6 cases were associated with graft failure or primary nonfunction (PNF).
Conclusions: Postoperative mortality of infants following LT has a significant correlation with not only preoperative condition of recipients but also graft-size mismatching. In terms of GRWR, large for size grafts to infant recipients maybe associated with graft failure or PNF in the postoperative state.

Univariate and multivariate analysis of factors predicting postoperative mortality in infants following LT

Variable	Category (if)	No.	Univariate			Multivariate		
			OR	95%CI	P value	OR	95%CI	P value
Age	<6mo/≥6mo	19/88	3.333	0.734-15.135	0.129	9.177	0.113-742.887	0.325
Height	≤65cm/>65cm	82/65	22.667	2.668-190.795	0.000*	1.711	0.021-138.281	0.811
Weight	≤8.5/>8.5	29/78	3.074	0.792-12.989	0.206	-	N/A	-
LBW	Yes/No	11/90	5.250	1.069-25.838	0.057*	0.092	0.000-23.884	0.400
CrCl	<97/≥97	30/71	10.900	2.889-54.172	0.000*	17.385	0.189-1600.582	0.216
Total bilirubin	>20/<20	44/57	5.203	1.024-26.442	0.039*	9.936	0.855-106.351	0.067
INR	≥1.5/<1.5	38/63	1.265	0.343-5.181	0.726	-	N/A	-
PELD	>22/<22	24/77	1.689	0.388-7.349	0.440	-	N/A	-
Mechanical ventilation	Yes/No	4/97	12.857	1.309-155.567	0.029*	22.629	0.523-978.646	0.105
Preoperative ICU location	Yes/No	19/82	2.375	0.557-10.507	0.364	-	N/A	-
Diagnosis	Genetic disorder/others	5/96	22.550	5.153-181.673	0.000*	26.804	1.392-496.959	0.029*
Donor BMI	>25/<25	23/78	3.139	1.252-21.098	0.027*	1.388	0.130-36.844	0.587
LT type	DDLT/LDLT	13/88	2.104	0.387-11.438	0.328	-	N/A	-
GRWR	≥4/<4	34/67	8.426	1.844-43.180	0.006*	19.872	1.669-236.674	0.018*
WIT	>25/<25	27/87	4.846	1.078-21.972	0.041	8.288	0.859-94.788	0.054
CIT	>48/<48	21/73	4.804	0.966-40.780	0.148*	5.711	0.312	0.385
PV diameter	>3.8/≤3.8	34/67	1.655	0.414-6.606	0.460	-	N/A	-
Era	97-06/07-15	51/50	0.766	0.198-3.025	0.741	-	N/A	-

Table1. baseline characteristics at transplantation

characteristics	Post operative expired inf ant (N=9)	Post operative Alive inf ant (N=92)	Total (N=101)	P value
Age at transplantation (mo)	7.2±3.5	8.5 ±2.3	8.3±2.4	0.000
0-3	1 (11.1%)	0 (0%)	1 (1%)	
3-6	2 (22.2%)	12 (13%)	14 (13.9%)	
6-9	2 (22.2%)	39 (42.3%)	41 (40.6%)	
9-12	3 (33.3%)	43 (46.7%)	46 (45.5%)	
Sex (male: female)	5:4 (55.6:44.4%)	56:36 (60.9:39.1%)	61:40 (60.4:39.6%)	0.480
Height (cm)	63.5	69.12	68.12	0.001
Weight (kg)	6.4±1.8	8.3±1.8	8.1±1.8	0.000
Body mass index (kg/m2)	16.4±2.6	16.9±1.5	16.8±1.6	0.322
Albumin (g/dL)	3.4±0.6	3.1±0.6	3.1±0.6	0.331
Total bilirubin (mg/dL)	20.7±7.8	18.4±10.0	18.6±9.9	0.046
Creatinine (mg/dL)	0.42±0.17	0.28±0.16	0.29±0.16	0.007
INR (ratio)	2.02±0.02	1.39±0.09	1.48±0.21	0.722
PELD score	26±18	18±10	19±11	0.260
Creatinine clearance (mL/min/1.73m2)	87±44	129±55	125±55	0.021*
Patient status at LT				
ICU/hospitalized home	9:0 (100.0:0.0%)	14:78 (15.2:84.8%)	14:78 (14.6:85.4%)	0.001
Mechanical ventilator (yes:no)	2:7 (22.2:77.8%)	2:90 (2.2:97.8%)	4:97 (4:96%)	0.077
Ascites (no control:refractory:controlled)	1:8:0 (11.1:88.9:0.0%)	6:42:44 (6.7:46.1:47.2%)	7:44:44 (6.9:43.5:49.5%)	0.472
Hepatic encephalopathy (no:G1-2:G3-4)	0:7:2 (0.0:77.8:22.2%)	58:31:3 (63.0:33.7:3.3%)	58:38:5 (57.4:37.6:5.0%)	0.000
Variceal bleeding (yes:no)	0:9 (0.0:100.0%)	7:85 (7.6:92.4%)	7:94 (6.9:93.1%)	1.000
Dialysis before LT (yes:no)	0:9 (0.0:100.0%)	7:85 (7.6:92.4%)	7:94 (6.9:93.1%)	1.000
SBP (yes:no)	0:9 (0.0:100.0%)	3:91 (3.2:96.8%)	3:98 (2.9:97.1%)	1.000

P.1833

Experience with two cases of living donor liver transplantation for neonatal hemochromatosis developed in siblings

Takumi Katano[1], Yukihiro Sanada[1], Yuta Hirata[1], Noriki Okada[1], Naoya Yamada[1], Yoshiyuki Ihara[1], Taizen Urahashi[1], Shinya Otomo[2], Kentaro Ushijima[3], Koichi Mizuta[1].
[1]Department of Transplant Surgery, Jichi Medical University, TOCHIGI, Japan; [2]Department of Pharmacy, Jichi Medical University Hospital, TOCHIGI, Japan; [3]Department of Clinical Pharmacology, Jichi Medical University, TOCHIGI, Japan.

Background: Neonatal Hemochromatosis (NH) is a disease presenting with fulminant hepatic failure in the neonatal period. While Whitington et al. have reported that the incidence of developing NH in siblings is more than 90%, only a few cases have been reported in siblings in Japan. Herein, we report on our experience with two cases of living donor liver transplantation (LDLT) for NH developed in siblings.
Case 1: The patient was born in a normal delivery at 38 weeks and 5 days of pregnancy (birth weight: 2634 g). Fulminant hepatic failure occurred on Day 1 after birth and an exchange transfusion was carried out, followed by an emergency LDLT carried out, with the father as a donor, on Day 13 after birth (weight at the time of LDLT: 2607 g). The isolated liver was 38 g, whereas the liver graft was 98 g (GV/SLV 73.1%, GRWR 3.8%, reduced S2 monosegment graft). The isolated liver was histopathologically diagnosed as NH. Following LDLT, radiological intervention was carried out twice due to hepatic arterial hypoperfusion, in addition to which, steroid pulse therapy was carried out to deal with acute cellular rejection. On Day 53 following LDLT, the patient was doing well and discharged from hospital. Seven years after LDLT have passed, and the patient is alive without any neurological sequela.
At the time of discharge of Case 1, the parents were informed of the possibility of the next child developing NH; however, their awareness was insufficient and the mother become pregnant with her next child. After delivery, it was revealed that this next child had developed NH, and they visited our hospital to consult us about liver transplantation.
Case 2: The patient was born at 32 weeks and 3 days of pregnancy through an emergency Caesarean section due to fetal distress (birth weight: 1594 g). Exchange transfusion (33 times) and high-dose γ-globulin therapy were administered for the fulminant hepatic failure developed on Day 10 after birth, and on Day 55 after birth, a LDLT with the mother as a donor was carried out (weight at the time of surgery: 2946 g). The isolated liver was 77.5 g, whereas the liver graft was 107 g (GV/SLV 83.5%, GRWR 3.6%, S2 monosegment graft). The isolated liver was histopathologically diagnosed as NH. Following the LDLT, although the patient suffered from abdominal compartment syndrome, catheter infection, and renal dysfunction, on Day 215 after LDLT, the patient was discharged from hospital. Four years after LDLT have passed, and the patient is alive without any neurological sequela.
Conclusion: We experienced two cases of LDLT for NH developed in siblings. While NH is suspected as the main cause of neonatal fulminant hepatic failure, since there is the possibility of NH developing in siblings, a differential diagnosis is important. Regarding the treatment for NH, the efficacy of high-dose γ-globulin therapy given to the mother as a therapy for the fetus has been reported; informed consent prior to delivery, and the preparation for delivery are of the utmost importance.

P.1834

Coordinating living donor liver transplantation for neonatal fulminant hepatic failure

Erika Onuma[1], Yukihiro Sanada[2], Sachiyo Yoshida[1], Taizen Urahashi[2], Yoshiyuki Ihara[2], Noriki Okada[2], Naoya Yamada[2], Yuta Hirata[2], Shinya Otomo[3], Kentaro Ushijima[4], Koichi Mizuta[2].
[1]Center for Department of Transplantation and Regeneration, Jichi Medical University Hospital, Simotuke, Japan; [2]Department of Transplant Surgery, Jichi Medical University, Simotuke, Japan; [3]Department of Pharmacy, Jichi Medical University Hospital, Simotuke, Japan; [4]Derpartment of Clinical Pharmacology, Jichi Medical University, Simotuke, Japan.

Purpose: There are two medical aspects regarding living donor liver transplantation (LDLT) for neonatal fulminant hepatic failure: neonatal and transplant medical care. Specifically, in the case of a first child, a diseased child places a great burden on parents with no child-rearing experience and the support system of the family is difficult to visualize. We evaluated the specificity and issues of neonatal LDLT based on experiences at our institution.
Materials and Methods: The study subjects included eight cases of neonatal fulminant hepatic failure that underwent LDLT at our institution during the period from October 2008 to September 2015. The original diseases were neonatal hemochromatosis in six patients, unknown etiology in one, and Niemann-Pick Type C in one.
Information on the family structure, social background, and psychological changes of the family member during the perioperative period was retrospectively collected from the medical records, after which the specificity and issues of neonatal LDLT were analyzed.
Results: The median period of hospitalization was 98 days (40-644 days), indicating a longer hospitalization period compared with pediatric LDLT in infants or older children. The donor was the father in seven cases and the mother in two, with the mother being the donor for cases in which the disease developed in siblings and in which the patient required re-transplantation. In two cases, the mother returned to the home of her parents to give birth, while in the other five cases, the mother was living together with her parents or living within the same prefecture. The patient was the first child three cases. Among the first child cases, overnight experience before a discharge for the purpose of child-rearing training and developing affection were required,and family support issues at the time of discharge were become clear. Regarding the non-first child cases, there was less anxiety regarding child-rearing as a system of family support was assured involving taking care of the siblings during hospitalization.
Conclusion: Regarding the families of patients undergoing neonatal LDLT, it is necessary to closely exchange information with the family from prior to the transplantation in addition to recognizing their mental and social support system; specifically, if the patient is the first child, intervention and support at an early stage is essential.

P.1835

Liver transplantation in a pediatric patient after treatment for yolk sac tumor

Taiichi Wakiya, Yoshikazu Toyoki, Keinosuke Ishido, Daisuke Kudo, Norihisa Kimura, Shinji Tsutsumi, Tadashi Odagiri, Akiko Suto, Chiaki Uchida, Kenichi Hakamada.
Department of Gastroenterological Surgery, Hirosaki University Graduate School of Medicine, Hirosaki, Japan.

Liver transplantation (LT) in patients with an extrahepatic malignant tumor is considered to be a contraindication. On the other hand, there is no established guideline regarding the indication and timing of LT for patients with a preexisting extrahepatic malignancy. Particularly in children, there have been few reports of LT for patients with preexisting extrahepatic malignancy. We herein report a pediatric case that underwent living donor LT (LDLT) after therapy for yolk sac tumor (YST), yielding a favorable outcome.
The patient, a 13-year-old female with biliary atresia, had undergone portoenterostomy at 2 months of age. She developed a left ovarian tumor with a high serum alpha-fetoprotein (AFP) concentration (101950 ng/mL, normal <7.0 ng/mL) at 10 years of age. She underwent left oophorectomy and was diagnosed with ovarian YST. After surgery, the patient received chemotherapy, and as a result her liver dysfunction progressed further. Thereafter, hepatopulmonary syndrome progressed gradually. She demonstrated an increasing intrapulmonary right-to-left shunt ratio by 99mTc-MAA pulmonary perfusion scintigraphy and there was concern regarding the progression to portopulmonary hypertension. She was examined carefully and exhibited no findings to suggest the recurrence of YST according to the laboratory tests (AFP concentration: 2 ng/mL) and imaging study. We decided to perform LDLT at 3 years and 6 months of age after the surgery for YST. She was discharged from the hospital without oxygenation therapy at 73 days after transplantation. Postoperative surveillance included the measurement of the AFP level every month and CT every 1-2 years. The patient is currently alive and doing well without recurrence of YST at 17 months after transplantation.
There is no significant difference between the recurrence rate of preexisting extrahepatic malignancy and the incidence of de novo malignancy if specific cases are selected. The indications and period from surgery for preexisting extrahepatic malignancy to LT should be determined according to the type and stage of cancer. A greater accumulation of cases is necessary to establish concrete guidelines for proper management after LT.

P.1836

Acquired diaphragmatic hernia following pediatrics living donor liver transplantation

Kai Wang, Wei Gao, Xingchu Meng, Nan Ma, Chao Sun, Chong Dong.
Transplant Surgery, Tianjin First Center Hospital, Tianjin, People's Republic of China.

Introduction: Diaphragmatic hernia (DH) is one of rare complications in pediatric liver transplantation (LT), with multiple factors in the pathophysiology. DH is usually emergent and be rescued by surgical treatment. DH in pediatrics following living donor liver transplantation (LDLT) has been reported with increased frequency in recent years, though the operational technics improving. We here report successful diagnosis and treatment of two pediatric cases with DH secondary to LDLT.
Materials and Methods: The primary disease was biliary atresia in two pediatric patients and both of the patients underwent LDLT using a left lateral graft. The first case was a 9-month-old male child who received LDLT from his father at 6-month-old. The second case was a 6-month-old female child who received LDLT from her mother at 5-month-old. The LDLT procedure and the postoperative course were uneventful.
Results and Discussion: The graft weight of first case was 170 g with graft to recipient body weight ratio (GRWR) of 2.7% and the second one was 170 g with GRWR of 2.5%. In clinical, the first case was manifested with urgent respiratory distress and long-time dyspepsia. Otherwise, the second one was manifested with intestinal obstruction for several days before diagnosis. Then DH was diagnosed by CT scan on 3 months and 1 month post-LDLT, respectively. The first case was diagnosed by right DH and the second one was diagnosed by left DH. The first case underwent prompt surgery to repair the DH. However, the second case was received surgical intervention of DH 13 month later because of light digestive symptom. The operations were performed successfully without any complications. increase of the intraabdominal pressure can result in diaphragm rupture. The diagnosis in pediatrics post-LDLT is difficult because of non-specific symptoms and is to be overlooked. It has been suggested that the use of a left lobe liver graft, malnutrition, thermal or mechanical injuries to the bare area, elevated intra-abdominal pressures, or mTor inhibitors may predispose to development of DH in infants.
Conclusion: DH post-LDLT is unusual but should be recognized as a possible complication when a left lateral segment graft is used. A high index of suspicion and prompt surgery could minimize complications. The unexplained respiratory or gastrointestinal symptoms after LDLT in pediatric patients, DH should be high in the differential diagnosis.

P.1837

Back to school and normality after lung transplantation in childhood and the need for psycho-social interventions

Andrea Caby[1], Christian Benden[2].
[1]Faculty of Health, Medical School Hamburg, Hamburg, Germany; [2]Department of Lung Diseases, University Hospital Zurich, Zurich, Switzerland.

Adolescence is a time of change, as teenagers move away from parental influence towards an increased independence testing their decision-making capabilities, even more so difficult in the setting of a teenager previously undergoing lung transplantation.
BM is a now 15-year-old male with a migration background diagnosed with cystic fibrosis (CF) in early childhood. Unfortunately, he developed end-stage CF lung disease with poor quality of life. Following a thorough transplant assessment including a psycho-social evaluation of patient and supporting family, he was placed on a transplant waiting list. At the age of twelve, lung transplantation was successfully performed.
Initially, BM was active in his care, with an excellent understanding of his medical requirements including an insulin-dependent diabetes mellitus. During the first post-transplant year, BM was kept out of school due to a higher infection risk taking triple immuno-suppressive therapy. Clinically, BM has been able to enjoy his improved health with a normal exercise tolerance and no evidence of graft dysfunction.
Two years post-transplant, BM wanted to move to a regular school. Suddenly, fluctuating levels of immunosuppression were detected on regular clinic visits, the development of de-novo donor-specific antibodies and poor diabetes control. Drug changes were inadequately recorded and home spirometry done infrequently.
A primary focus of our time with BM has been maintaining a fine balance between his medical needs to maintain a good long-term survival post-transplant and the re-integration into regular school and normal life. Input from our psychologist and social worker was required, and school visits carried out. Since ressource and solution focussed methods applied in multidisciplinary teams may support patients coping with chronic illness and may introduce new perspectives concerning the bio-psycho-social complexity of a case, a further need for psycho-social interventions could be stated.
With the help of adequate psycho-social interventions, we enabled the boy to reduce his overall stress, to minimize symptoms, and to strengthen his coping skills, maintaining adherence to treatment, and thus improve quality of life. Some intervention methods in particular were used in a psycho-educative manner in order to improve interaction and access to adequate information or to decrease distress and disability, from coping strategies to better communication and understanding within the family.
As health care professionals, we aimed to ensure that the adolescent fully understands the impact of his decisions; it would be the aim of further research to investigate the impact of such interventions in pediatric solid organ transplant patients.

P.1850

Effect of different glutathione-related compounds on survival of hypoxic human islets

Heide Brandhorst, Samuel Acreman, Niamh Mullooly, Daniel Brandhorst, Paul R.V.Johnson.
Nuffield Department of Surgical Sciences, University of Oxford, Oxford, United Kingdom.

Introduction: Hypoxia remains one of the major influences on islet isolation outcomes and subsequent islet survival. In addition, the increasing interest in islet macroencapsulation devices has shown that intra-capsular hypoxia is the main obstacle preventing the translation of this technology from bench to bedside.
While mitochondria-generated radical oxygen species (ROS) are the main contributors to hypoxia-induced islet cell death, glutathione is an important component of the islet anti-oxidative defence mechanisms. The present study assessed the effect of the potential glutathione precursors N-acetyl-cysteine (NAC), taurine (TAU) and L-glutamine (GLN) on survival of hypoxic human islets.
Materials and Methods: Isolated human islets were incubated in 1-80 mM NAC, 1.25-40 mM TAU and 1-8 mM GLN and cultured for 2-3 days at 1.5% O_2 prior to islet quality assessment (n=6). After defining the most efficient concentration of compounds, islets were assessed for the intracellular content of reduced glutathione (GSH) and glucose-stimulated insulin release in a separate set of experiments performed at 0.75% O_2 (n=6). All data were normalised to untreated hypoxic controls and expressed as mean±SEM.
Results: Compared with hypoxic controls (100%) 10 mM TAU was most efficient to reduce islet fragmentation (70±6.9%,P<0.05) and to increase islet recovery after hypoxia (168±25%, P<0.01). Four mM GLN was similarly effective to prevent islet fragmentation (59±6.2%, P<0.001) and to enhance post-culture islet recovery (185±23, P<0.001). The most effective concentration of 5 mM NAC reduced fragmentation (71±5.6, P<0.001) but did not improve post-culture islet recovery (135±5.6%, NS). Although 5 mM NAC was effective to reduce ROS generation (54±2.9%, P<0.05), normalized viability was not significantly increased (106±1.8%, NS). In contrast, islet ROS generation was reduced when 10 mM TAU (60±5.6%, P<0.05) or 4 mM GLN (51±6.4%, P<0.01) was used for treatment and correlating inversely with normalized viability (TAU: 114±3.5%, P<0.05; GLN: 120±3.0%, P<0.05). Because of the relatively low overall survival of NAC-treated islets (143±5.7%, NS vs. controls) considering both recovery as well as viability, NAC was excluded from further studies. Measurement of the intracellular GSH content revealed that the anti-oxidative effect of TAU was stronger (183±17%, P<0.01) compared to GLN (160±18%, NS) and controls. This correlated with increased islet overall survival of TAU-treated islets (258±26.3%) at 0.75% O_2 in comparison with GLN (220±23.6%, P<0.001). In addition, islet insulin secretory response to glucose was best preserved after severe hypoxia when islets were treated with 10 mM TAU.
Conclusion: These data demonstrate that GLN and TAU can improve survival of hypoxic islets. The protective characteristics of GLN and TAU may be considered for nutrition of islets during isolation, culture and after transplantation in a macrodevice.
This work was performed as a part of a collaborative project to develop a bioartificial pancreas to treat type 1 diabetes (BIOSID). BIOSID is supported at the level of 5,469,603 € through the Cooperation Program of the European Community's FP7 (HEALTH-F2-2012-305746)

P.1851

Mesenchymal stem cell-preconditioned medium increases survival of hypoxic human islets

Heide Brandhorst[1], Samuel Acreman[1], Niamh Mullooly[1], Simen W.Schive[2], Hanne Bjørnson Scholz[2], Daniel Brandhorst[1], Paul R.V.Johnson[1].
[1]Nuffield Department of Surgical Sciences, University of Oxford, Oxford, United Kingdom; [2]Department of Transplantation Medicine and Institute for Surgical Research, Oslo University Hospital, Oslo, Norway.

Introduction: Mesenchymal stem cells (MSC) produce a number of growth factors that seem to be protective for islets when co-transplanted in a hypoxic environment. This effect appears to particularly pronounced in MSC isolated from adipose tissue. However, the potential risk of neoplasia and teratoma formation is increased when MSC are transplanted into immuno-suppressed patients. The aim of this initial study was therefore to investigate whether the production of growth factors from MSC can be stimulated by different culture conditions and utilized to benefit human islets suffering from hypoxia. The effect of potentially protective growth factors was assessed using MSC-preconditioned medium.
Materials and Methods: MSC were isolated from surgically resected human adipose tissue and expanded using MEMα supplemented with 10% FCS and glutamax. Each batch of culture medium was preconditioned with MSC cultured for 2 days in normoxia (21% oxygen) or hypoxia (1%). Unused supplemented medium served as control. After harvesting of MSC, cell-depleted media were frozen at -20ºC until use.
Eight human pancreases retrieved with appropriate consent and ethical approval were processed using standard techniques as previously described. Prior to quality assessment isolated islets were cultured for 48-72 hours in hypoxia (2% oxygen) in either unused or MEMα preconditioned at 21% or 1% oxygen. Data were normalized to control islets cultured in hypoxia in non-conditioned medium and are presented as mean ± SEM.
Results: Compared with controls, recovery increased to 117±12% (P=0.078) and 138±12% (P<0.05) when islets were cultured in medium preconditioned at 21 or 1% oxygen, respectively. Viability assessed by FDA-PI did not change after culture in MSC-medium either unused (59±2%), preconditioned at 21% (59±3%) or 1% (61±3%) oxygen. In contrast, compared to controls (19±7 µU/ng DNA/45 min) glucose-stimulated insulin release was significantly increased when islets were cultured in MSC-medium preconditioned at 21% (49±30 µU/ng DNA/45 min, P<0.01) or 1% (27±9 µU/ng DNA/45 min, P<0.05). Calculation of islet overall survival, considering both recovery and viability, suggests that MSC-medium preconditioned at 1% oxygen is more protective (143±14%) than MSC-medium preconditioned at 21% oxygen (119±14%) or non-conditioned MEMα (P<0.05).
Conclusion: This study demonstrates that culture medium preconditioned by MSC from adipose tissue increases survival and in vitro function of human islets maintained in hypoxic conditions. These findings indicate that hypoxic MSC seem to produce factors that improve survival of islets suffering from a lack of oxygen. An assessment of gene expression, focussing on genes related to apoptosis and hypoxia, is currently ongoing.
This work was performed as a part of a collaborative project to develop a bioartificial pancreas to treat type 1 diabetes (BIOSID). BIOSID is supported at the level of 5,469,603 € through the Cooperation Program of the European Community's FP7 (HEALTH-F2-2012-305746)

P.1852

Different effects of neutral proteases on human islet viability

Heide Brandhorst[1,2], Paul R.V.Johnson[1], Olle Korsgren[2], Daniel Brandhorst[1,2].
[1]Nuffield Department of Surgical Sciences, University of Oxford, Oxford, United Kingdom; [2]Department of Immunology, Genetics and Pathology, Uppsala University Hospital, Uppsala, Sweden.

Introduction: The efficient release of islets from the pancreas requires the synergistic interaction between collagenase class I, class II and a supplementary neutral protease extracted from *Clostridium histolyticum* (Neutral Protease, NP) or *Bacillus thermoproteolyticus rokko* (Thermolysin, TL). Recently, it has been shown that Clostripain (CP) also seems to play a significant role for efficient islet release from the human pancreas. This study aimed to evaluate the impact of different concentrations of NP, TL and CP on human islet integrity. To minimise the influence of donor-related variables already isolated islets were treated with these proteases.
Materials and Methods: Isolated human islets were precultured for 3-4 days at 37ºC to clear preparations (n=10) from predamaged islets. Aliquots of 500 IEQ were incubated for 90 min with enzyme concentrations calculated by assuming a 100 gram pancreas perfused with a volume of 300 mL HBSS supplemented with 2000 PZ-U of collagenase activity plus either 120 DMC-U of NP, 70,000 caseinase units of TL or 200 BAEE-U of CP (1x-level). To evaluate the toxic potency of proteases, these experience-based activities were increased 5x and 10x. Islets incubated in plain HBSS served as controls (CTR). After 24 h-culture in enzyme-free medium quality assessment of treated and control islets was performed. Data were normalised to controls and expressed as mean±SEM.
Results: Viability of CTR (100%) was significantly reduced by using the 1x- and 5x-level of NP (91.1±1.3%, $P<0.01$) or TL (88.5±2.0%, $P<0.001$), respectively. CP affected viability only when used at the 10x-level (93.3±1.4%, $P<0.05$).
While NP (57.5±3.7%) and TL (58.6±7.7%) significantly reduced intracellular insulin at the 5x-level ($P<0.01$) compared to CTR, this occured only when a 10x-concentration of CP was used (62.8±6.6%, $P<0.01$).
Semi-quantitative scoring (0-3) revealed that islet fragmentation became evident at the 5x-level of NP (0.70±0.15, $P<0.05$ vs. CP) and TL (0.40±0.16, NS). Compared to CTR, fragmentation reached significance using the 10x-concentration of NP (1.20±0.13, $P<0.001$) or TL (0.80±0.13, $P<0.05$) but not when CP was used at the same level (0.20±0.13).
Mitochondria function of CTR was not reduced by CP even when used in 10x-concentration (88.1±3.8%). In contrast, NP and TL significantly affected mitochondria ($P<0.01$) at the 1x (83.8±3.9%) and 5x (80.8±4.9%) level, respectively.
A detrimental effect ($P<0.05$) on islet overall survival, considering both recovery and viability, was noted when using the 1x level of NP (43.5±6.6%) or TL (42.5±5.7%). An adverse effect of CP on overall survival became evident not until the 10x concentration was used (42.0±4.3%, $P<0.01$).
Conclusion: These data clearly suggest that CP has a significantly lower toxic potency compared to NP and TL which have a similar harmful effect on human islets. CP may serve as useful enzymatic supplement to reduce the amount of NP or TL needed for efficient pancreas digestion.

P.1853

Tetrahydrocurcumin enhances islet cell function and attenuates apoptosis in mouse islets

Hyuk Jang[1], Seong Su Kim[3], Mee Young Oh[1], Hee Ju Kim[1], Ji Hwan Lee[1], Duck Jong Han[2].
[1]Surgery, University of Ulsan, Gangneung Asan Hospital, Gangneung, Korea; [2]Surgery, Asan Medical Center, Seoul, Korea; [3]Anesthesia, University of Ulsan, Gangneung Asan Hospital, Gangneung, Korea.

Background: The transplantation of isolated pancreatic islets is a promising treatment for diabetes. Curcumin has been used for its pharmacologic effects, such as antidiabetic and antiinflammatory activities. Tetrahydrocurcumin (THC), one of major metabolites of curcumin, has been reported to have antioxidant and anti-inflammatory activities. This study examines the hypothesis that preoperative THC treatment can attenuate ischemic damage and apoptosis before islet transplantation.
Methods: Islets isolated from Balb/c mice were randomly divided into 2 groups, and cultured in medium supplemented with or without THC. In vitro islet viability and function were assessed. After treatment with a cytokine cocktail consisting of tumor necrosis factor-α, interferon-β, and interleukin-1β, islet cell viability, function, and apoptotic status were determined. Proteins related to apoptosis were analyzed using Western blotting.
Results: There was no difference in cell viability between the 2 groups. Islets cultured in the medium supplemented with THC showed 1.3-fold higher glucose-induced insulin secretion than the islets cultured in the medium without THC. After treatment with a cytokine cocktail, glucose-induced insulin release and the total insulin content of the islets were significantly improved in THC-pretreated islets compared with islets not treated with THC. Apoptosis was significantly decreased, and Bcl-2 was elevated in the THC-pretreated group. Cytokine-only treated islets produced significantly higher levels of BAX, caspase-3 and UCP 2 than islets pretreated with THC before cytokine treatment.
Conclusions: These results suggest that preoperative THC administration enhances islet function before transplantation and attenuates the cytokine-induced damage associated with apoptosis.

P.1854

How about heparin-pegylated islets in allo-islet transplantation in cynomolgus monkeys

Hyojun Park, Sung Joo Kim, Kyo Won Lee, Jae Berm Park, Ji Soo Lee, Kyung Sik Kim, Jong Hwan Lee, Seung Hyun Kim.
Department of Surgery, Samsung Medical Center, Seoul, Korea.

Much effort is devoted to the protection of islets during transplantation for clinical allo-islet transplantation to be successful with smaller number of transplanted islets. Pegylation is an effective way to protect islets by wrapping islets with various materials including heparin rapidly, with minimal loss of islets. Islets 10,000 to 13,000 EIN/kg were transplanted into 12 cynomolgus monkeys (2 control group, 10 heparin pegylation group). Induction immunosuppression was done with antithymocyte globulin 5mg/kg 4 times or ATG with rituximab (375mg/m2) and and tacrolimus was given as maintenance immunosuppression. Basal c-peptide at 2 months post-transplant was 1.76 ± 1.1 ng/ml in the heparin pegylation group, while it was 0.9 ± 0.8 ng/ml in the control group and 0.1 ± 0.1 ng/ml in the pegylation group. Monkeys transplanted with allo-islets undergone heparin pegylation were able to achieve partial insulin independence at 2 months post-transplant with mean 12,000 EIN/kg islets. This protocol requires more long-term follow-up.

P.1855

Australia's first successful remote centre paediatric islet auto-transplant after pancreatectomy in a 7 year old for hereditary pancreatitis

Toni Radford[1], Myfanwy Geyer[4], Thomas Loudovaris[5], Tom Kay[5], Balarumugan Appakalai [3], John Chen[2], Sanjeev Khurana[4], Christine Russell[1], Richard Couper[4], Shane T.Grey[1], Christopher Drogemuller[1], Patrick T.Coates[1].
[1]Central Northern Adelaide Renal and Transplantation Service, Royal Adelaide Hospital, Adelaide, Australia; [2]Hepatobilliary Surgery, Flinders Medical Centre, Adelaide, Australia; [3]Department of Surgery, University of Louisville, Louisville, KY, United States; [4]Department of Endocrinology, Women's and Children's Hospital, Adelaide, Australia; [5]Islet Transplant Program, St. Vincent's Institute, Melbourne, Australia.

Introduction: Chronic pancreatitis is a debilitating condition that can arise from mutations in the PRSS1 gene encoding the pancreatic protein cationic trypsinogen. It can be managed with bile duct stenting and pain management. However, these provide only temporary relief. The main objective of pancreatectomy is to provide pain relief, however the patient will become insulin dependent. Combining this procedure with an islet auto-transplant (IAT) can improve blood glucose control with possible insulin independence.
Methods: Here we present a case report of the first Australian paediatric IAT. GW is a 7 year old male with chronic pancreatitis due to a mutation in the PRSS1 gene. Ethics approval for the procedure was given by the paediatric ethics department at the Women's and Children's Hospital.
Results and Discussion: GW first presented as a 5 year old male with hereditary pancreatitis and severe pain managed by oxycodone and amitriptyline. Preliminary tests showed normal c-peptide levels, elevated lipase 4014U/L and amylase 504U/L Magnetic resonance cholangiopanctreatography identified a tortuous, dilated pancreatic duct and significant parenchymal thinning. Cannulation of the duct and stenting had been unsuccessful. He was taking opioids 4 times/day until the day of surgery for pain control. The pancreatectomy followed standard practice but was difficult due to adhesions and severe scarring. Once retrieved, the pancreas was couriered by our islet scientist using a commercial airline, interstate to the isolation centre in Melbourne (760 km distance). Cold ischaemic time was 05hr:21m. GW returned to the paediatric intensive care unit (PICU) and insulin was commenced. The islet isolation was hampered by un-dissolvable fibrous tissue and calcification of the pancreas. This required the following changes, firstly the pancreas was dissected into ~20 fragments with each individually injected with collagenase. Digestion was significantly extended (2hr: 21m) and involved a second batch of enzyme, standard procedure then continued. This resulted in a final islet yield of 1,149 IEQ/kg (28,656IEQ total). The cells were couriered back to the transplant centre with a cold ischaemic time of 3hr:35m. Islets were infused over a 30 minute period via percutaneous puncture of the portal vein. Portal pressures remained at 13mm/H2O throughout, indicating no thrombosis. GW was discharged after 4 weeks, on oxycodone, paracetamol, insulin and pancreatic replacement enzymes.
Conclusion: Australia's first paediatric IAT with donor pancreas shipping, remote isolation and reinfusion is reported. GW has weaned from all pain relief, is active, back at school, with good appetite and normal weight gain.C-Peptide levels of 0.2nmols/l indicate islet cell function and exogenous insulin use has declined from 1.0U/kg/day to 0.25U/kg/day units with blood glucose levels between 4-8mmol/L.This demonstrates that IAT can provide metabolic control after total pancreatectomy.

P.1856

Evaluation of donor characteristics including histological analysis of pancreases allocated for islet isolations

Frantisek Saudek, Zuzana Berkova, Peter Girman, Jan Kriz, Tomas Koblas, Ludek Voska, Slavek Lipar, Ivan Leontovyc, David Habart, Lucie Kosinova, Sarka Loukotova, Tomas Neskudla.
Institute for Clinical and Experimental Medicine, Praha, Czech Republic.

Aims of the Study: Precise and effective donor selection is needed to avoid elimination of potentially successful donors and acceptance of donors with inadequate islet mass. The purpose of this study was to evaluate donor and pancreas characteristics influencing the islet isolation outcome and to investigate the contribution of histological examination of donor pancreases for organ selection.
Methods: Donor characteristics were evaluated in successful (n=61) and failed (n=98) islet isolations. Samples of donor pancreatic tissue were taken for histological examination. The islet isolations with more than 250 000 islet equivalents were considered as successful. Selected donor characteristics as age, BMI, cold ischemia time, the cause of death, intensive care hospitalization, serum amylase, usage of vasopressors, blood glucose levels, organ procurement, social and medical history, edema, fibrosis and potential organ damage were evaluated according to the Edmonton scoring system. Tissue samples from the head or neck of the pancreas were fixed in 10% formaldehyde and stained with hematoxylin & eosin. For insulin detection immunohistological staining was used. Slices were were scanned by EVOS FL Auto imaging system and the images were analyzed ImageJ software. The percentage of insulin positive area to total pancreatic area was calculated. To predict the isolation outcome based on histological evaluation and donor score, receiver operating characteristic (ROC) analysis was performed and area under the curve (AUC) was calculated. Multivariate logistic regression analysis was used to determine isolation success based on histological estimation and scoring system.
Results: The average islet yield from all 159 isolations was 223 289 ± 131 276 IE while 61 successful isolations produced 356 328 ± 87 356 IE. We confirmed that BMI less than 25 kg/m2 ($p<0.001$), cold ischemia time more than 8 hours ($p<0.01$), hospitalization longer than 96 hours ($p<0.05$) and higher catecholamines doses ($p<0.05$) as well as edematous pancreases ($p<0.01$) can unfavorably affect the isolation outcome. The percentage of insulin positive area in successful group was 1.43 ± 0.6 whereas in failed group it was significantly lower 1.02 ± 0.7 ($p < 0.001$). ROC analysis confirmed statistically significant relation: AUC = 0.796; 95% confidence interval: 0.689 to 0.879; $p < 0.001$. The optimal cutoff point for insulin positive area in pancreas > 1.02% had 89% sensitivity and 76% specificity for success and revealed significantly better prediction of isolation outcome then if only the scoring system was used ($p < 0.05$).
Conclusion: Histological examination prior to islet isolation an represents important information for donor selection. While its evaluation as a single variable cannot sufficiently predict the isolation outcome, its use in combination with the already established scoring system might be helpful for better organ selection.
This study was supported by grant NT 13099 IGA MZ CR

P.1857

Evaluation of a newly developed pancreas preservation solution, F6H8S5, for clinical islet isolation and transplantation

Magnus Ståhle[1], Hanna Schierbeck[1], Johan Olerud[1], Aksel Foss[2], Bengt Gustafsson[3], Marko Lempinen[4], Torbjörn Lundgren[5], Ehab Rafael[6], Gunnar Tufveson[7], Bastian Theisinger[8], Daniel Brandhorst[1], Olle Korsgren[1], Andrew Friberg[1].
[1]Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden; [2]Transplantation Surgery, Rikshospitalet, Oslo, Norway; [3]Department of Transplantation, Sahlgrenska University Hospital, Göteborg, Sweden; [4]Division of Transplantation, Surgical Hospital, Helsinki, Finland; [5]Division of Transplantation Surgery, Karolinska Institute, Stockholm, Sweden; [6]Nephrology and Transplantation, University Hospital, Malmö, Sweden; [7]Surgical Sciences, Division of Transplantation, Uppsala University Hospital, Uppsala, Sweden; [8]Novaliq GmbH, Heidelberg, Germany.

I**ntroduction:** Pancreas transportation is associated with ischemia and hypoxia; two of the most decisive factors negatively influencing outcomes associated with human islet isolation and islet transplant engraftment. In a pilot study, using non-clinical grade pancreases, a newly developed pancreas preservation solution, polydimethylsiloxane 5 (F6H8S5), showed increased islet yield compared to standard preservation solutions. This study aimed to evaluate pancreases in a clinical setting compared with standard solutions for 1) cold ischemia-time (CIT) <10 hour and 2), an extended CIT > 20 hour.
Materials and Methods: ***Part 1:*** Procured clinical grade pancreases were shipped from procurement hospitals within the Nordic Network for Clinical Islet Transplantation to a central isolation facility in either F6H8S5 or in standard preservation solutions, i.e., University of Wisconsin (UW) or Custodiol followed by islet isolation. All solutions were pre-oxygenated for at least 5 minutes charge at a flow rate of 2000 mL/min oxygen. ***Part 2:*** Pancreases were procured and shipped in UW or Custodiol. Upon arrival at the islet isolation facility, duodenum was removed followed by rough trimming while F6H8S5 was oxygenated for 15-20 minutes. Trimmed pancreases were immersed into oxygenated F6H8S5 and stored at 4°C over night followed by subsequent islet isolation resulting in a CIT >20 hours. In both parts of the study, islet yield, fulfillment of transplantation criteria, isolation and purification variables were evaluated as well as islet quality control.
Results and Discussion: Pancreas preservation using F6H8S5 proved as effective as UW and Custadiol when used within CIT up to 10 hour, both in terms of isolation outcome, islet functionality and recovery. Preservation in F6H8S5 of pancreases with extended CIT (>20 hours) resulted in increased IEQ/gram pancreas compared to controls with CIT< 10 hours whereas no difference was found concerning isolated islet functionality, isolation outcome or recovery.
Conclusion: This study of clinically obtained pancreases preserved for islet isolation and transplantation show that F6H8S5 is a suitable preservation solution for pancreases intended for islet isolation. Furthermore, it seems to allow extended cold ischemic time without inflicting islet function and numbers.

P.1858

The investigation on target matrix of collagenase G for achieving tailor-made islet isolation

Satoru Yoshida[1], Youhei Yamagata[2,3], Kanako Nakagawa[4], Kazuhisa Maeda[4], Kazutaka Murayama[5], Kimiko Watanabe[2], Takehiro Imura[6], Akiko Inagaki[6], Yasuhiro Igarashi[6], Shigehito Miyagi[1], Kazuo Ohashi[7], Noriaki Ohuchi[1], Susumu Satomi[1], Masafumi Goto[1,6].
[1]Division of Advanced Surgical Science and Technology, Tohoku University School of Medicine, Sendai, Japan; [2]New Industry Creation Hatchery Center, Tohoku University, Sendai, Japan; [3]Department of Applied Biological Chemistry, Graduate school of Agricultural Science, Tokyo University of Agriculture and Technology, Tokyo, Japan; [4]School of Bioscience and Biotechnology, Tokyo University of Technology, Tokyo, Japan; [5]Division of Biomedical Measurements and Diagnostics, Graduate school of Biomedical Engineering, Tohoku University, Sendai, Japan; [6]Division of Transplantation and Regenerative Medicine, Tohoku University School of Medicine, Sendai, Japan; [7]Laboratory of Drug Development and Science, Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan.

Introduction: We recently revealed that one of the crucial target matrices of Collagenase H (ColH) is collagen III in the field of islet transplantation. However, the target matrix components of Collagenase G (ColG), which is the other collagenase subtype, remain uncertain. The characterization on the target matrices of both collagenase subtypes is necessary for achieving tailor-made islet isolation. Therefore, we examined the molecular composition of the target extracellular matrix (ECM) of ColG using highly purified recombinant enzymes.
Materials and Methods: We performed in vitro substrate digestion assays and immunofluorescence analysis in the present study. In the in vitro substrate digestion assays, the digestion pattern of each substrate (collagen types I-VI, laminin and fibronection) using each collagenase subtype (ColG and ColH provided by Meiji Seika Pharma Co., Ltd.) was evaluated on the sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) by comparing the bands between samples obtained before and after reactions. In the immunofluorescence analysis, tissue sections were prepared to react with each collagenase subtype, and were then evaluated for the presence of type IV collagen on the rat pancreatic tissues.
Results: In vitro substrate digestion assays revealed that both collagenases digested collagens, but not laminin or fibronectin. Of particular interest, collagen type IV and V were digested by ColG, but not by ColH. In the immunofluorescence analysis, the rat pancreatic islet sections were positively stained for type IV collagen. After incubating them with ColG, the rat pancreatic tissue sections displayed weak reactions for collagen IV, whereas no difference was observed when ColH was added. These results of immunofluorescence analysis against collagen IV support the findings obtained from in vitro substrate digestion assays. Furthermore, we performed immunofluorescent staining with the antibody against type IV collagen on the rat hepatic tissues as well to examine as an application for the different type of tissues, and the same results as the rat pancreatic tissues were obtained.
Discussion: These results clearly demonstrated that the collagen IV expressed on the tissues was effectively digested by ColG, but not by ColH. Furthermore, our novel findings were applicable for not only pancreatic islet isolation but also for other types of cell isolation such as hepatic tissue dissociation. This type of tailor-made islets isolation according to the ECM of pancreatic tissues would be of particular importance to improve the efficiency of clinical islet isolation, since an individual donor source which is available for human islet transplantation is broadly different.
Conclusions: Our present study demonstrates that one of the targets of ColG is collagen IV in pancreatic tissue dissociation. Furthermore, these results support that one of crucial targets of ColG appears to be collagen IV in other cell isolation such as hepatic tissue dissociation as well. Therefore, we speculate that semiquantitation of the collagen IV present in pancreatic/hepatic tissues may contribute to achieving tailor-made islet/hepatocyte isolation using individually optimized amount of ColG.
Kozue Imura; Megumi Goto

P.1859

The availability of marmoset diabetes modeling as a transplantation model

Wenji Yuan[1], Satsuki Fukuda[1], Takashi Inoue[2], Hitoshi Okochi[1], Erika Sasaki[2], Masayuki Shimoda[1].
[1]National Center for Global Health and Medicine, Tokyo, Japan; [2]Central Institute for Experimental Animals, Kawasaki, Japan.

Background: nonhuman primates (NHPs) are considered as the useful models for both diabetes pathogenesis researches and preclinical studies of islet transplantation.
Common marmosets as a small primate model, has been attracting much attention in the research field of biomedical science.
Methods: We demonstrate marmoset diabetes model establishment by the combination of partial pancreatectomy and streptozotocin administration. Fifteen animals were used. We performed partial pancreatectomy (Resection of about 60-70% of pancreatic), and streptozotocin (STZ) 100mg/kg to 200mg/kg of body weight were given multiple intravenously as we previously reported in 2015 American Transplant Congress. After the marmosets shows stable high blood glucose level, (nonfasting blood glucose level > 200 mg/dl), We transplanted 1x107 of min6 cells under renal capsule of diabetic marmoset with several immunosuppressant protocols (1. Cyclosporin A 10-20mg/kg/day, 2. Tacrolimus 0.05-0.1mg/kg/day). Blood glucose determination and a blood biochemistry inspection were conducted periodically.
Results: in this preliminary study, 5 marmosets were used. No adverse events were observed in all marmosets. After min6 cells transplantation, the non-fasting blood glucose levels were decreased for a few days but then elevated to the pre-transplant levels within a week.
Discussions: the immunosuppressant protocol must be improved. We are examining the transplant study with modified immunosuppressive drug regimens.

P.1860

Factors effecting islet isolation outcomes over the past 15 years for the Westmead Islet Transplant Program

WayneJ. Hawthorne[1,2], YiVee Chew[1], Lindy Williams[1], Sussan Davies[1], David Liuwantara[1], Philip J.O'Connell[1,2].
[1]The Centre for Transplant & Renal Research, The Westmead Institute for Medical Research, Westmead, Australia; [2]Westmead Clinical School, Sydney Medical School, University of Sydney, Westmead, Australia.

Aims: There are a large number of patients suffering from Type 1 diabetes but not all patients are suitable to undergo islet cell transplantation, those patients with recurrent severe hypoglycemia is the main indication for islet cell transplantation. Islet cell transplantation for type 1 diabetes relies heavily on successful isolation outcomes to ensure successful transplantation. There are a great number of influential co-factors that can effect the isolation. As such we aimed to evaluate our islet isolation outcomes and identify factors during donor selection, organ procurement and islet isolation influencing the preparation leading to release of the islet preparation for subsequent transplantation in an effort to improve our ongoing outcomes.
Methods: Heart beating deceased donor pancreata were used in evaluation of these cofactors. Islets were isolated from these pancreata using Collagenase enzyme and Neutral Protease (SERVA). Donor characteristics, pancreas procurement data, isolation yield and outcomes were collected and compared to determine correlation between each variable. Isolations were also divided into Transplanted (Tx) VS Non-transplanted preparations (Non-Tx) to identify variables significantly influencing isolation outcomes.
Results: A total of 235 islet isolations have been performed at Westmead but only data from 209 islet isolations collected between July 2000 and December 2015 were evaluated. We excluded data from Donation after Cardiac Death (DCD) donors and pancreata that were not taken for clinical transplantation.
Overall over the entire 15 year period an average of 25.4% of islet preparations were transplanted. However, with significant improvements seen over the last few years we have been able to improve isolation outcomes with 50.0% of islet isolations in the 2013-2014 and 2014-2015 periods reaching release criteria. Transplantable yields (defined as >300,000 IEQ; >4,000 IEQ/kg for a 75kg recipient) were obtained from donors aged between 20-60 years of age, with BMI >20kg/m2, and weight >55kg. Compared to non-tx (n=158), Tx (n=51) had significantly higher total IEQ (605,792±257,950 VS 329,905±202,249 IEQ) and IEQ/g pancreas (8,268±6,419 VS 5,229±3,792 IEQ/g).
We have found that the most influential co-factors to release of an isolation for transplantation revolve around the donor and the logistics of obtaining the pancreas. In particular the quicker the pancreas can be transported to the isolation facility has the most significant correlation with the CIT being significantly correlated ($p<0.05$). the other most influential factors were relating specifically to the choice of donor with higher donor BMI, donor weight and pancreas weight being significantly correlated ($p<0.05$) to transplant as an outcome. Transplantation also exhibited significantly higher viability, purity and beta cell viability indices compared to Non-tx.
Conclusions: We found that increased donor BMI/weight and lower CIT all have significant effects on outcomes. In particular these influence which islet isolations resulted in transplantable yields/outcomes. Optimising logistics of donor procedures to obtain the shortest transport times can significantly improve outcomes with little additional effort.

P.1861

Variable hernia rates following transplantation: The role that transplant type, incision and immunosuppression play

Sara Shahrestani[1,2], Hanh M Tran[1], Henry CPleass[1,2], Wayne J Hawthorne[1,2].
[1]Department of Surgery, Westmead Hospital, Sydney, Australia; [2]Westmead Clinical School, Sydney Medical School, Sydney, Australia.

Background: The type of incision used in organ transplantation may increase the likelihood of hernia or wound infections. This, in combination with immunosuppression use, can increase wound complications, due to impaired immune protection and delayed wound healing.
Methods: We reviewed the available literature in order to conduct a meta-analysis examining the rate of hernia in solid organ transplant. We were also interested in examining whether this rate differed by type of organ transplanted into the abdomen, the type of incision and immunosuppression.
Results: 80 articles met our criteria for inclusion in the systematic review, and 55 contained the statistics necessary for inclusion in the meta-analysis. Of these articles, 10 were case reports or series, 13 were prospective cohort studies, and 57 were retrospective reviews. This included over 33,000 solid organ transplant recipients who received kidney, liver, pancreas or intestinal transplants. There were 20, 537 kidney transplant recipients, 12, 075 liver transplant recipients, 171 pancreas transplant recipients and 208 intestinal transplant recipients. The quality of reporting of studies was generally high, as assessed by the Newcastle-Ottawa scale for bias in systematic reviews. Studies tended to select participants effectively and employ adequate follow-up. We found the rate of hernia in solid organ transplants was around 6% (n=64, CI 95% 0.05-0.08, $p<0.001$) and that liver transplants were associated with the highest rate of incisional hernia at 10% (n= 31, $p<0.001$). Kidney transplants were associated with the lowest rate of incisional hernia at 2% (n= 22, $p<0.001$) and hernia in pancreas transplants occurred at a rate of 3% (n=3, $p<0.001$). Quite markedly, the use of the immunosuppressant sirolimus was associated with a five-fold increase in the rate of incisional hernia ($Q(1)=15.99$, $p<0.001$). Studies did not consistently report the type of incision used. Eight out of 31 studies of liver transplants reported using a Mercedes incision, and this was associated with a 9% rate of hernia ($p<0.001$). Only two out of 22 studies in kidney transplantation reported the use of paramedian incision, and this was associated with hernia at a rate of 5% ($p=0.003$). Four out of 22 studies in kidney transplantation included the use of the midline incision and this was associated with a 4% ($p=0.001$) hernia rate. By reviewing the literature, we found that the lowest rate of recurrence after repair occurs with the use of laparoscopic hernia repair.
Conclusions: The rate of incisional hernia in solid organ transplants is highest following liver transplantation and the use of the Mercedes incision appears to contribute to this high rate. Sirolimus should be used with care in patients with healing wounds as it is associated with the highest rate of hernia. Evidence supports laparoscopic hernia repair as the most effective form of repair and this should be attempted where possible.

P.1862

Duodenoduodenostomy as a preferred intestinal anastomosis during pancreas transplantation

Katarzyna Baumgart[1,2], Marek Durlik[1,2].
[1]Department of Gastrointestinal and Transplantation Surgery, Central Clinical Hospital of the Ministry of Interior, Warsaw, Poland; [2]Department of Surgical Research and Transplantology, Mossakowski Medical Research Centre, Warsaw, Poland.

Introduction: Since the first pancreas transplantation in 1966, surgical techniques have vastly evolved. Nowadays, in most transplant centres the preferred type of anastomosis is the one with the small intestine. Since 2007, when the duodenoduodenal anastomosis was introduced, it has become a valuable type of anastomosis.
Materials and Methods: From 2004 untill January 2016, 154 patients underwent 134 SPK, 18 PTA and 2 PAK pancreas transplantations in our Centre. We performed 29 duodenojejunal anastomoses and 125 duodenoduodenal anastomoses. We carried out the postoperative endoscopic surveillance of the anastomosis, including confocal laser endomicroscopy (CLE) and EUS examinations when necessary.
Results: Duodenduoduodenal anastomosis provides easy, direct and noninvasive access to the transplanted duodenum via an endoscope. When needed, it's extremely useful in endoscopic treatment while stating the symptoms of anastomotic bleeding and there's no need for further operation. Thanks to its location it is very easy to monitor the duodenum and obtain biopsy samples from the anastomosis, as well as to perform a confocal endomicroscopy or an endoscopic ultrasound (EUS) examination. By January 2016 we have performed 28 endoscopies due to anastomotic bleeding. In 10 endoscopic examinations we detected atypical duodenum mucosa suggesting graft rejection. We carried out 19 EUS examinations and 11 confocal laser endomicroscopy examinations.
Conclusion: Duodenoduodenostomy is a safe and beneficial type of anastomosis in pancreas transplantation and may provide additional therapeutic and diagnostic options in postoperative care.

P.1863

Delayed pancreas graft function after pancreas transplantation - single centre experience

Katarzyna Baumgart[1,2], Dorota Zielińska[1], Marek Durlik[1,2].
[1]Department of Gastrointestinal and Transplantation Surgery, Central Clinical Hospital of the Ministry of Interior, Warsaw, Poland;
[2]Department of Surgical Research and Transplantology, Mossakowski Medical Research Centre, Warsaw, Poland.

Introduction: Delayed pancreas graft function (DGF) is a poorly defined condition that may occur after pancreas transplantation. It is defined by the need for exogenous insulin at the time of hospital discharge. It is described as a common problem in most centres.
Materials and Methods: From 2004 untill January 2016 154 patients underwent 134 SPK, 18 PTA and 2 PAK pancreas transplantations in our Centre. Amongst the 125 patients who didn't require graftectomy in the early postoperative course, DGF was stated in only 4 patients (0,03%). All patients had undergone Simultaneous Pancreas-Kidney Transplantation (SPK). We retrospectively analysed the donor and recipient factors.
Results: Insulin requirement 6 months after pancreas transplantation was a criteria between DGF and primary graft nonfunction. Amongst the 4 patients with DGF, 1 patient didn't require exogenous insulin after 3 months. 2 patients didn't achieve insulin independence, however the total cumulative insulin requirement was smaller than before the transplantation. 1 patient required a pancreas graftectomy due to duodeno-arterial fistula 10 months after transplantation.
Conclusion: DGF was not a common condition in our centre and our material is too small to provide valuable statistical analysis. In both groups, the donor and recipient parameters were comparable e.g. donor age of 29 years (DGF group) vs 33 years, CIT 9,5h vs 9,62h, donor BMI 24,26 vs 25,72 kg/m2, recipient BMI 23,7 vs 23,14 kg/m2.

P.1864

The inflammatory parameters and blood gases obtained from the pancreatic graft's vein and the postoperative course in patients after pancreas transplantation - preliminary study

Katarzyna Baumgart[1,2], Marek Durlik[1,2].
[1]Department of Gastrointestinal and Transplantation Surgery, Central Clinical Hospital of the Ministry of Interior, Warsaw, Poland;
[2]Department of Surgical Research and Transplantology, Mossakowski Medical Research Centre, Warsaw, Poland.

Introduction: Pancreas transplantation is an established method in the treatment of patients with diabetes, which restores proper insulin secretion and helps to achieve a metabolic balance and eliminate the need for exogenous insulin. Unfortunately, pancreas transplantation is a procedure with a still relatively high complication rate. The analysis of the markers in the graft's vein might be helpful in predicting the postoperative course in patients after transplantation.
Materials and Methods: From 2004 untill January 2016, 154 patients underwent pancreas transplantations in our Centre. We obtained graft blood samples from 16 patients from May 2015 untill January 2016 and correlated them with the postoperative course.
The blood samples from the graft's vein were obtained right after the revascularisation and again 30 minutes later. The measurement of the blood gases and inflammatory markers like C-reactive protein, procalcitonin, amylase and lipase serum were analysed. We performed a statistical analysis on the influence of these factors on the graft thrombosis, graft pancreatitis, graft rejection and early pancreas graft fibrosis.
Results: Preliminary results and statistical analysis show that HCO3 activity after 30minutes and hematrocrit after revascularisation might have an influence on the incidence of the graft thrombosis. Early graft fibrosis might be influenced by O2Hb both immediately after and again 30 minutes after revascularisation. None of the factors did reach statistical significance in the graft rejection group.
Conclusion: The graft vein blood sample results might be helpful in predicting the graft quality and might have an influence on some postoperative complications.

P.1865

Pancreas rejection with graft necrosis presenting with episodic massive intestinal bleeding

Shih-Chin Chen[1,2], Yi-Ming Shyr[1,2], Shin-E Wang[1,2].
[1]Division of General Surgery, Department of Surgery, Taipei Veterans General Hospital, Taipei, Taiwan; [2]National Yang Ming University, Taipei, Taiwan.

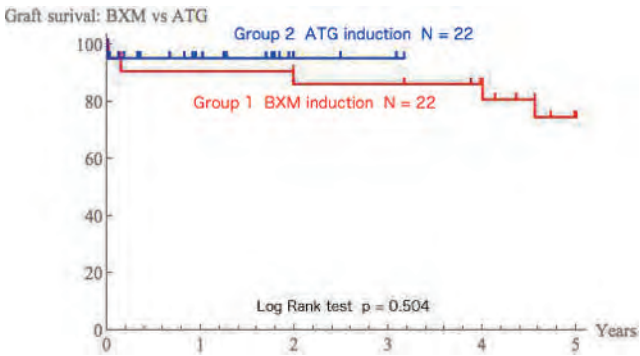
Purpose: This study is to present our unusual experience of episodic massive intestinal bleeding due to pancreas rejection with graft necrosis.
Materials and Methods: A case of NIDDM with uremia underwent simultaneous pancreas and kidney transplant in 2005. With 3 times of acute rejection on the kidney and pancreas grafts, he eventually went back to hemodialysis 7 years later.
Results: The pancreas graft failed due to acute rejection on the pancreas graft 9 years after SPK transplant. Therefore, all immunosuppressants were discontinued. Unfortunately, the pancreas graft became necrotic, and thereafter, intermittent gastrointestinal (GI) bleeding occurred. The angiography detected bleeding from arterial Y-graft, and the extravasated blood flew through the graft duodenojeunostomy anastomosis into the bowels and presented with GI bleeding. The bleeder was controlled by coil embolization. Hemorrhagic shock due to massive re-bleeding happened 1.5 months after coil embolization. The emergent angiography showed coil migration into the necrotic pancreas graft and active re-bleeding again from the same arterial Y-graft. The bleeder was temporarily controlled by a covered-stent in recipient common iliac artery, and emergency explant of the failed and necrotic pancreas graft was performed and the arterial Y-graft was ligated securely to prevent re-bleeding.
Conclusion: Bleeding from arterial Y-graft could occur after rejection and necrosis of pancreas graft due to sudden withdrawal of immunosuppressants, which might present with intermittent massive GJ bleeding. The coil embolization might fail due to coil migration into the necrotic pancreas graft.

P.1866

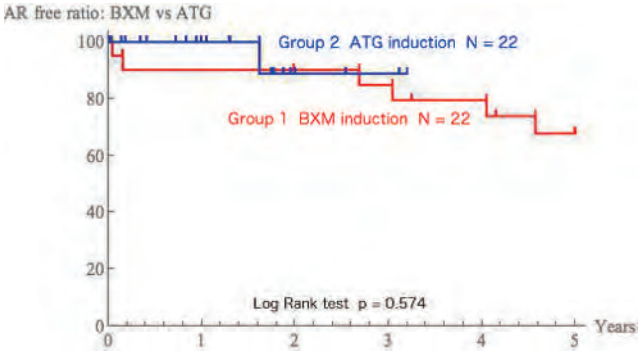
ATG induction markedly reduced acute rejection and improved graft survival in pancreas transplantation

Kazuhiro Iwadoh, Akira Kondo, Noriyuki Masaki, Takeshi Hachisuka, Katsuyuki Miki, Yuichi Ogawa, Kotaro Kai, Akihito Sannomiya, Toru Murakami, Kumiko Kitajima, Ichiro Koyama, Ichiro Nakajima, Shohei Fuchinoue.
Department of Surgery III, Tokyo Women's Medical University, Tokyo, Japan.

Introduction: Once acute rejection occurs in pancreas transplantation (PTx), it is difficult to manage and complicates the clinical course, so it is essential to prevent acute rejection in PTx. Ever since we commenced PTx from brain-dead donors in 2001, we had used anti-CD25 monoclonal antibody (basiliximab: BXM) for induction. Anti-thymocyte globulin (ATG) has been widely used in renal transplantation, especially in sensitized patients. In 2012 we started to use ATG as an induction in PTx to ameliorate clinical outcomes of PTx. Here, we discuss the efficacy of the two types of induction on PTx.
Materials and Methods: 44 cases of PTx from brain-dead donors were performed at our institute from 2001 to 2015. 37 cases (84.1%) were simultaneous pancreas kidney transplantation (SPK), and the rest were pancreas after kidney transplantation (PAK). We divided them into 2 groups based on induction therapy: Group 1; BXM (G1; n=22) and Group 2: ATG (G2; n=22). The induction therapy in G1 was used until 2012 and that of G2 was used thereafter. Immunosuppressants consisted of tacrolimus, mycophenolate mofetil and methylprednisolone. Patient and graft survival rates, acute rejection (AR)-free ratios, frequency of AR, and parameters for infection were compared.
Results and Discussion: The average donor age (G1 vs. G2) was 50.7±12.5 vs. 37.3±13.6 (p>0.05) and the mean recipient age was 40.7±5.8 vs. 44.1±9.5 (p>0.05). There were 19 cases (86.4%) of SPK in G1 vs. 18 (81.8%) in G2 (p>0.05). There was a significant difference in the type of drainage method used. The ratio of bladder drainage to enteric drainage was 19 / 3 in G1, vs. 6 / 16 in G2 (p<0.001). 5-year patient survival rates were 100% in both groups. 1, 3, 5-year graft survival rates in G1 were 90.1%, 86.4% and 74.7% in G1 while 1, 3-year graft survival rates in G2 were 95.5% and 95.5% (p>0.05).



There were 6 cases of graft loss in G1, while there was only 1 case of graft loss in G2, which was due to thrombosis. 1, 3, 5-year AR-free ratios in G1 were 90.2%, 84.9% and 67.8% while 1, 3-year AR-free ratios in G2 were 100% and 88.9% (p>0.05).



There were 6 cases of AR episodes in G1,while there was only 1 in G2 ($p>0.05$). Average levels of CRP, CMV antigenemia, urinary WBC, and b-D-glucan for 1 year after PTx were comparable between the two groups ($p>0.05$).

Conclusion: ATG induction in PTx markedly reduced AR and graft loss frequencies compared to BXM induction, though statistical significance was not found, probably due to lack of statistical power. Furthermore, no increase in infectious complications was recognized in the ATG induction group.

P.1867

Exocrine drainage in pancreas transplantation: medium-term results of enteric conversion and significance of bladder drainage

Ichiro Koyama, Ichiro Nakajima, Akira Kondo, Noriyuki Masaki, Ken Hachisuka, Katsuyuki Miki, Yuichi Ogawa, Kazuhiro Iwadoh, Makoto Tonsho, Kotaro Kai, Akihito Sannomiya, Toru Murakami, Kumiko Kitajima, Shohei Fuchinoue.
Department of Surgery, Kidney Center, Tokyo Women's Medical University, Tokyo, Japan.

Background: The management of exocrine pancreatic secretion has a considerable influence on the morbidity and mortality of pancreas transplant recipients. Although enteric drainage (ED) is now the most common procedure in simultaneous pancreas-kidney transplantation (SPK), bladder drainage (BD) is still favorable in pancreas transplant alone (PTA) or after previous kidney transplant (PAK). The aim of the present study is to evaluate survival and complication of BD compared to ED.

Patients: We performed 47 pancreas transplants between 2001 and 2015 (40 SPK and 7 PAK). In the first half of 2000s when pancreas transplant was scarce in Japan, we initially employed BD (n=25) for the safety of recipients. Subsequently, exocrine drainage was gradually shifted to ED (n=22) except for PAK, because of increase in the number of transplants and progress of immunosuppression.

Results: Pancreas graft survival rate was almost equivalent: ED 91.7 % vs BD 92.0 % at 1 year. Nine out of 25 BD patients (36%) developed urological complications and underwent enteric conversion (EC). The mean transplant-EC interval was 23 months (1-55 months). Indications for EC were reflux pancreatitis (n=4), hematuria (n=3), bladder irritation (n=1), leakage of anastomotic site (n=1). No surgical complication was seen associated with the conversion procedure and long-term resolution of symptoms were obtained. Three ED patients developed complications relating to exocrine drainage: pancreatic fistula, GI bleeding, perforation of graft duodenum.

Conclusions: Although ED is a preferred approach in the majority of transplant centers, BD is still beneficial option in terms of urinary amylase measurement monitoring for pancreas graft rejection especially in PAK. Enteric conversion resulted in long-term solution of urinary symptoms and excellent graft function without surgical complications.

P.1868

Hybrid laparoscopic distal pancreatectomy of living donor using Shuriken shaped umbilicoplasty

Yoshinobu Sato[1], Yoshiaki Hara[2], Shin-Ei Kudo[2], Fumio Ishida[2], Junichiro Tanaka[1].
[1]General and Digestive Surgery, Showa University Fujigaoka University, Yokohama, Japan; [2]Digestive Center, Showa University Yokohama Northern Hospital, Yokohama, Japan.

Introduction: Donor safety is the most serious matter in a living donor transplantation, although a cosmetic advantage is also important for living donor laparoscopic surgery. We have already reported about the living donor surgery in liver and pancreas. Moreover we have reported about the complete untinatectomy by Hybrid laparoscopic with Shuriken shaped umbilicospasty (Hepatogastroenterol 2014;61:1486). In this study we would demonstrate the Hybrid laparoscopic distal pancreatectomy with shuriken shaped umbilicosplasty for living pancreatic donor in the view points of cosmetic economic, and safety procedures.
Patient and Method: A 76-year-old weman was hospitalized for surgery of intraductal papillary mucinous neoplasm located in the body of pancreas. Under general anesthesia, a Shuriken shaped umbilical skin incision was made by 7 cm in horizontal and 4cm longitudinal width with 3cm round skin incision. The intermediate skin between outside and inside skin incision was removed. Subcutaneous tissue around the umbilicus and the upper abdominal subcutaneous region was dissected, and the 8cm of upper abdominal minilaparotomy was performed. The complete resection of distal pancreatectomy with spleen was performed by hybrid laparoscopic procedure with the hand-assisted or the laparo-assisted manner. The manipulations of splenic artery and vein, and pancreatic stump could be done under direct vision.The umbilicoplasty of only 2cm round skin wound was made by the reefing of subcutaneous suture with 5-0 absorbable suture. The postoperative course was uneventful.
Results: This operative procedure could be undergone with Hybrid laparoscopic techniques. The operative time was 4 hours without blood transfusion. The operative course was uneventful and the patient discharged on the 10 postoperative days.
Discussion: Our new procedure could be used for pancreato-duodenectomy, distal pancreatectomy under direct vision. Therefore this means that our procedure might bring the pancreatic organ perfusion for preservation. It might be considered that our method is good for both cosmetic and safety benefits in pancreatic donor surgery.

P.1869

Pancreas transplant at Taipei Veterans General Hospital

Yi-Ming Shyr, Shin-E Wang.
Surgery, Taipei Veterans General Hospital, Taipei, Taiwan.

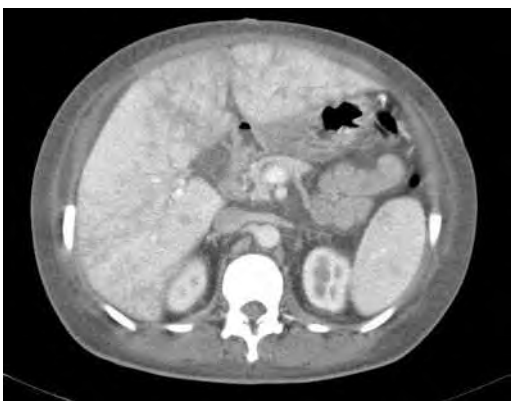
Background: Type 1 diabetes eventually leads to nephropathy, neuropathy, retinopathy and angiopathy after 10 – 30 years. Currently, pancreas transplant is the treatment of choice in tight control of blood sugar for IDDM patients, and further to stabilize, prevent or even to reverse the diabetic complications.
Material and Methods: We will present our experience in pancreas transplant which was initiated on September 19, 2003. From September 2003 to Sept. 2015, there were 115 pancreas transplants performed for 110 patients at Taipei Veterans General Hospital, with 39 SPK, 12 PAK, 46 PTA and 18 PBK. Most (80%) of our pancreas transplants were for IDDM patients.
Results: The blood sugar usually returned to normal level within 5 hours (median) after revascularization of the pancreas grafts. The fasting blood sugar maintained within normal range thereafter throughout the whole clinical course in most cases. There were 2 surgical mortality. The technical success rate was 96.0%. Excluding the 4 cases with technique failure, overall 1-year pancreas graft survival is 98.5% and 5-year is 94.1%, with 100% 1-year for SPK, 97.1% 1-year for PTA, 100% 1-year for PAK and 100% 1-year for PBK.
Conclusion: Pancreas transplant provided an ideal insulin-free solution for DM, especially IDDM. Pancreas transplant could be performed with similar successful rate irrespective of the type of pancreas transplant at our hospital.

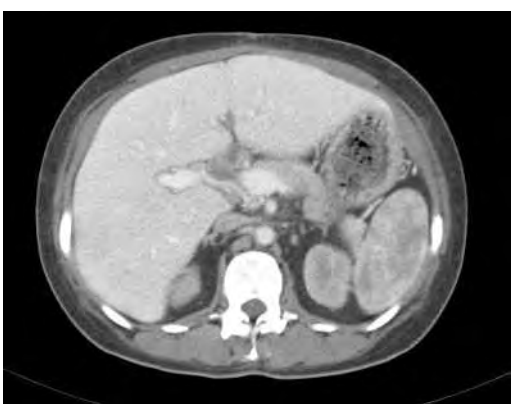
P.1870

Hepatic veno-occlusive disease related to tacrolimus after pancreas transplantation

Yi-Ming Shyr, Shin-E Wang.
Surgery, Taipei Veterans General Hospital, Taipei, Taiwan.

Background: Hepatic veno-occlusive disease (HVOD) describes the nonthrombotic, fibrous obliteration of the small centrilobular hepatic veins by connective tissue and centrilobular necrosis in zone 3 of the acini.
Materials: We describe a case of HVOD occurring after pancreas transplantation, in which tacrolimus might have played a causative role since complete recovery was observed after discontinuation of tacrolimus.
Results: A 25-year-old female with NIDDM and uremia. She underwent SPK transplantation. Nine months after transplantation, she reported development of fever, mild right abdominal pain and an increase in abdominal girth. The CT scan showed pictures of HVOD with hepatomegaly, massive ascites, periportal edema, diffuse mottled hepatic enhancement and patent hepatic veins (Fig. 1b). The periportal edema and diffuse mottled hepatic enhancement, in addition to the signs of portal hypertension, might suggest sinusoidal stasis. Tacrolimus was discontinued and replaced by cyclosporine. Three months after discontinuing tacrolimus, there was resolution of the patient HVOD demonstrated by CT scan (Fig .1c).
Conclusion: This is the first case of HVOD after pancreas transplantation in the literature. HVOD should be suspected when a recipient presents with hepatomegaly, ascites or jaundice after pancreas transplantation under tacrolimus.





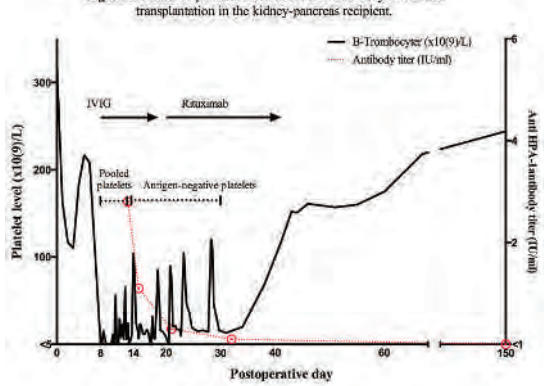
P.1871

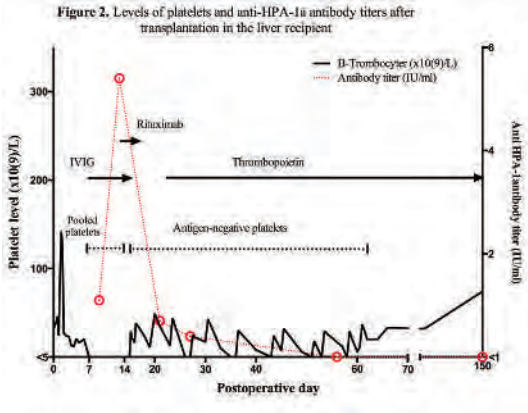
Severe transplantation-mediated alloimmune thrombocytopenia in two recipients of organs from the same donor

Isabella Sönnerborg[1], Petter Höglund[2], Johan Nordström[1], Lars Wennberg[1], Greg Nowak[1].
[1]Department of Transplant Surgery, Karolinska University Hospital, Stockholm, Sweden; [2]Department of Transfusion Medicine, Karolinska University Hospital, Stockholm, Sweden.

Introduction: Organ transplantation results in passive transfer of donor lymphocytes[1],[2]. We describe passenger lymphocyte syndrome with severe acute thrombocytopenia in two recipients, of a kidney-pancreas and liver graft respectively, from a single organ donor. The donor had no history of thrombocytopenia but donor serum contained an alloantibody against human platelet antigen 1a (HPA-1a).
Method: HPA-1a antibodies in donor and recipient sera titers were identified and quantified using MAIPA. Genotypes and microchimerism were determined by PCR.
Results and discussion: Both recipients developed acute thrombocytopenia with bleeding complications within two weeks post-operatively. The kidney-pancreas recipient also developed concomitant hemolytic anemia. Donor-derived HPA-1a antibodies were detected in serum from both recipients. In the liver recipient, thrombocytopenia was more severe, antibody titers higher and duration longer. Whereas the kidney-pancreas recipient responded partially to HPA-1a-positive platelet transfusions, the liver recipient required HPA-1a-negative transfusions for several weeks despite treatment with high-dose corticosteroids, immunoglobulin, rituximab and thrombopoietin. After two months, both recipients became negative for HPA-1a antibodies followed by increasing platelet counts, which is in line with previous observations on passenger lymphocyte syndrome. Two additional recipients, of a heart and a kidney, from the donor did not develop thrombocytopenia, suggesting that a larger inoculum of lymphoid cells in e.g. a liver or pancreatico-duodenal graft could increase the risk of transferring immune diseases.
Conclusion: Thrombocytopenia due to passenger lymphocyte syndrome is rare but potentially life-threatening[1]-[6]. To our knowledge, this is the first time transfer of alloimmune anti-platelet antibodies is reported in kidney-pancreas transplantation, and only the second time in liver transplantation[1]. These cases highlight the risk of transferring immune disease in organ transplantation and exemplify possible treatment approaches to transplantation-mediated alloimmune thrombocytopenia. Our observations support a cautious approach to using donors with known immune thrombocytopenia

Figure 1. Levels of platelets and anti-HPA-1a antibody titers after transplantation in the kidney-pancreas recipient.





References:
[1] West KA, Anderson DR, McAlister VC, Hewlett TJ, Belitsky P, Smith JW, et al. Alloimmune thrombocytopenia after organ transplantation. N Engl J Med. 1999;341(20):1504-7.
[2] Friend PJ, McCarthy LJ, Filo RS, Leapman SB, Pescovitz MD, Lumeng L, et al. Transmission of idiopathic (autoimmune) thrombocytopenic purpura by liver transplantation. N Engl J Med. 1990;323(12):807-11.
[3] de la Torre AN, Fisher A, Wilson DJ, Harrison J, Koneru B. A case report of donor to recipient transmission of severe thrombocytopenia purpura. Transplantation. 2004;77(9):1473-4.
[4] Diaz GC, Prowda J, Lo IJ, Arepally GM, Evans N, Wheeless Y, et al. Transplantation-mediated alloimmune thrombocytopenia: Guidelines for utilization of thrombocytopenic donors. Liver Transpl. 2008;14(12):1803-9.
[5] Kaestner F, Kutsogiannis DJ. Successful kidney and liver transplantation from a donor with immune thrombocytopenia. Transplant Proc. 2013;45(7):2838-40.
[6] Pereboom IT, de Boer MT, Haagsma EB, van der Heide F, Porcelijn L, Lisman T, et al. Transmission of idiopathic thrombocytopenic purpura during orthotopic liver transplantation. Transpl Int. 2010;23(2):236-8.
[7] Rozman P. Platelet antigens. The role of human platelet alloantigens (HPA) in blood transfusion and transplantation. Transpl Immunol. 2002;10(2-3):165-81. 16. Warkentin TE, Smith JW. The alloimmune thrombocytopenic syndromes. Transfus Med Rev. 1997;11(4):296-307.
[8] Hurtarte-Sandoval AR, Navarro-Cabello MD, Alvarez-Rivas MA, Robles-Lopez AI, Salmeron-Rodriguez MD, Aguera-Morales ML, et al. Passenger Lymphocyte Syndrome After Simultaneous Pancreas-Kidney Transplantation: A Case Report of an Unusual Cause of Alloimmune Hemolytic Anemia. Transplant Proc. 2015;47(9):2667-8.

P.1872

Successfull simultaneous pancreas kidney transplantation in a patient with congenital partial lipodystrophy

Pablo Uva, Luis Leon, Elena Minue, Roxana Pilotti, Ignacio Cabrera, Alejandro Giunippero, Antonio Gallo, Francisco Osella, Fernanda Toniolo, Eduardo Chuluyan, Domingo Casadei.
Pancreas Transplantation, Instituto de Nefrología - Nephrology, Buenos Aires, Argentina.

Introduction: Partial congenital lipodystrophy is a genetic disorder characterized by reduction or absense of adipose tissue with regional lipoatrophy combined with zones of lipohypertrophy and is associated with hypertriglyceridemia, insulin resistance and diabetes mellitus. Patophysiology remains unclear, however, decreased subcutaneous deposits of adipocytes and leptine could lead to hyperphagia. This could produce high caloric intake favouring ectopic fat deposits leading to insulin resistance. This patients could present with high insulin requirements, hypertriglyceridemia, steatosis and cardiomyopathies among others. Leptin replacement could ameliorate insulin resistance and hypertriglyceridemia. We here present a case of partial congenital lipodystrophy with end stage renal disease with a successfull simultaneous pancreas kidney (SPK) transplant. Case Report This is a 41 years-old female with partial congenital lipodystrophy. She was diagnosed with diabetes mellitus at age 15 and was started wih insulin therapy with high insulin requirements (115U/day). Her case was complicated with hypertriglyceridemia, bilateral retinopathy and end stage renal disease due to biopsy proven membranoproliferative glomerulonephitis type 1, on hemodialysis for the past 3 years. Her pre transplant studies revealed bad glucose control (HbA1c 7.9%) and a low C-peptide (2.7ng/mL). Leptin analogues were not available in the Country for treatment. On September 7th, 2015 she received a SPK transplant from an ideal donor. Induction therapy included Solumedrol and Antithymocyte Globulin (ATG Fresenius 15mg/kg). Maintenance immunosuppression included tacrolimus, sodium mycophenolate, steroids and belatacept. Belatacept was included to use lower tacrolimus levels. Both grafts function immediatly and required no postoperative insulin therapy and no hemodialysis. Her postoperative course was complicated by an intestinal obstruction leading to an intestinal anastomotic leak requiring conversion to a new Roux en Y duodenojejunostomy. She then presented a low output pancreatic fistula that closed with no other complications. Currently, at 5 months postransplant she has normal pancreatic and kidney function tests with a fructosamine of 237umol/L, HbA1c of 4.6, creatinine of 0.8mg/dL and no proteinuria. Tacrolimus level is kept at 6-8 ng/mL.
Discussion: This is to our knowledge the first report of a successful SPK transplant in a patient with Congenital Partial Lipodystrophy.

P.1873

En bloc simultaneous pancreas and kidney composite graft transplant with limited vascular access

Shin-E Wang, Yi-Ming Shyr.
Surgery, Taipei Veterans General Hospital, Taipei, Taiwan.

Purpose: Limited vascular access could be encountered in an obese or re-transplant patient. We described modifications that facilitated an en bloc simultaneous pancreas and kidney (SPK) composite graft transplant in an obese type 2 diabetic patient with renal failure under hemodialysis.
Materials and Methods: At the back-table, the superior mesenteric artery and splenic artery of the pancreas graft were reconstructed with a long "Y" iliac artery graft. The smaller left renal artery is anastomosed end-to-side to the larger and longer common limb of the arterial Y graft and the shorter portal vein is anastomosed end-to-side to the longer graft left renal vein. Thus, this en bloc composite graft allowed to facilitate "real" SPK transplant using single common graft artery and vein for anastomosis to one recipient arterial and venous site. The en bloc pancreas and kidney composite graft was implanted by suturing the graft left renal vein to IVC and graft common iliac artery the recipient distal aorta. Exocrine drainage was provided by anastomosis of the graft duodenum to a roux-en-y jejunum limb in an side-to-side fashion. Immunosuppressants included basiliximab, tacrolimus, mycophenolate mofetil, and methylprednisolone.
Results: The operative time was 7 hours with cold ischemic time of 6 hours and 25 min. and warm ischemic time of 47 min. The patient was discharged on postoperative day 20, with a serum creatinine level of 1.4 ng/ml and a blood glucose level of 121 mg/dL. He has not had any rejection episodes or postoperative complications in the following 12 months after the en bloc SPK transplant.
Conclusion: En bloc pancreas and kidney composite graft might be an option for patients with limited vascular access. This technique (1) facilitates "real" simultaneous pancreas and kidney (SPK) transplant with only single common artery and vein for implanting the composite graft; (2) minimizes dissection of vessels and conserves recipient vessels.

P.1874

Lymphadenopathic Kaposi's sarcoma mimicking PTLD after pancreas transplant

Shin-E Wang, Yi-Ming Shyr.
Surgery, Taipei Veterans General Hospital, Taipei, Taiwan.

Background: Kaposi's sarcoma currently comprises more than 5% of all de novo neoplasms in this group. The average time to development of Kaposi sarcoma following transplantation is 15-30 months. Human herpesvirus 8 (HHV-8) genomic sequences have been identified by polymerase chain reaction in more than 90% Kaposi sarcomas.
Materials: From 2003 to 2015 Kaposi's sarcoma was identified for study from 100 patients with 104 pancreas transplants performed at Taipei Veterans General Hospital. Literature review was also done.
Results: Only one case of Kaposi's sarcoma was identified, with an incidence of 1%. The patient suffered from varicellar zoster Infection (chicken pox) 11 months after pancreas transplant alone (PTA). Four months later (15 months after PTA), lymphadenopathy with enlargement of multiple lymph nodes in neck, around celiac trunk, along the superior mesenteric artery and abdominal aorta, which mimicked posttransplant lymphoproliferative disorder (PTLD). The biopsy for pathology turned out to be Kaposi's sarcoma. HHV-8 viral gene was detected by the molecular (PCR) assay. The lymphadenopathic Kaposi's sarcoma regressed 3 months after treatment by adding sirolimus, reducing the dose of tacrolumus and discontinuing mycophenolate mofetil. There has been no evidence of tumor recurrence for almost 5 years, and he has been enjoying an insulin-free life with euglycemia for more than 5 years.
Conclusion: This is an unusual HHV-8 associated Kaposi's sarcoma mimicking PTLD presenting as lymphadenopathic form, instead of usual cutaneous form. Sirolimus is recommended for the treatment of Kaposi's sarcoma, in addition to reduction, cessation or modification of immunosuppressive regimen.

P.1875

Optimization of procedures for short-time hepatocyte preservation prior to transplantation

Kengo Fukuoka[1], Satoru Yoshida[1], Takehiro Imura[2], Akiko Inagaki[2], Yasuhiro Igarashi[2], Muneyuki Matsumura[1], Shigehito Miyagi[1], Kazuo Ohashi[3], Noriaki Ohuchi[1], Susumu Satomi[1], Masafumi Goto[1,2].
[1]Division of Advanced Surgical Science and Technology, Tohoku University School of Medicine, Sendai, Japan; [2]Division of Transplantation and Regenerative Medicine, Tohoku University School of Medicine, Sendai, Japan; [3]Laboratory of Drag Development and Sciences, Graduate School of Pharmaceutical Science, Osaka University, Osaka, Japan.

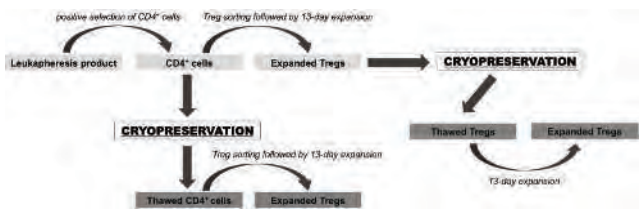
Background: In clinical hepatocyte transplantation, a short-time hepatocyte preservation prior to transplantation is inevitable. However, no optimal method for hepatocyte preservation has so far been established. We have recently developed a prominent oxygen-permeable bag (Tohoku Device (TD)) for both pancreatic islet culture and transplantation. Therefore, in this study, we first investigated whether TD is also effective for hepatocyte preservation. Moreover, we optimized suspended solutions and supplementations for short-time hepatocyte preservation.
Materials and Methods: Isolated hepatocytes were suspended in various solutions (200 ml) and were applied for 3 types of examinations. In the first study (n=8), suspended hepatocytes were preserved at room temperature for 2 hours under 3 different procedures: (I: Non-TD) ordinary standing tube preservation, (II: TD-shaking) TD with shaking (10 times per minute), and (III: TD-quiet) TD without any shaking. In the second study (n=8), hepatocytes were suspended in (I) Medium (common hepatocyte culture medium), (II) ET-Kyoto (ET-K), and (III) Wisconsin University solution (UW) and preserved in TD at 4 degrees for 4 hours with or without fetal bovine serum (FBS) supplementation. In the third study (n=6), hepatocytes were suspended in the culture medium supplemented with FBS or albumin (Alb) and preserved in TD at 4 degrees for 4 hours. After the preservation, survival rate of hepatocytes was calculated and trypan blue exclusion assay as hepatocyte viability tests was performed. The plating efficiency (PE) assay and deoxyribonucleic acid (DNA) quantitation of the cultured hepatocytes were measured.
Results and Discussion: In both the TD groups, the survival rate of hepatocytes was considerably higher than that of the tube group (P=0.39) and the hepatocyte viability was significantly higher than that of the tube group (P=0.03). The PE in the TD-quiet group was significantly higher than that of the ordinary tube group (P=0.027), whereas the DNA quantitation of the cultured hepatocytes was almost the same among the 3 groups. Thus TD-quiet is considered to be an effective procedure. In the second study, viabilities and PE were similar among the 3 groups under FBS supplementation, while the survival rate of hepatocytes in the Medium group was significantly higher than that of the other groups (P<0.001). Survival rates of hepatocytes preserved in any solutions without FBS were extremely low. Therefore, organ preserving solutions seems to be ineffective in case of short-time hepatocyte preservation. In the third study, survival rates, viabilities, and PE were comparable between the FBS and Alb groups.
Conclusions: TD-quiet together with suspending in the common medium is considered to be an effective procedure for a short-time hepatocyte preservation. Although FBS is an essential factor to maintain the quality of isolated hepatocytes, albumin might be a useful substitute for FBS in the clinical settings.
Megumi Goto; Kozue Imura
References:
[1] Goto M, Yoshikawa Y, Satomi S, et al. Optimization of a prominent oxgen-permeable device for pancreatic islets. *Transplant Proc*. 2008 Mar;40(2):411-412

P.1876

New strategies for cell banking for regulatory T cell (Treg)- based therapy in order to overcome compromise of cell viability and Treg altered phenotype during cryopreservation

Karolina Golab[1], Randall Grose[2], Veronica Placencia[3], Amittha Wickrema[3], Martin Tibudan[1], Sabarinathan Ramachandran[1], Sabrina Matosz[1], Julia Solomina[1], Natalia Marek-Trzonkowska[4], Piotr Trzonkowski[5], Piotr Witkowski[1].
[1]Department of Surgery, University of Chicago, Chicago, IL, United States; [2]South Australian Health and Medical Research Institute, University of Adelaide, Adelaide, Australia; [3]Department of Medicine, Hematology-Oncology, Cancer Research Center, University of Chicago, Chicago, IL, United States; [4]Department of Family Medicine, Medical University of Gdańsk, Gdańsk, Poland; [5]Department of Clinical Immunology and Transplantology, Medical University of Gdańsk, Gdańsk, Poland.

Introduction: Several clinical trials have been initiated testing safety and efficacy of T regulatory cell based therapy in organ/cell transplantation. Feasibility of such therapy would be improved, if cells could be cryopreserved until optimal timing for clinical infusion[1]. In our study, we tested two cell banking strategies for Treg therapy: 1) cryopreservation of $CD4^+$ cells for subsequent Treg isolation with expansion and 2) cryopreservation of expanded Tregs. We assessed impact of cryopreservation and thawing on cell viability and phenotype, and performed subsequent clinical-grade Treg isolation/expansion.
Materials and Methods: 1) Human $CD4^+$ cells were isolated from leukapheresis products on CliniMACS® device, frozen in clinical grade conditions, in control-rate freezer in PlasmaLiteTM with 5% human serum albumin, 10% DMSO and kept in liquid nitrogen. After 1 year, cells were thawed and cultured overnight. Cell viability/apoptosis and expression of CD25, CD127, FoxP3 markers were checked by flow cytometry. Next, Tregs ($CD4^+CD25^{hi}CD127^-$ cells) were isolated by FACS and expanded for 13 days upon stimulation with anti-CD3/CD28 beads in medium with interleukin-2.
2) Another portion of fresh $CD4^+$ cells was used directly to sort/expand Tregs. Expanded Tregs were cryopreserved and processed for thawing in the same manner as $CD4^+$ cells. As Tregs recovery was low, they were subsequently expanded again. All procedures were performed accordingly to cGMP guidelines and expansions were concluded with testing Treg final product to fulfill release criteria for the clinical application.



Results and Discussion: The average cell recovery was 75.6% and 45.4% just after thawing, but decreased to 38.2% and 19.9% after overnight culture for $CD4^+$ and Treg cells, respectively. That might be associated to relatively high apoptosis/necrosis rate just after thawing 24.2% for $CD4^+$ cells and 41.1% for Tregs. We observed that Treg phenotype upon cryopreservation was unstable: percentage of $CD4^+CD25^{hi}CD127^-$ cells in thawed $CD4^+$ cells decreased from 6.7% to 5.5% and increased to 7.4% after overnight culture. Similar observation was noticed for $CD4^+FOXP3^+$ population. After Treg thawing, % of $CD4^+CD25^{hi}CD127^-$ cells decreased from 98.4% to 80.2% and from 83.3% to 59.5% for $CD4^+FOXP3^+$ cells, and dropped after overnight to 57.4% and 54.1%, respectively. During the subsequent expansion, Treg also expanded well with the average fold increase in cell number – 130 and presented stable phenotype.

Conclusion: Our results indicate that cryopreservation affects cell recovery, viability and Treg marker expression. This impact should be considered when designing clinical protocols involving freezing, thawing and subsequent Treg infusions. Further optimization of Treg cryopreservation is warranted. In the meantime, Tregs recovered after thawing can be successfully re-expanded in vitro to overcome cell loss, impaired viability and instability of key Treg markers expression caused by cryopreservation.

References:

[1] Golab, K. et al. Challenges in cryopreservation of regulatory T cells (Tregs) for clinical therapeutic applications. *Int. Immunopharmacol*. 2013; 16:371–375.

P.1877

A parallel comparison of suppressive capacity in immune responses: regulatory T cells versus regulatory macrophages

Fei Guo[1], Dandan Huang[1], Wayne J.Hawthorne[1], Min Hu[1], Yuanfei Zhao[1], Joanne Hawkes[1], Heather Burns[1], Shounan Yi[1], Philip O'Connell[1].

[1]Centre for Transplant and Renal Research, The Westmead Institute for Medical Research, Westmead, Australia.

Introduction: Both human regulatory T cells (Treg) and regulatory macrophages (Mreg) are shown to be important in regulation of immune responses and have potential as a promising cell therapy for immunomodulation in both allo- and xeno-transplantation. However, which of these two types of regulatory cells are superior to the other in suppression of a particular immune response are yet to be shown. In this study we compared the suppressive capacity of human Treg and Mreg in different immune environments in vitro, thereby providing information to select an appropriate type of regulatory cells for suppression of a particular immune response when required.

Materials and Methods: Human PBMC were used for isolation of CD4+CD25+CD127lo Treg and CD14+ monocytes, respectively. Treg were polyclonally expanded with anti-CD3/CD28 beads, IL-2 and rapamycin and Mreg were induced from CD14+ monocytes with macrophage colony-stimulating factor (MCSF) and IFN-γ. Mix lymphocyte reaction (MLR) were performed to assess suppressive capacity of both regulatory cells in different immune responses using anti-CD3/CD28 beads and irradiated human and porcine PBMC as polyclonal, allogeneic and xenogeneic stimulators, respectively, human PBMC and CD4+CD25- T cells as responder cells and autologous Treg and Mreg as suppressor cells. Viability of regulatory cells and responder cells in MLR cultures was analyzed using annexin v apoptosis detection kit.

Results: Both Treg and Mreg demonstrated great capacity to suppress proliferation of PBMC and CD4+ T cells in response to polyclonal, allogeneic and xenogeneic stimulation. Despite no noticeable difference in their potency in suppression of either polyclonal or allogeneic response, Treg exhibited stronger suppression of proliferating xenoreactive responder PBMC, CD4+ and CD8+ T cells at ratios of 1:4 through to 1:16 of Mreg or Treg: responder cells when compared to that shown by Mreg. More Mreg than Treg were found undergoing apoptosis detected in the all three MLR settings.

Discussion: Compared to polyclonally expended human Treg, Mreg were less efficient in suppression of the xenogeneic response, which however, may not be correlated with their reduced viability in all MLR settings as they demonstrated similar suppressive potency to that Treg showed in the polyclonal and allogeneic responses.

Conclusion: Human Treg and Mreg have similar capacity to suppress either polyclonal or allogeneic response. However Treg are superior to Mregwhen required for suppression of the xenogeneic response. This different suppressive capacity between Treg and Mreg needs be further tested in vivo.

P.1878

Comparison of three transplantation techniques in a mouse model of hepatocyte transplantation

Carl Jorns[1], Masaaki Watanabe[1], Kristina Kannisto[2], Oscar Wiklander[2], Helen Zemack[1], Stephen Strom[2], Bo-Göran Ericzon[1], Ewa Ellis[1].
[1]Transplantation Surgery, Clintec, Karolinska Institutet, Stockholm, Sweden; [2]Department of Laboratory Medicine, Karolinska Institute, Stockholm, Sweden.

Hepatocyte transplantation is a promising treatment for patients with metabolic liver disease. Intraportal injection of isolated hepatocytes is currently the preferred transplantation techniques. However, portal administration is associated with significant immediate cell loss. Direct intrahepatic injection has been suggested as an alternative technique potentially attenuating this complication. The aim of this study was to compare three different transplantation techniques. Hepatocytes isolated from C57bl/6 mice were transplanted into ApoE (-/-) mice by intrasplenic (n=7), intrahepatic (n=8) and intraportal injection (n=6).
Analysis of engraftment by weekly serum ApoE concentration showed significantly lower serum ApoE concentration in the intrahepatic group compared to both intrasplenic and intraportal for each time point (0.06%±0.03 vs 0.56% ± 0.33 vs 0.33%±0.19; % of wildtype serum concentration). Six weeks after transplantation animals were sacrificed and analysis of hepatic mRNA expression showed significantly lower ApoE mRNA expression in the intrahepatic group compared to intrasplenic and intraportal injection (0.08%±0.04 vs 1.0%±0.4 vs 1.4%±0.3). Short term in vivo and ex vivo organ biodistribution studies using donor hepatocytes labeled with a near-infrared fluorescent lipofil dye were performed. While hepatocytes transplanted via the portal route mainly accumulated in liver a large proportion of intrahepatic transplanted hepatocytes were detected in the lungs 3 and 20 hours after infusion. Accumulation of hepatocytes in lungs could not be inhibited by clamping of liver veins. In conclusion, transplantation of hepatocytes by direct intrahepatic injection results in lower overall engraftment possibly caused by translocation of hepatocytes to the venous system with eventual accumulation in the lungs.

P.1879

Neonatal livers produce high quality hepatocytes with lower immunogenicity compared to adult-liver derived hepatocytes

Charlotte Lee[1], Emer Fitzpatrick [1], Sharon Lehec [1], Ragai RMitry[1], Shirin-Elizabeth Khorsandi[2], Paradzai Chitongo[3], Simon Walker[1], Raquel Fernandez-Dacosta [1], Celine Filippi [1], James Hardie [4], Nigel Heaton [2], Anil Dhawan [1].
[1]Dhawan Lab, Institute of Liver Studies, King's College London, London, United Kingdom; [2]Liver Transplantation, Institute of Liver Studies, King's College Hospital, London, United Kingdom; [3]Viapath, King's College Hospital, London, United Kingdom; [4]NHS Blood and Transplant, St Mary's Hospital, London, United Kingdom.

Introduction: Hepatocyte transplantation is a promising alternative to orthotopic liver transplantation (OLT) for children with liver-based metabolic disease and acute liver failure. A major hurdle to its success is early cell loss (up to 70% of hepatocytes) due to the instant blood mediated inflammatory response (IBMIR). Neonatal livers are generally not used for OLT in view of their low weight and small vessel size however may be an excellent source of good quality hepatocytes for clinical transplantation purposes. In addition, they may potentially be less immunogenic and thus may not elicit the IBMIR to the same extent as adult-liver derived hepatocytes. We set out to compare the viability, function and immunogenicity of neonatal and adult hepatocytes.
Methods: Neonatal hepatocytes were isolated as described by Dhawan et al 2012[1]. Cell viability was assessed using an MTT assay and hepatocyte function was determined with albumin and urea immunoassays. A Chandler loop model using heparin-coated PVC tubing was incubated at 37°C and rotated at 24rpm to mimic portal vein blood flow. This was used to compare neonatal and adult hepatocyte immunogenicity when cells were in contact with ABO-matched blood. Samples were taken at 0, 15 and 30 min following perfusion and full blood count measured on a haematology analyser. Plasma was analysed for cytokine expression using a Randox Array Chip.
Results: Neonatal hepatocytes had a high viability upon isolation 86% ± 3.7 (n=3). Hepatocytes were cultured overnight and MTT assays revealed neonatal hepatocytes had a significantly higher viability than hepatocytes isolated from adult livers (OD: mean ± SEM; 0.99 ±0.24 (n=3) Vs 0.34 ± 0.077 (n=6), $P<0.05$). Neonatal hepatocytes secreted significantly higher albumin than adult hepatocytes one day post plating (1028 ng/ml ±573 (n=3) Vs 139.7 ng/ml (n=6) $P<0.05$). There was no significant difference in urea production (3.2ng/ml ± 0.61 Vs 6.8 ng/ml ± 3.5), most likely due to lack of mitochondrial function. The Chandler loop model showed adult hepatocytes elicited a significant drop in platelet count compared to the control (55.5 x109 cell/L ± 12.5 Vs 178.8 x109 cell/L ± 17.6 n=6 $P<0.001$). Neonatal hepatocytes did not cause a drop in platelet count compared to the control (165 x109 cell/L). Cytokine expression in plasma from the loop was analysed in one neonatal dataset. This showed adult hepatocytes (N=3) eliciated higher concentrations of the pro-inflammatory cytokines IL-6, IL-1a, IL-IRA and VEGF and lower concentrations of the anti-inflammatory cytokine IL-10 than neonatal hepatocytes.
Conclusion: Hepatocytes isolated from neonatal livers have very high viability and albumin production. Initial results suggest neonatal hepatocytes do not activate the immune response compared to adult hepatocytes. Neonatal hepatocytes may engraft more successfully and function at a higher rate, improving the clinical efficacy of hepatocyte transplantation.
NIHR Biomedical Research Centre at Guys and St Thomas' Foundation Trust
References:
[1] Hughes R, Mitry RR, and Dhawan A. Current Status of Hepatocyte Transplantation. *Transplantation*. 2012;93: 342–347

P.1880

Coming home: Hepatocyte transplantation to the liver via the splenic artery in a juvenile large animal model

Nathanael Raschzok[1], Jeffrey Siefert[1], Petr Podrabsky[2], Karl H.Hillebrandt[1], Juliane K.Unger[3], Birgit Sawitzki[4], Steffen Lippert[1], Peter Tang[1], Anja Reutzel-Selke[1], Benjamin Struecker[1], Dominik Geisel[2], Mehmet H.Morgul[1,5], Andreas W.Reske[6], Sabine Kafert-Kasting[7], Wolfgang Rüdinger[7], Jörg Rennecke[7], Philipp Pickerodt[8], Safak Gül-Klein[1], Timm Denecke[2], Jens Oetvoes[3], Johann Pratschke[1], Igor M.Sauer[1].
[1]General, Visceral, and Transplantation Surgery, Experimental Surgery and Regenerative Medicine, Charité – Universitätsmedizin Berlin, Berlin, Germany; [2]Klinik für Radiologie, Campus Virchow-Klinikum, Charité – Universitätsmedizin Berlin, Berlin, Germany; [3]Department of Experimental Medicine, Charité – Universitätsmedizin Berlin, Berlin, Germany; [4]Institute of Medical Immunology, Charité – Universitätsmedizin Berlin, Berlin, Germany; [5]Visceral-, Transplantation-, Thoracic and Vascular Surgery, University of Leipzig, Leipzig, Germany; [6]Department of Anesthesiology and Intensive Care Medicine, University Hospital Leipzig, Leipzig, Germany; [7]Cytonet, GmbH & Co. KG, Weinheim, Germany; [8]Dept. of Anesthesiology and Intensive Care Medicine, Campus Virchow Klinikum and Campus Mitte, Charité – Universitätsmedizin Berlin, Berlin, Germany.

Introduction: Hepatocyte transplantation (HcTx) has shown promising results for treatment of metabolic liver disease in newborns and children. The most common route for application is the portal vein, which however is difficult to access in newborns. Easier accessible routes are needed to establish HcTx for clinical routine. The spleen has already been studied as alternative implantation and application site for hepatocytes. We here investigate the feasibility of HcTx via an interventionally placed catheter in the splenic artery in a juvenile pig model.
Methods: Göttingen minipigs (6 – 13 kg) were repetitively infused with human hepatocytes into the splenic artery via catheterization of the femoral artery. The animals were sacrificed either directly after cell infusion (n=2), two days after infusion (n=1) or 14 days after infusion (n=1). The splenic and portal venous blood flow was controlled via color-coded Doppler sonography. Computed tomography was performed after the second cell infusion and at the end of follow up in the animal scarified 14 days after HcTx. Blood samples for clinical chemistry were taken before and after transplantation. Tissue samples from the liver, spleen, and lung were stained for human CK18.
Results: Catheter placement was feasible in all cases. Repetitive transplantation of 1,00E+08 cells per kg BW per session was possible without adverse effects on blood flow in portal vein or splenic artery. In one animal, partial thrombosis was observed in the distal splenic artery at day two after infusion, completely resolving during the follow-up period. Immunohistochemistry demonstrated cell translocation through the portal-venous system into the liver, beginning directly after infusion. Smaller numbers of cells relocated into the lung.
Conclusions: Our results demonstrate that HcTx via interventional access to the splenic artery is feasible in juvenile minipigs. The cells are able to pass through the spleen to relocate in the liver. The splenic route should therefore be further evaluated as interventional approach for HcTx.

P.1881

Humoral diagnosis of acute rejection in myogenic cell allotransplantation in skeletal muscles: A study in nonhuman primates

Daniel Skuk, Jacques P.Tremblay.
Axe Neurosciences, Research Center of the CHUQ, Québec, QC, Canada.

Introduction: Transplantation (Tx) of cells with myogenic properties has potential applications in the treatment of several muscle disorders. Excluding purely autologous cell Tx, graft survival depends on a good control of acute rejection (AR). Monitoring AR is essential to treat this condition and to preserve the graft. Since there were no established criteria to diagnose AR in clinical cell Tx in the skeletal muscle, we conducted studies in nonhuman primates to fill this void. We have already determined the essential elements for the histopathological diagnosis[1]. In this study, we analyze the value of the detection of antibodies against the donor cells in the recipient's serum.
Material and Methods: We allotransplanted muscle precursor cells (MPCs) in macaques immunosuppressed with tacrolimus. To induce a clear rejection of the myofibers expressing allogeneic proteins we immunosuppressed monkeys at optimal levels for a month (to allow complete myofiber regeneration by the grafted MPCs in a stable graft) and then we withdraw tacrolimus (week 0). MPC-grafted sites were biopsied at every 2 weeks after tacrolimus withdrawal and analyzed by histology until the resolution of rejection (week 12-16). Blood samples were taken before Tx and at every 2 weeks after tacrolimus withdrawal to detect antibodies against the donor's MPCs by flow cytometry, using a goat anti-monkey antibody conjugated to FITC.
Results and Discussion: As previously determined [1], the histological feature enabling to diagnose AR in this context was the presence of dense focal accumulations of CD8+ and CD4+ lymphocytes, partially or completely surrounding myofibers. All monkeys showed an increase of antibodies against the donor's MPCs, taking as a reference the pre-Tx and pre-rejection values. This increase was highly variable in intensity, and preceded (~ 2 weeks), coincided or followed (~ 2 weeks) the focal lymphocyte accumulations and/or the graft loss, and remained thereafter. Levels of antibody detection after the start of immunosuppression and Tx were the same or slightly higher or lower than the pre-Tx values.
Conclusion: The development of antibodies against the donor's MPCs was consistently associated with AR. A clear increase would indicate current or past AR. However, smaller increases in the level of detection of these antibodies are not necessarily associated with AR.
Jesse's Journey Foundation for Gene and Cell Therapy of Canada
References:
[1] Skuk D. Acute rejection of myofibers in nonhuman primates: Key histopathologic features. *J. Neuropathol. Exp. Neurol.* 71: 398-412; 2012.

P.1882

Evaluation of potential of umbilical cord blood-derived regulatory macrophages for cellular immunotherapy

Xiaoqian Ma[1], Jia Wang[1], Cejun Yang[1], Juan Zhang[1], Pengfei Rong[1], Shounan Yi[1,2], Wei Wang[1].
[1]The Third Xiangya Hospital of Central South University, Cell Transplantation & Gene Therapy Institute, Changsha, People's Republic of China; [2]Center for Transplant & Renal Research, The Westmead Institute for Medical Research, University of Sydney, Sydney, Australia.

Background: Regulatory macrophages (Mreg) have been experiencing a clinical trial in the One Study as a potential cell therapy for immunomodulation in transplantation. However the limited number of Mreg induced from individual adult peripheral blood mononuclear cells (PBMC) may restrict their application. Thus, exploring an alternative source for Mreg is necessary for the development of Mreg-based therapy. In this study we investigated the potential of cord blood derived Mreg for cellular immunotherapy.
Materials and Methods: CD14+ monocytes isolated from adult PBMC (APB) and cord blood (CB) were cultured with macrophage colony-stimulating factor (M-CSF) for 7 day with IFN-γ added at day 6 for Mreg induction. Mreg phenotype was characterized by flow cytometry, and their suppressive function was assessed by mixed-lymphocyte reaction (MLR) using anti-CD3/CD28 microbeads as stimulators, CFSE-labelled CD14- PBMC as responder cells and autologous and third-party APB- and CB-Mreg as suppressor cells. The non-specific effect of third-party (allo) CB-Mreg on naïve T cell proliferation and their immunogenicity were evaluated by coculturing T cells in the presence and absence of anti-CD3/CD28 microbeads with non- or irradiated CB-Mreg, respectively, followed by flow cytometry analysis.
Results: Mreg induced from both APB and CB showed no difference in their yield and phenotype. As a result, both APB- and CB-Mreg demonstrated similar potent suppression of proliferating polyclonal- and third-party (allo)-reactive CD14- PBMC, CD4+ and CD8+ T cells at ratios of 1:1 and 1:2 of Mreg: responder cells. Interestingly, CB-Mreg exhibited stronger suppression of proliferation of alloreactive CD14- PBMC and CD8+ T cells at a 1:4 ratio of Mreg: responder cells when compared to APB-Mreg. The proliferation of anti-CD3/CD28 microbeads stimulated but not non-stimulated allo T cells was impaired substantially by coculturing with CB-Mreg. However allo T cells did not proliferate when cultured with irradiated CB-Mreg.
Discussion: Compared to APB-Mreg, CB-Mreg had similar or stronger capacity to suppress the allogeneic response with low immunogenicity and no significant effect on naïve allo T cell proliferation in an in vitro alloimmune environment.
Conclusions: Our shows third-party CB-Mreg as a potential source for sufficient number of human Mreg used for clinical cellular therapy and may meet the demands of setting up a cell bank for transplantation immune tolerance.
This work was supported by NSFC 81201171

P.1883

Necroptosis plays a central role in hypoxia-induced islets cell death

Longlong Xie, Wei Li, Xiaoqian Ma, Cejun Yang, Juan Zhang, Bin Ye, Shen Liu, Wei Wang.
The Third Xiangya Hospital of Central South University, Cell Transplantation & Gene Therapy Institute, Changsha, People's Republic of China.

Objective: Xenotransplantation of pancreatic islets represents a promising therapeutic alternative for the treatment of type 1 diabetes mellitus. However, hypoxia-induced injury or death of islets graft was often observed after transplantation, and the underlying molecular mechanism has not yet been completely elucidated. Programmed necrosis (necroptosis) is a caspase-independent form of regulated cell death which implicated in the development of a range of diseases, including inflammatory, autoimmune and neurodegenerative. It's widely accepted that apoptosis and necroptosis are the main manners involved in cell death regulation. In this study we investigated the role of necroptosis in hypoxia-induced islets cells death.
Methods: 3-to 5-old new born pigs were supplied by Xeno company. The NICCs were isolated from the donor pancreas and divided into four groups followed by pre-treated with vehicle, Nec-1, zVAD and Nec-1/zVAD in a hypoxia incubator with 5% CO2 and 1% O2, respectively. Cell viability was assessed by MTS assay at various time points, and protein markers involved in necroptosis were tested via immunoblotting. The variation of inflammation factors was evaluated by q-PCR array and ELISA. Treatment with Etanercept, a specific inhibitor for TNFR, was conducted to further confirm the effect of TNFα in hypoxia-induce necroptosis and inflammation.
Results: We identified that necroptosis plays a crucial role in hypoxia-induced islets cell death in vitro. We demonstated that the activation of RIPK1/RIPK3/MLKL signaling pathway was involved in islets cells necroptosis. More importantly, TNFα and other inflammation factors were dramatically up-regulated in hypoxia-induced necroptosis, pre-treated with Nec-1 or Etanercept substantially inhibited necroptosis and TNFα secretion, which implied hypoxia-induced necroptosis was inflammation-dependent.
Conclusions: Taken together, our results suggest necroptosis was the main manner of hypoxia-induced islets cell death in vitro, and we unexpectedly found TNFα and other inflammatory factors plays pivotal role in this procedure.
This work was supported by NSFC 81201171

P.1884

Induced pluripotent stem cells play an immunomodulatory role in skin allograft acceptance

Charles Yuen Yung Loh[1,2,3], Aline Yen Ling Wang[1], Gerald Brandacher[4], Sheng-Hao Chuang[1], Fu-Chan Wei[1,3].
[1]Department of PRS, Vascularized Composite Allotransplantation (VCA) Center, Chang Gung Memorial Hospital, Linkou, Taiwan; [2]Division of Surgery and Interventional Science, University College London, London, United Kingdom; [3]College of Medicine, Chang Gung University, Linkou, Taiwan; [4]Department of PRS, Vascularized Composite Allotransplantation (VCA) Laboratory, Johns Hopkins University School of Medicine, Baltimore, MD, United States.

Authors 1 & 2: Equal contribution

Introduction: Vascularized composite allotransplantation (VCA) is a promising new field in plastic surgery. Skin is known to be the most immunogenic component of a composite tissue and therefore is the primary target of alloreactive lymphocyte-mediated rejection.

Induced pluripotent stem cells (iPSCs) have pluripotent capabilities comparable to that of embryonic stem cells (ESCs) but do not come with the ethical considerations that ESCs have[1]. iPSCs have been suggested to possess immunosuppressive effects in mixed lymphocyte reaction assays[2]. However, iPSCs and their immunomodulatory effects both in vivo and in transplantation have never been investigated.

This novel study explores the potential of iPSCs as cell therapy in prolonging skin allograft survival in a murine model. We utilize a transient episomal vector reprogramming method of generating iPSCs in this study which increases its safety profile and harnesses its potential for clinical application.

Material and Methods: Mouse Embryonic Fibroblasts (MEFs) were transfected with pCX-OKS-2A plasmids, which were a gift from Shinya Yamanaka (Addgene plasmid # 19771). The reprogrammed MEFs were then cultured in iPSC medium. The resulting iPSCs were harvested and injected intravenously (iv) in the experimental group on POD 0 whilst the control group had PBS iv.

Skin grafts from the tails of C57BL/6 mice were transplanted to BALB/c mice. Skin graft survival and immune cell profiles were evaluated. Rejection was determined when necrosis was first observed.

Results and Discussion: iPSCs generated in our lab were characterized in order to confirm the level of pluripotency. Pluripotent gene expression levels such as Nanog, Klf4, Sox2 and Oct3/4 in iPSCs were similar to ESCs when quantified using immunocytochemistry, real-time quantitative polymerase chain reaction and flow cytometry.

iPSCs demonstrated immunosuppressive effects on in vitro proliferation assay testing with lymphocyte mitogen Concanavalin A, monoclonal antibody T cell receptor stimulator anti-CD3 and mixed alloreactive lymphocyte proliferation assays.

The control group consistently demonstrated rejection with skin allograft necrosis around postoperative day 7 whereas iPSC mice had significantly prolonged skin allograft survival.

This correlated with histological findings of a lesser degree of skin allograft lymphocytic infiltration in iPSC mice. The skin allograft architecture in the iPSCs group also was better preserved compared to the control group, which was consistent with a lower degree of inflammation and tissue destruction.

Conclusion: The evidence presented provides preliminary evidence that iPSCs prolong allograft survival via orchestrating an anti-inflammatory cell-cytokine composition, both systemically and locally, which in turn results in the promotion of skin allograft survival through the creation of a 'graft protective microenvironment'.

References:

[1] Lu X, Zhao T. Clinical therapy using iPSCs: hopes and challenges. Genomics Proteomics Bioinformatics. 2013 Oct;11(5):294-8.

[2] Schnabel LV, Abratte CM, Schimenti JC, et al. Induced pluripotent stem cells have similar immunogenic and more potent immunomodulatory properties compared with bone marrow-derived stromal cells in vitro. Regen Med. 2014;9(5):621-35.

P.1885

Generation of novel Cag-Luc-EGFP transgene-expressing murine embryonic stem cell derived hepatoblasts as a transplantable cell source

Trinidad Cisneros, Justin Arredondo-Guerrero, Xiumei Qu, Sheri M.Krams, Carlos Esquivel, Olivia Martinez.
Surgery/Abdominal Transplantation, Stanford University School of Medicine, Stanford, CA, United States.

Introduction: Challenges to the clinical use of stem cell technology in transplantation include the low survival and poor engraftment of transplanted cells. The purpose of this study is to: 1) derive embryonic stem cell (ESC) lines expressing the CAG-luc-eGFP (L2G85) reporter genes that will allow for in vivo monitoring of the survival and engraftment of transplanted cells, and 2) use the ESC to generate ESC-derived hepatoblasts (ESC-DH) in vitro for transplantation.

Materials and Methods: ESC expressing the L2G85 reporter genes were derived from FVB-Tg(CAG-luc,-GFP)L2G85Chco/J blastocysts. ESC were cultured in 2i Media and their pluripotency was characterized by expression of Oct4, Nanog and SSEA1 by flow cytometry and gene expression analysis. Flow cytometry and bioluminescent imaging (BLI) was used to determine the expression levels of L2G85. Teratoma formation assay was used to determine in vivo pluripotency, and chromosomal counts were determined by karyotype analysis. In vitro differentiation of ESC into ESC-DH was done using a 13-day, modified Gouon-Evans two-step differentiation protocol. Differentiation was monitored by flow cytometry, gene expression and microscopy. Transplantation of 2x106 ESC-DH under the renal capsule of syngeneic FVB mice (H-2kq) was conducted to monitor the engraftment and survival of graft cells by BLI.

Results and Discussion: Thirteen ESC clones were successfully derived with >90% OCT4, Nanog and SSEA expression. Karyotype analyses revealed ESC contain normal chromosomal counts. At day 13 of differentiation, our modified protocol produces cells that are >90% positive for the hepatoblast marker CD326+cKIT, and display morphologic and functional features of the hepatocyte lineage including albumin and glycogen production, and expression of hepatoblast-associated genes. Furthermore, transplanted ESC-DH survive for over 3 weeks in vivo and can be monitored by BLI.

Conclusion: We have developed novel mESC lines expressing the CAG-luc-eGFP L2G85 transgene that can be used to generate cells from all three germ layers. Moreover, we have established a reproducible in vitro protocol for generating ESC-DH in the absence of a co-culture system with a high degree of survival following differentiation and transplantation. This strategy can be used for elucidating the immune response to ESC-derived hepatoblasts and, potentially, for cell replacement therapy for liver disease.

P.1886

Intravascular transplantation of CD34+ stem cells inhibit intimal hyperplasia after vascular injury

Piotr Religa, Sharan Ananthaseshan .
Medicine, Solna, Karolinska Instiutet, Stockholm, Sweden.

Introduction: Vascular progenitor cells contribute to repair of injured vasculature. In this study, we aimed to investigate the role of bone marrow derived cells in the intimal formation after arterial injury.
Methods and Results: Balloon injury of wild type mice femoral artery was followed by local delivery of bone marrow-derived cells from GFP transgenic mice. The arteries were collected 1, 4, 7 and 14 days after injury and studied for morphology, localization and phenotypes of delivered cells. Bone marrow-derived cells were present in the intima only at the early stages of arterial injury and expressed endothelial progenitor cell markers (CD31, CD34, VEGFR-2). In the areas where intima was thicker, bone marrow-derived cells differentiated to intimal smooth muscle cells but they did not fuse with intimal cells. Delivery of these cells contributed to a 1.4 fold inhibition of intimal hyperplasia.
Conclusion: Bone marrow-derived endothelial cells differentiated but not fused with vascular smooth muscle cells at the early stages of intimal formation and inhibited intimal hyperplasia.

P.1887

Tunnelling nanotube-based mitochondrial transfer from MSCs to human hepatocytes as a potential mechanism for cell functionality enhancement in hepatocyte transplantation

Raquel Fernandez Dacosta, Celine Filippi, Charlotte Lee, Valeria Iansante, Simon Walker, Sharon Lehec, Emer Fitzpatrick, Anil Dhawan.
Dhawan Lab - Hepatocyte Biology and Transplantation Group- at the Institute of Liver Studies, King's College London, London, United Kingdom.

Introduction: Intraperitoneal transplantation of encapsulated primary human hepatocytes is an emerging therapy for children with acute liver failure[1]. However sustaining the function of cryopreserved hepatocytes isolated from marginal grafts remains a challenge. Mesenchymal stromal cells (MSC) enhance hepatocyte viability and metabolism, through mechanisms not yet fully understood[2]. We hypothesized that MSC rescue hepatocyte function via the transfer of mitochondria through tunneling nanotubes (TNT). Therefore, the aims of this study were to (i) assess if a TNT-based transfer of mitochondria occurs between MSC and hepatocytes; (ii) to study TNT composition and formation inhibition, and (iii) to determine if TNT inhibition stops mitochondria transfer and affects MSC trophic effects on hepatocytes.
Materials and Methods: Mitochondrial transfer from MSC to hepatocytes was followed over 24h of co-culture using FACS as well as live- and fixed-cell microscopy, after staining of MSC mitochondria with mitotracker red. TNT presence in the co-cultures was studied by fluorescent microscopy. Characterization of TNT was performed by immunofluorescence to detect actin and tubulin expression. TNT-disruption was tested by dosing actin polymerization inhibitors: latrunculin A (LatA) and cytochalasin D (CytD). MTT and Anexin V/PI assays were used to assess LatA/CytD cytotoxicity. Mitochondrial transfer after TNT inhibition was quantified by FACS.
Results: Hepatocytes containing MSC mitochondria were detected as early as 2h after co-culture onset, using live microscopy (n=3) and confirmed by FACS (n=3), with statistical significance at 4 and 6h of co-culture (21±6 and 27.6±10%, $p<0.05$ vs monocultures). TNT containing red-labelled MSC mitochondria were observed between co-cultured MSC and hepatocytes. Actin was identified as the main TNT structural component. 0.5µM LatA achieved TNT inhibition without cytotoxic effect on the cells, leading to a decrease in the number of hepatocytes with MSC mitochondria.
Discussion: MSC seems able to transfer mitochondria to primary human hepatocytes through F-actin-based tunnelling nanotubes. However, full disruption of TNT-formation by 0.5µM LatA inhibited mitochondria migration from MSC to hepatocytes but only partially, leading to the idea of a potential overestimation of mitochondria transfer due to the additional cell-to-cell tranfer of mitotracker. The transfection of MSC with a mitochondria fluorescent protein will resolve this issue. The importance of mitochondrial transfer will be confirmed by the study of TNT inhibition effects on MSC trophic effects.
Conclusion: Our preliminary studies show that MSC do transfer mitochondria to primary hepatocytes through actin-based TNT, which might be the responsible mechanism for the beneficial effects of MSC on hepatocyte function. This data is helpful to further advance our knowledge to use co transplantation of MSC and hepatocytes in the treatment of liver disease.
References:
[1] Jitraruch, S.; Dhawan, A.; Hughes, R. D.; Filippi, C.; Soong, D.; Philippeos, C.; Lehec, S. C.; Heaton, N. D.; Longhi, M. S.; Mitry, R. R. Alginate microencapsulated hepatocytes optimised for transplantation in acute liver failure. PLoS One. 9:e113609; 2014.
[2] Fitzpatrick, E.; Wu, Y.; Dhadda, P.; Hughes, R. D.; Mitry, R. R.; Qin, H.; Lehec, S. C.; Heaton, N. D.; Dhawan, A. Co-culture with mesenchymal stem cells results in improved viability and function of human hepatocytes. Cell Transplant. 1–29; 2013.

P.1888

Relevance of Immunomodulatory factors: HLA-G, Treg cells and cytokine levels in hematopoietic stem cell transplantation

Uma Kanga[1], Manish Mourya[1], Tulika Seth[2], Kaustav Chowdhury[1], Dipendra K. Mitra[1], Poonam Coshcic[3], Manoranjan Mahapatra[2].
[1]Dept of Transplant Immunology and Immunogenetics, All India Institute of Medical Sciences, Delhi, India; [2]Dept of Hematology, All India Institute of Medical Sciences, Delhi, India; [3]Dept of Tansfusion Medicine, All India Institute of Medical Sciences, Delhi, India.

Introduction: HLA-G is a non classical HLA-class I molecule which has immunemodulatory role. HLA-G interacts with the receptors Immunoglobulin-Like-Transcript (ILT2 and ILT4) and mediates long-term induction of several immune cells including HLA-G-expressing CD4, CD8, Treg cells, and IL-10 producing Tr1 cells. The increased IL-10 production promotes differentiation of DC to DC-10 cells which further enhance IL-10 production and Treg cell generation. These events create a micro-environment that induces tolerance.
Material Methods: The present study was conducted on 50 patients (28 leukaemia and 22 aplastic anaemia) who underwent Hematopoietic Stem Cell Transplantation (HSCT). In this study, we evaluated the levels of soluble HLA-G, Treg cell frequency and levels of Th1 (IL-2, IL-17, TNF-α, and IFN-λ) and Th2 (1L-4 and IL-10) type cytokines at pre transplant and different post transplant time points (day 15, day 30, day 90 and at GvHD event).
Results: We observed that levels of soluble HLA-G1/G5 and Treg cell frequency was significantly lower at the time of GvHD, as compared to the pre transplant and post transplant time points. Further, we observed significantly lower levels of IL-4, IL-10 and higher levels of Th1 cytokines at the time of GvHD, in comparison to other time points.
Conclusion: we can conclude that HLA-G molecule creates an immunosuppressive micro-environment which modulates Treg cell frequency and Th2 cytokine levels that correlate well with development of GvHD and thus impacts the outcome of HSCT.

P.1889

HLA-G molecules: Possible predictor of GvHD

Manish Kumar Mourya[1], Tulika Seth[2], Poonam Coshcic[3], Manoranjan Mahapatra[2], Uma Kanga[1].
[1]Dept of Transplant Immunology and Immunogenetics, All India Institute of Medical Sciences, Delhi, India; [2]Dept of Hematology, All India Institute of Medical Sciences, Delhi, India; [3]Dept of Transfusion Medicine, All India Institute of Medical Sciences, Delhi, India.

Introduction: HLA-G is a non classical HLA-class I molecule which has immunomodulatory role. The polymorphism at HLA-G 3'UTR region controls post transcriptional regulation through the interaction with different miRNA. HLA-G interacts with several immune cells and creates a micro-environment that induces tolerance.
Aim and Objective: The present study was conducted on 50 patients (28 leukaemias and 22 aplastic anaemia) who underwent Hematopoietic Stem Cell Transplantation (HSCT). In this study, we investigated HLA-G 3'UTR 14pb polymorphism and miRNA interaction site within 3'UTR. Additionally, we evaluated the levels of soluble HLA-G, at pre transplant and different post transplant time points.
Results: The Kaplan Meier survival curve revealed that recipients with 14bp ins/ins genotype had lower incidence of GvHD in comparison of those with 14bp del/del genotype. We also observed that incidence of the GvHD was higher in recipients with HLA-G 3'UTR +3035CC, in comparison to +3035TT/TC. Among recipients carrying +3142 GG/CC genotype, incidence of GvHD was lower. Haplotype analysis of HLA-G 3'UTR revealed presence of 7 UTR haplotypes in North Indian population. UTR1 and UTR 3 were significantly associated with GvHD. Further, we observed that levels of soluble HLA-G1/G5 was significantly lower at the time of GvHD, as compared to the pre transplant and post transplant time points.
Conclusion: Considering these factors together, we can conclude that soluble HLA-G levels and/or HLA-G 3'UTR polymorphism can serve as possible biomarker for predication of GvHD.

P.1890

Development of an anti-HLA antibody-producing humanized mouse model

Senichiro Yanagawa, Hiroyuki Tahara, Hideki Ohdan.
Gastroenterological and Transplant Surgery, Hiroshima University, Hiroshima, Japan.

Chronic antibody-mediated rejection due to anti-human leukocyte antigen (HLA) antibodies (Abs) is an important problem in organ transplantation. The spleen has been reported to be the source of donor-specific antibody (DSA)-producing cells in the anti-donor Ab-producing mouse model; however, the source of anti-HLA Ab-producing cells has not been identified in most humanized mouse models. Therefore, to investigate such anti-HLA Ab-producing cells, we attempted to develop an anti-HLA Ab-producing humanized mouse model.
Severely immunodeficient (NSG) mice were humanized by injection of human cord blood- or bone marrow-derived hematopoietic stem cells (HSCs). After the appearance of T, B, and myeloid cells in the peripheral blood of the humanized mice, peripheral blood mononuclear cells (PBMCs) derived from healthy volunteers were injected into the mice as alloantigens (allo-PBMCs). Allo-PBMCs were checked by flow cytometry to distinguish HLA-Ab type between recipient and donor cells. Before injection of allo-PBMCs, human total IgG and anti-HLA antibodies were not detected in the sera of all mice (n = 15). At 2–4 weeks after injection of allo-PBMCs, we measured human total IgG and anti-HLA class I antibody levels in sera by using a multiple antigen bead-based assay (WAKFlow; n = 15). Non-DSA anti-HLA-Abs, but no DSA anti-HLA-Abs, were detected in 6 of 15 mice. Although these detected anti-HLA antibodies were not donor-specific or cross reactive group (CREG), we succeeded in developing a HSC-humanized mouse model that produced anti-HLA antibodies.
The same approach was employed in naïve NSG mice (n = 12) humanized by PBMCs and then injected with allo-PBMCs; 4 mice were injected with allo-PBMCs four times, while the remaining mice were injected only once. Non-DSA HLA-Abs were detected in only 3 of 12 mice. Levels of human total IgG in the sera of these mice were not increased, suggesting that the tolerance for donor antigen was due to multiple antigen injections. We are now attempting to develop a humanized mice model that can produce anti-HLA-antibodies.
We believe that the successful development of such anti-HLA Ab-producing humanized mouse models will enable the further development of in vivo patient-specific therapies, including DSA desensitization, for use during organ transplantation.

P.1895

Generation of GalT-KO pig with expression of human CD55, human endothelial protein C receptor and human transmodulin for xenotransplantation

Jongki Cho[1,2], Ghangyong Kim[1,2], Xun Fang[1,2], Pantu Kumar Roy[1,2], Euncheol Lee[1,2], Kyuhong Min[1,2], Youngkwang Yu[1,2], Seungwon Na[1,2], Bahia Mahamed Saliah Hassan[1,2], Byeongchun Lee[3], Youngtae Kim[2,4], Chungkyu Park[2,4].
[1]College of Veterinary Medicine, Chungnam National University, Daejeon, Korea; [2]Xenotransplantation Research Center, Seoul National University, Seoul, Korea; [3]College of Veterinary Medicine, Seoul National University, Seoul, Korea; [4]College of Medicine, Seoul National University, Seoul, Korea.

This study was performed to generate the a-1,3-Galactosyltransferase knockout (GalT-KO) pig with expression of human CD55 (hCD55), human endothelial protein C receptor (hEPCR) and human transmodulin (hTM) for xenotransplantation into primates by somatic cell nuclear transfer (SCNT). Donor cells (GalT-/-×hCD55×hEPCR×hTM) for SCNT were kindly provided by Dr Galli (Perota et al., 2011) and used as donor cells after proliferation by subculture. After SCNT, 100-200 reconstructed oocytes were transferred into 12 recipients with natural estrous heat and 28 recipients with synchronized estrus. In case of recipients with natural estrus, 7 recipients (77.8%) were diagnosed as pregnant by ultrasonography on 35 days after ultrasonography. However, pregnancy rates (25.0%) in the recipients with synchronized estrus were significantly lower that of natural estrus ($P<0.05$). After genotyping in the cloned piglets after parturition, GalT-KO was confirmed and gene of hCD55, hEPCR and hTM were confirmed.
In conclusion, we produced transgenic pig with GalT-KO and introduction of multiple human gene to decrease the immune-rejection reaction after xenotransplantation.
This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI13C0954)

P.1896

Preformed IgM antibody target in non-human primate received α-GTKO pig heart and artery

Yun Shin Chung[1,2,3], Sunghoon Hurh[1,2,3], Eun Mi Lee[1,2,3], Dohyun Han[2], Hyeon Keun Chee[4], Hyeon Sook Yang[5], Keon Bong Oh[6], Ka Hee Cho[7], Su-Cheol Han[8], Zahid Alam[1,2,3], Min Jung Kim[1,2], Young June Kim[3], Jaeseok Yang[1,2,9], Ik Jin Yun[10], Sang-Il Min[11], Curie Ahn[1,2,3,9,12].
[1]Transplantation Research Institute, Seoul National University College of Medicine, Seoul, Korea; [2]Center for Medical Innovation, Biomedical Research Institute, Seoul National University Hospital, Seoul, Korea; [3]Designed Animal & Transplantation Research Institute, Green Bio Science & Technology, Seoul Nationa University, Pyeongchang, Korea; [4]Cardiovascular Surgery, Konkuk University Hospital, Seoul, Korea; [5]Internal Medicine, Konkuk University Hospital, Seoul, Korea; [6]Animal Biotechnology, National Institute of Animal Science, Wanju, Korea; [7]Animal Research Center, Orient Genia Inc., Sungnam, Korea; [8]General Toxicology Research Center, Jeonbuk Department of Inhalation Research, Korea Institute of Toxicology, Jeongeup, Korea; [9]Transplantation Center, Seoul National University Hospital, Seoul, Korea; [10]Surgery, Konkuk University Hospital, Seoul, Korea; [11]Surgery, Seoul National University College of Medicine, Seoul, Korea; [12]Nephrology, Seoul National University College of Medicine, Seoul, Korea.

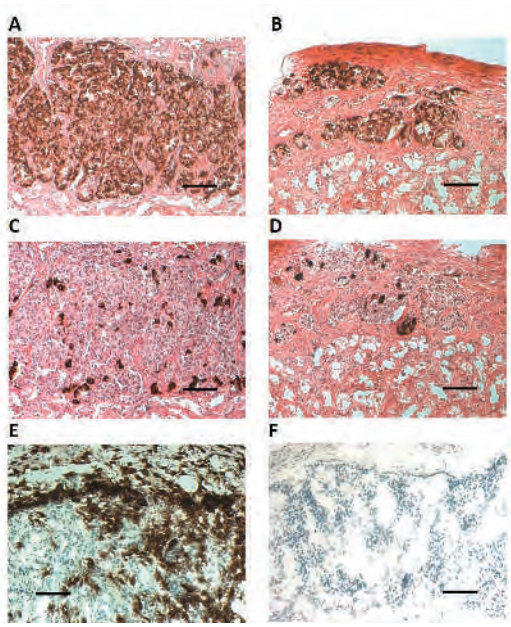
Background: Although α-galactosyltransferase knock out (GTKO) pigs overcome the hyper-acute rejection in pig organ xenotransplantation to non-human primate (NHP), preformed antibodies (Abs) are still problematic to porcine antigens on xenografts of NHP. We evaluated the potential reactivity of preformed monkey IgM and IgG Ab and identified preformed monkey Ab targets on GTKO pig xenografts of NHP received GTKO pig heart and artery.
Methods: The concentration of monkey IgM, IgG and IgA Ab of naïve monkey sera was determined by ELISA. Binding affinity of preformed monkey IgM and IgG Ab of naïve monkey sera to GTKO pig aortic endothelial cells (PAECs) was analyzed and compared with those to GT wild type (WT) PAECs by flow cytometry. Complement-mediated cytotoxicity of naïve monkey sera to WT and GTKO PAECs was analyzed by flow cytometry. GTKO porcine heart and artery were transplanted into NHP and monkey IgM and IgG Ab were observed in the xenografts of the monkey received GTKO pig heart and artery by confocal microscopy. Targets of preformed IgM Ab of naïve monkey sera to GTKO PAECs were identified by immunoprecipitation and proteomics analysis by using LC/MS/MS technology.
Results: Naive monkey sera contained 1.4~4.6mg/ml of IgM, 26.4~91.2mg/ml of IgG and 6.4~40.4mg/ml of IgA Ab. Complement-mediated cytotoxicity of naïve monkey sera to WT vs GTKO PAECs showed 45~90% vs ~10%. While both preformed IgM and IgG Abs of monkey sera bind to WT PAECs, preformed IgM Ab of monkey sera remarkably binds to GTKO PAECs. Binding affinity of preformed IgM Ab to GTKO PAECs was closely related to complement-mediated cytotoxicity of monkey sera and the captured monkey IgM Ab on the xenograft tissues of NHP received GTKO heart and artery. In the current study, we identified 216 protein candidates as preformed monkey IgM Ab-binding targets of GTKO PACEs by proteomics analysis.
Conclusions: Our data demonstrate that GTKO porcine heart and artery xenograft could be captured by preformed IgM Ab of monkey sera. Binding affinity of preformed IgM Ab might be closely related to complement-mediated cytotoxicity of monkey sera to GTKO PAECs and captured monkey IgM Ab of the xenografts of NHP received GTKO heart and artery. For further studies, Ab-mediated rejection to the preformed NHP IgM Ab target antigens will be addressed.
This study was supported by the Agriculture Science & Technology Development (# PJ011363) and the Ministry of Science, ICT & Future Planning (#2014M3A9D3034013)

P.1897

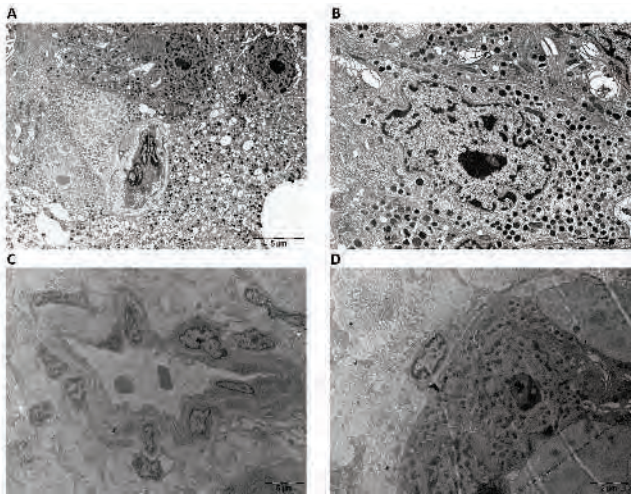
Histological and ultra-structure characterization of human immune cells against neonatal porcine islet xenograft

Wenlong Huang[1], Ping Wu[1], Nasser Tahbaz[2], Gina R.Rayat[1].
[1]Alberta Diabetes Institute, University of Alberta, Edmonton, AB, Canada; [2]Department of Cell Biology, University of Alberta, Edmonton, AB, Canada.

Background: Islet transplantation is being considered as an alternative treatment for Type 1 Diabetes Mellitus; however, the shortage of human donors limits its wide application in the clinic. Hence, the use of porcine islets as an alternative source has been an active area of research in the field of xenotransplantation. The immune mediated rejection of porcine islets by human immune cells is a major hurdle for the successful application of porcine islet xenotransplantation in patients with type 1 diabetes. Therefore, the aim of this study is to understand the immune mediated rejection of porcine islets by initially characterizing the human immune cells reacting against porcine islets in vivo.
Methods: Wild type neonatal porcine islets were isolated and cultured for 7 days, and then 2,000 IEQ were transplanted under the kidney capsule of Streptozotocin-induced diabetic NOD.SCID gamma mice (n=10). Blood glucose levels were monitored from the tail-vein blood of mice once a week for >100 days post-transplantation. Four weeks after mice have achieved normoglycemia, they were injected with 15 million human PBMCs from donors with or without Type 1 Diabetes Mellitus. Kidneys bearing the islet xenograft were harvested 1-4 weeks post-cell reconstitution. The islet grafts were analyzed for the presence of porcine endocrine cells as well as human immune cells by immunohistochemistry and Transmission Electron microscopy.
Results: Eight of the 10 mice became normoglycemia (Blood Glucose Level < 8.4 mmol/L) at 71-135 days post-transplantation. One mouse achieved normoglycemia at 175 days post transplantation while the other one mouse did not achieve normoglycemia until the end of the study. However the Blood Glucose Level of this mouse was 10.3 mmol/L, suggesting that the islet xenograft was functioning. Islet grafts of mice that received human PBMCs from non-diabetic and type 1 diabetic donors as well those that were not reconstituted showed for insulin and glucagon positive staining cells. Only the islet grafts from reconstituted mice had positive human CD45 staining, which was not observed in the grafts from non-reconstituted mice.



There was no difference in intensity of human CD45 immunohistochemistry staining for the mice that were reconstituted with human PBMCs from donors with or without Type 1 Diabetes Mellitus (p=0.51). Porcine endocrine cells were also identified using Transmission Electron microscopy. Mouse innate immune cells were seen to extravasate from the blood vessels and lodged where the porcine islets are located in non-reconstituted control mice.



In the reconstituted mice, human immune cell infiltration, damage of hormone secreting cells and formation of collagen were observed, which revealed the rejection of the islet graft by the human immune cells. In addition, active autophagy was observed in some of the cells in the graft.

Conclusion: Both innate and adaptive human immune cells are involved in the rejection of neonatal porcine islet xenograft. Further characterization of the identity of these cells warrants further investigation.

The work is supported by Canadian Institutes of Health Research

P.1899

Student opinions in xenotransplantation

Kyeonghee Jang[1], Wayne Paris[1], Ruth Bargainer[2], Leah Colsch[1], D.K.C. Cooper[3].
[1]School of Social Work, Abilene Christian University, Abilene, TX, United States; [2]School of Nursing, Texas Tech University Health Sciences Center, Abilene, TX, United States; [3]Thomas E. Starzl Transplantation Institute, Pittsburgh, PA, United States.

Introduction: The importance of psychosocial assessment and treatment within organ transplantation has been well established. Given recent immunologic progress in the laboratory, xenotransplantation (XTP) has the potential to provide an unlimited supply of donor organs, but at the same time will present the medical team with unique psychosocial challenges in their work with the patient and their family. To date there have been numerous opinion papers published about XTP but only a limited amount of data that explores perceptions and beliefs among the general public. The current work is the first report of an ongoing multi-disciplinary research team exploring the psychosocial beliefs and attitudes about XTP (i.e., pig to human) among various populations. This study reports the attitudes of students about XTP from a private faith-based university in the Southwestern US.

Methodology: The current study used an exploratory descriptive design. After university IRB approval, an email was forwarded to all juniors, seniors and graduate students informing them of the study. Those interested were directed via weblink to an informed consent document. Those who anonymously agreed to participate were then forwarded the 35 item survey. All initial statistical analysis was done with the latest version of SPSS software with procedures that were appropriate for parametric and non-parametric data.

Results: Of the 1,100 emails sent, 298 (27%) students completed the informed consent, and 260 completed the survey. Respondents were primarily female (70%; 181/260), single – never married (77%; 200/260) and Caucasian (79%; 206/260). They identified their religious beliefs as primarily Christian (73%; 190/260), Protestant (21%; 54/260) or Catholic (6%; 16/260). Seventy-two percent (188/260) had discussed organ donation with family and/or friends and 68% (176/260) of them were organ donors. The vast majority were in favor of organ donation from a religious perspective (93.5%; 243/260), although significantly fewer ($p<0.01$) would accept a pig organ transplant even if the expected results were the same as when a human donor organ had been transplanted (78%; 202/260) while significantly fewer ($p<0.01$) (19%; 49/260) of those who supported organ donation would consider it if the results were likely to be worse. Only 9% (23/260) were aware that pig donors must be genetically modified (to prevent rejection) before they could be used as sources of organs for transplantation into humans. After having been informed of the modification, 11% (28/260) changed their attitudes towards the procedure, 22% (26/260) felt uncertain, and 64% (167/260) did not change their attitudes. The majority (71.6%, 207/260) remained in favor of XTP even after being informed, this proportion was NS different from the attitudes with the same results expected (78%). There were NS demographic or religious differences for those who would or would not accept a pig organ.

Conclusions and Implications: The findings suggest that the significant factors in reducing the student's willingness to consider a XTP include anticipated poorer medical outcome from the transplant for those who favored organ donation and the source of organ donation. The knowledge about the modification was not a significant factor. It will be important to determine whether the attitudes and beliefs found exist with students at other private and public universities as well as with the general population. The implications of the current findings in relation to future research efforts in the development of psychosocial assessment and treatment for human clinical trials will be discussed.

P.1900

No PERV infection in pig islets recipients after 15 years of xenotransplantation

Xiaofeng Jiang, Lehong Zhang, Ping Xue, Heping Peng, Xuewei Yang, Haiwu Lu, Dawei Zhang, Liangqi Cao.
Department of surgery, The second affiliated hospital of Guangzhou Medical University, Guangzhou, People's Republic of China.

Five type 1 diabetic patients were transplanted by encapsulated pig islets in our department from 1999 to2000. To investigate the PERV infection,the patients' blood were collected for detecting PERV using PCR and RT-PCR. No positive results were found by the method. Pig islet xenotransplantation is a safety method to treat the diabetic patients.

P.1901

Levels of anti-Gal IgG2 negatively correlate with the survival of porcine islet graft in non-human primates under immunosuppression of anti-CD154

Hee Jung Kang[1], Haneulnari Lee[1], Eun Mi Park[1], Jong Min Kim[2], Chung-Gyu Park[2].
[1]Laboratory Medicine, Hallym University College of Medicine, Anyang-si, Korea; [2]Xenotransplantation Research Center, Seoul National University College of Medicine, Seoul, Korea.

Background: Anti-CD154 is the most successful immunosuppression (IS) regimen in non-human primate (NHP) models of porcine islet transplantation (PITx). Recently, we have shown that adult porcine islets survived and maintained normoglycemia in NHPs for longer than 6 months under use of anti-CD154 Ab[1] but the survivals of the grafts were variable. In this study, to address what determines the survival of grafts in these recipients, the levels of anti-Gal antibodies and biomarkers for systemic inflammation were measured in rhesus monkeys of PITx treated with anti-CD154, and their relationship with the graft survival was investigated.
Methods: Intraportal PITx was performed in 15 diabetic monkeys: 3 received various IS without CD40 pathway blockade (Tx-control); 12, IS with anti-CD154 (Tx-anti-CD154). Blood samples were weekly obtained from the monkeys after PITx until the graft ceased to function, and additionally from 3 monkeys without transplantation (nonTx-control) for 6 months. Levels of anti-Gal IgG, IgG1, IgG2 and IgM in the samples were analyzed by in-house ELISA using anti-human IgG1 and anti-human IgG2 antibodies cross-reacting to those of rhesus macaque (Sigma-Aldrich, St. Louis, MO, USA)[2] and levels of D-dimer and high-sensitive CRP by turbidimetry.
Results: Intraportal PITx led to strong induction of anti-Gal IgG1 response, not IgG2, in Tx-control, which was suppressed in Tx-anti-CD154. The level of anti-Gal IgG2 revealed negative association with the levels of CRP and D-dimer in nonTx-control, not in Tx-anti-CD154. The time-weighted average levels of anti-Gal IgG2 in Tx-anti-CD154 were significantly lower than in nonTx-control. Additionally, the levels of anti-Gal IgG2 and D-dimer negatively correlated with the graft survival in Tx-anti-CD154.
Conclusions: The strong correlation between the suppression of anti-Gal IgG2 response and the graft survival in Tx-anti-CD154 suggests that IgG2 response is implicated with the effector mechanism mediating rejection.
This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number : HI13C0954). Anti-CD154 (5C8) antibody was made available through the "Nonhuman Primate Reagent Resources funded by the US National Institutes of Health contract HHSN272201300031C"
References:
[1] Shin JS, Kim JM, Kim JS, Min BH, Kim YH, Kim HJ, et al. Long-Term Control of Diabetes in Immunosuppressed Nonhuman Primates (NHP) by the Transplantation of Adult Porcine Islets. Am J Transplant 2015
[2] Ruiz W, McClements WL, Jansen KU, Esser MT. Kinetics and isotype profile of antibody responses in rhesus macaques induced following vaccination with HPV 6, 11, 16 and 18 L1-virus-like particles formulated with or without Merck aluminum adjuvant. J Immune Based Ther Vaccines 2005;3:2

P.1904

Enhancement of CRISPR-mediated homology-directed repair using small molecule in porcine fibroblasts

Young June Kim[1], SungHoon Hurh[2], Eun Mi Lee[1,2], Yun Shin Chung[2], Hwa Jung Kim[2], Jaeseok Yang[2], Byeong Chun Lee[1,4], Hosup Shim Shim[5], Curie Ahn[1,2,3].
[1]Designed Animal & Transplantation Research Institute, Institute of Green Bio Science and Technology, Pyeong Chang, Korea; [2]Transplantation Research Institute, Seoul, Korea; [3]Department of Internal Medicine, College of Medicine, Seoul National University, Seoul, Korea; [4]Department of Theriogenology and Biotechnology, College of Veterinary Medicine, Seoul National University, Seoul, Korea; [5]Department of Nanobiomedical Science and BK21+ NBM Global Research Center for Regenerative Medicine, Dankook University, Cheonan, Korea.

Background: Clustered regularly interspaced short palindromic repeats(CRISPR) has recently emerged as a powerful tool for generating targeted genomic mutations with highly efficiency. This enzyme binds to specific DNA sequences and induces double-strand breaks of DNA that are repaired by either non-homologous end joining (NHEJ), an error-prone process leading to loss-of-function alleles, or homology-directed repair (HDR) for precise insertion of point mutations or a fragment of desired sequence at the targeted locus. However, precise introduction of mutations directed by HDR has remained inefficient. Here we showed that CRISPR-mediated HDR efficiency could be increased in porcine somatic cells using small molecule that can regulate DNA repair pathways.
Methods: CRISPR/Cas9 and conventional targeting plasmid designed to target exon 9 of α1,3-galactosyl transferase(GGTA1) were simultaneously delivered into GGTA1 mono-allelic Knockout(KO) porcine fibroblasts via electroporation. Cells were then recovered in 10% FBS supplemented DMEM medium with or without 5 μM of L755507, a β3-adrenergic receptor agonist, for 48 hours after electroporation. At day 6, galactose-α-1,3-galactose(α-Gal) epitope negative cells were separated using a biotin-labeled IB4 lectin attached to streptavidin-coated magnetic beads. After 2 weeks of additional culture with G-418, colonies were analyzed by PCR and Flow cytometry.
Results: After selection of α-Gal epitope negative cells using IB4-lectin and antibiotic, 2 colonies from L755507 treated group were identified as carrying the expected mutations via PCR analysis. Flow cytometric analysis revealed that α-Gal antigens were not present in these colonies. In contrast, colonies in which CRISPR-mediated HDR event had been occurred were not isolated from control group.
Conclusion: Through this study, we demonstrated that L755507 could be used to increase the efficiency of CRISPR-mediated HDR in porcine fibroblasts. This method may be useful for more efficient and precise gene insertion or point mutation in porcine somatic cells for biomedical research and xenotransplantation.
This research was supported by Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2015R1C1A1A02037507); This research was supported by the Bio & Medical Technology Development Program of the NRF funded by the Korean government, MSIP (2014M3A9D3034034)

P.1905

The analysis of C5 inhibitor efficacy in antibody mediated xenogeneic immune responses modulation using a microfluidic system

Eun Mi Lee[1,2], Zahid Alam[2], Hyori Kim[3], Sunghoon Hurh[1], Won Jun Yang[3,4,5], Yun Shin Chung[2], Young June Kim[2], Min Jung Kim[1], Jaeseok Yang[6], Byeong Chun Lee[7], Junho Chung[3,4], Curie Ahn[1,6,8].
[1]Transplantation Research Institute, Seoul National University College of Medicine, Seoul, Korea; [2]Designed Animal & Transplantation Research Institute, Institute of Green Bio Science & Technology, Seoul National University, Pyeongchang, Korea; [3]Cancer Research Institute, Seoul National University, Seoul, Korea; [4]Department of Biochemistry and Molecular Biology, Seoul National University, Seoul, Korea; [5]Department of Cancer Biology, Seoul National University College of Medicine, Seoul, Korea; [6]Transplantation Center, Seoul National University Hospital, Seoul, Korea; [7]Department of Theriogenology and Biotechnology, College of Veterinary Medicine, Seoul National University, Korea; [8]Division of Nephrology, Seoul National University College of Medicine, Seoul, Korea.

Background: Glycan antigens are considered critical targets for transplant rejection in xenotransplantation. Therefore, we validated antibody mediated and innate immune cells mediated responses against glycan using a microfluidic system. We demonstrate the potential of C5 inhibitor as a modulator of complement activation in xenotransplantation.
Materials and Methods: We established a Bioflux 200 system for the shear-flow assay in live cells. Wild type (WT) pig and aGal knockout (GTKO) pig endothelial cells (ECs) were seeded in microfluidic channels. Channels were perfused with pooled human serum of various concentrations. We analyzed human IgG and IgM binding to pig ECs and complement-dependent cytotoxicity by immunofluorescence assay. In addition, to test the efficacy of C5 inhibitor, we treated C5 inhibitor in human pooled serum then we perfused into the microfluidic channels which contained pig ECs. Finally we measured complement activities in the serum stimulated with WT or GTKO pig ECs by ELISA.
Results: The binding of human xenoreactive antibodies to the pig ECs were reduced GTKO pig ECs, demonstrating that GTKO did interfere with antibodies binding to the pig ECs. Complement activations (C3a, C4a, C5a etc.) increased in human serum against WT pig ECs compared to GTKO pig ECs group. In addition, pig ECs cytotoxicity reduced in 200 nM C5 inhibitor treatment group. They showed basal level such as control. We could also confirm the C5a production and activation effectively were reduced by C5 inhibitor treatment.
Conclusion: C5 inhibitor may be a valuable component to protect the xenogeneic immune responses by the reduction of C5a production and activities.
This study was supported by the Ministry of Science, ICT & Future Planning (#2014M3A9D3034034); This study was supported by Ministry of Trade, Industry and Energy of Korea (#10048948)

P.1906

The analysis of αGal antigen in tree shrew

Wang LuMin[1], Fu JiaZhao[1], Li YaGuang[1], Li Cheng[2], Yanping Li, Tong CaiLing[2], Xia JunJie[2], Qi ZhongQuan[2].
[1]Xiamen University, The Organ Transplantation Institution, Xiamen, Hong Kong; [2]Xiamen University, The Organ Transplantation Institution, Xiamen, Hong Kong.

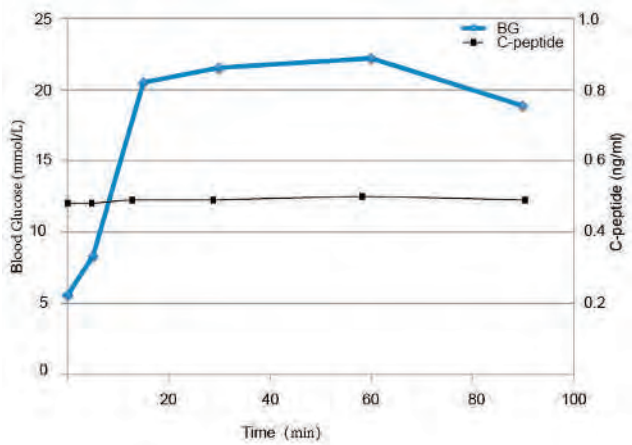
Introduction: Tree shrew is considered to be the most approximate mammal with primate. At the same time, it is considered to have the most potential to take the place of mammal for human disease research.This research gives a test about the αGal antigen in tree shrew to see its potential on development of xenotransplantation model research.
Materials and Methods: This research use FCM analyses αGal antigen in tree shrew cells matched with rat various visceral organ cells, HUVEC, porcine endothelial cell and macaque endothelial cells. First of all,we hocus the tree shrew with 10% chloral hydrate at the dose of 4.5g/Kg and then we take out the heart, liver, spleen, lung and kidney from tree shrew. Grind them into pieces in the base of Red Blood Cell Lysis Buffer separately. Digest them into single cells by CollagenaseII and pancreatic enzymes and then turn these cells into RPMI 1640 medium. These cells are set as control group. The matched Group1 is designed as cells of heart, liver, spleen, lung, kidney from rat in the same preparation method of control group.The matched Group2 is designed as HUVEC, porcine endothelial cell and macaque endothelial cells which are bought by research laboratory.
Results: The control group showed that cells from heart, liver, lung, kidney are αGal weak positive and cells from spleen are αGal strong positive. Group1: cells from heart, spleen, lung, kidney of rat are αGal strong positive, and cells from liver of rat are weakly positive. Group2:porcine endothelial cells are αGal strong positive, HUVEC and macaque endothelial cells areαGal negative.
Discussion: Cells from tree shrew express αGal antigen. With the development of αGal gene knockout technology more and more popular, it is easy to set up a xenotransplantation model which makes rat donor and tree shrew receptor.This model can avoid the interference of anti-Gal antibody so to reduce the cost.Tree shrew is small shaped,low cost breeding and easy to operate thus the most ideal animal of choice for xenotransplantation model.
Qi Zhongquan; Kuang Xiaocong; Qin Qing
References:
[1] Bone-marrow mesenchymal stem cell transplantation to treat diabetic nephropathy in tree shrews
[2] Genome of the Chinese tree shrew
[3] Gene Expression Signatures in Tree Shrew Sclera in Response to Three Myopiagenic Conditions

P.1907

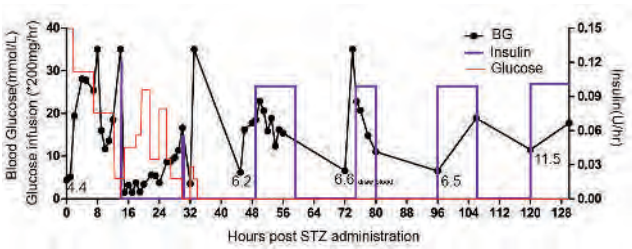
Induction of diabetes in cynomolgus monkey with one injection of streptozotocin

Zhengzhao Liu[1], Lisha Mou[1], Ying Lu[1], Wenbao Hu[1], Tian He[1], Zhicheng Zou[1], Huidong Zhou[1], Rita Bottino[2], David K.C.Cooper[3], Zhiming Cai[1].
[1]Shenzhen Xenotransplantation Medical Engineering Research and Development Center, Shenzhen Second People's Hospital, Shenzhen, People's Republic of China; [2]Thomas E. Starzl Transplantation Institute, University of Pittsburgh Medical Center, Pittsburgh, PA, United States; [3]Institute of Cellular Therapeutics, Allegheny-Singer Research Institute, Pittsburgh, PA, United States.

Introduction: Islet cell transplantation has long been considered as a potential cure for type I diabetes. The shortage of human donors has drawn the attention to xenotransplantation. The preclinical study in pig-to-Non human primate xenotransplantation provide valuable model to study the immunorejection. Streptozotocin (STZ) induced diabeitc monkey is a wide used pre-clinical animal model for islet xenotransplantation. It has a variety of adverse effects, ranging from nausea, emesis, and weight loss to liver damage, renal failure, and metabolic acidosis[1]. Although it has been found difficult to achieve complete diabetes without serious adverse effects. We have found that 100mg/kg STZ i.v. can safely induce complete diabetes in cynomolgus monkey.
Materials and Methods: After carrying indwelling catheter in the carotid artery and vein[2], cynomolgus monkey received 100 mg/kg STZ (Sigma) i.v. dissolved in normal saline and infused from vein line in 5 min[3].
Results and discussion: The monkey that received 100 mg/kg STZ become fully diabetic (C-peptide, 0.33 ng/mL) with no apparent adverse effects. The stimulated C-peptide level (IVGTT) is less than 0.5 ng/mL.



A triphasic response in blood glucose was observed during the first 36 hours after STZ administration.



An initial decrease of insulin release as a result of inflammation and an inability of the beta cells to respond to glucose because of streptozotocin enters the B cell via a glucose transporter (GLUT2) and causes alkylation of DNA[4]; Further destruction and disruption of the beta cells results in a massive release of insulin leading to a fall in blood glucose level; Finally, there is a critical loss of beta cells resulting in diabetic states[2].This optimal dosage of STZ provides a useful model for islet xenotransplantation[2].

Conclusions: (1) 100mg/kg STZ induced diabetes safely in cynomolgus monkey without adverse effects. (2) A triphasic blood glucose response suggested the complete induction of diabetes. We have found that 100mg/kg STZ i.v. can safely induce complete diabetes in cynomolgus monkey.

References:

[1] Nagaraju, S., et al., Streptozotocin-associated lymphopenia in cynomolgus monkeys.*Islets* 2014; 6(3): p. e944441.
[2] Rood, P.P., et al., Induction of diabetes in cynomolgus monkeys with high-dose streptozotocin: adverse effects and early responses. *Pancreas*, 2006. 33(3): p. 287-92.
[3] Koulmanda, M., et al., The effect of low versus high dose of streptozotocin in cynomolgus monkeys (Macaca fascilularis). *Am J Transplant*, 2003. 3(3): p. 267-72.
[4] Szkudelski, T., The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiol Res*, 2001. 50(6): p. 537-46.

P.1908

Gene editing techniques in cardio-xenotransplantation

Meisam Naeimi Kararoudi.
Internal Medicine, University of Perugia, Perugia, Italy.

Xenotransplantation is a potential solution for the urgent and steadily increasing world-wide persisting donor organ shortage. Several studies have shown that the pigs are the best choice of source animal for providing the limitless heart for humans due to their availability, Breeding potential, Size of adult organs, cost of maintenance, Anatomic and physiologic similarity to human, Relationship of immune system, etc. Since 1968 several efforts have been made in clinical heart xenotransplantation using Pig heart, but none of them were success.The most 4 important and profound barriers to cardiac xenotransplantation are the immunologic responses to the porcine grafted heart, namely, hyper acute rejection(HAR), acute humeral xenograft rejection (AHXR), immune cell-mediated rejection and chronic rejection. For Understanding the mechanism of xenograft rejection and overcoming the above mentioned barriers, several investigations using a clinically relevant large animal model (Pig-to-baboon) have been done. Fortunately significant improvement have enhanced heterotropic cardiac xenograft and intrathoracic heterotopic cardiac xenograft survival rate. Undoubtedly genetic engineering has played the most crucial role in these achievements by producing genetically modified pigs which are more compatible and acceptable for human immune system. Since 1992 different techniques have been used for making genetically modified pigs for using as heart donor in cardiac-xenotransplantation such as Microinjection of randomly integrating transgenes, Somatic cell nuclear transfer (SCNT), Homologous recombination, Zinc finger nucleases (ZFN), Transcription activator-like effector nucleases(TALENs) and the most recent technique CRSIPR/Cas9.Thanks to these methods, Xenotransplantation is not just Future anymore as great Norman Shumway used to say. This review provides an overview and description of several precise methods of the most frequent transgenic approaches (SCNT, ZFN, TALENs, CRISPR/Cas9) and techniques that have been used to generate donor pigs for cardio-xenotransplantation such as deleting Gal antigen expression on the surface of all porcine cells, Downregulation the human complement system (h-CD46 or h-CD55 or h-CD59), h-TM or human, HO-1, A20, LEA 29Y, HLA-E and also several Multi-genetically modified pigs. The exceptional character about this review is its accurate study of gene maniupulating in several recent approaches.

References:

[1] Johannes Postrach,et al. Heart xenotransplantation in primate models, chapter 10: Methods Mol Biol. 2012;885:155-68.
[2] D.K. C. Cooper. Xenotransplantation Advisory Committee of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant. 2000 Dec;19(12):1125-65. Review.…..
[3] Hardely JD,Kurrus FE. Heart Transplantation in man : development studies and report of a case. JAMA 1946; 188: 1132-40.
[4] Klymiuk N, Genetic modification of pigs as organ donors for xenotransplantation. Mol Reprod Dev. 2010 Mar;77(3):209-21.
[5] Mohiuddin MM, Current status of pig heart xenotransplantation. Int J Surg. 2015 Nov;23(Pt B):234-9.
[6] Cooper DK. The role of genetically engineered pigs in xenotransplantation research. J Pathol. 2016 Jan;238(2):288-99.
[7] Polejaeva, I.A., et al., Cloned pigs produced by nuclear transfer from adult somatic cells. Nature, 2000. 407(6800): p. 86-90
[8] Lai, L., et al., Production of alpha-1,3-galactosyltransferase knockout pigs by nuclear transfer cloning. Science, 2002. 295(5557): p. 1089-92.
[9] Carol L. Keefer. Artificial cloning of domestic animals, 8874–8878, PNAS ,July 21, 2015 ,vol. 112, no. 29
[10] Hicham Bouabe and Klaus Okkenhaug, Gene Targeting in Mice: a Review. Methods Mol Biol. 2013 ; 1064: 315–336.
[11] Thomas Gaj,. ZFN, TALEN and CRISPR/Cas-based methods for

genome engineering, Trends Biotechnol. 2013 July ; 31(7): 397–405.
[12] Xiaoyu Chen and Manuel A.F.V. Gonçalves, Engineered viruses as genome editing devices, Molecular Therapy accepted article preview online 04 September 2015.
[13] Y. Kim, J. A library of TAL effector nucleases spanning the human genome, Nat. Biotechnol. 31 (2013) 251–258.
[14] Benjamin P. Kleinstiver. High-fidelity CRISPR–Cas9 nucleases with no detectable genome-wide off-target effects, Nature (2016).

P.1909

Comparison of islet isolation yields from three different wild type adult pig breeds

Jun-Seop Shin[1,2], Byoung-Hoon Min[1,2], Jong-Min Kim[1,2], Jongwon Ha[2], Sang-Joon Kim[2], Chung-Gyu Park[1,2].
[1]Microbiology and Immunology, Seoul National University College of Medicine, Seoul, Korea; [2]Xenotransplantation Research Center, Seoul, Korea.

Background: Pig islets are alternative source for the allogeneic human islets to treat type 1 diabetes and clinical islet xenotransplantation under an appropriate regulatory oversight will be started in the near future. However, there is still no consensus on the best pig breed as adult islet donor. Here we compared islet isolation yields from three different pig breeds and sought to find the best parameter to predict the highest islet yield.
Methods: Three different adult pig breeds were used as islet donors; the Seoul National University (SNU) miniature pig (n=14), the Prestige World Genetics (PWG) miniature pigs (n=20), and Durok×Yorkshire×Landrace (DYL) breed (n=9). All isolations were done by same personnel and procedure using same collagenase blend (Vitacyte collagenase MA plus BP protease). Isolation yields indicated as total islet equivalent per gram of the digested pancreas (IEQ/g) were compared before, after purification and after 1 day culture. To find the reason why there were the differences of islet yields between pig breeds, extensive histomorphometrical analyses were conducted.
Results: Islet yield was significantly higher in SNU miniature pigs (7711.3±3926.8 IEQ/g pancreas) than those in PWG and DYL pigs (2545.5±1871.5 and 3213.7±2066.4 IEQ/g pancreas, respectively), although the weight of pancreas was significantly smaller in SNU miniature pig than those from two other pigs (47.9±9.9 g vs. 72.8±14.3 g and 113.1±18.6 g). Histomorphometric analyses on the pancreatic samples taken from similar location of the pancreases from each animals showed that islet distribution was similar among three pig breeds, but individual islet size of SNU miniature and DYL pigs was significantly larger than that of PWG pig.
Conclusion: These results suggest that SNU miniature pigs are the best source pig for providing higher yield of islet, but cost effective due to smaller size of the pancreas.

	Age (M)	Body Weight (kg)	Pancreas weight (g)	Remnant pancreas (g)	Total IEQ at Pre-COBE	Total IEQ at post-COBE	Yield (IEQ/g)
D×Y×L	4.4±0.7*	81.3±3.7*	113.1±18.6*	20.8±6.9* (18.4%)	344,041± 257,195*	297,930± 194,395	3213.7± 2066.4*
PWG	28.8±8.4	64.5±3.5*	72.8±14.3*	16.8±6.3* (23.1%)	169,558± 88,940	133,354± 89,991*	2545.5± 1871.5*
SNU	30.6±16.9	73.9±15.7	47.9±9.9	7.2±4.6 (15.0%)	205,125± 144,730	368,687± 240,480	7711.3± 3926.8

This study was supported by a grant from the Korea Healthcare Technology R&D Project, Ministry for Health & Welfare, Republic of Korea (Project No. HI13C0954)

P.1910

Immunosuppressive effect of arsenic trioxide (A2O3) on xeno-islet transplantation

Bin Zhao, Lumin Wang, Junjie Xia, Zhongquan Qi.
Medical College, Xiamen University, Organ Transplantation Institute, Xiamen, People's Republic of China.

Background: The immunorejection of islet transplantation has been a widely concerned problem in clinical practice, which hinder its popularization. And our previous research has been demonstrated that arsenic trioxide (As2O3) has the effect of immunosuppression and prolonging islet allograft survival. Therefore, according to previous research, we studied whether As2O3 has the similar effect and mechanism on islet transplantation.
Method: Apply streptozocin (STZ) in C57BL / 6 mice to induce with type 1 diabetes, then establish heterogeneous islet transplantation model. These model rice are divided into different groups by therapeutic regimen, and recycle graft, blood and tissue samples of recipient rice, then conduct cross comparison between groups for vitro and vivo experiment results. Finally, analyze effect of As2O3 for allgograft and receptor's immune system in the heterogeneous islet transplantation.
Conclusion: The result has shown that As2O3 can lower posttransplantation cellular immune level, improve the level of Foxp3+ regulatory T cells and lower part of the serum antibody level to prolong allograft survival. At the same time, our study has found As2O3 has obvious synergistic effect with leflunomide.
Keyword: Heterogeneous islet transplantation;Immunosuppressants; As2O3
References:
[1] Cooke, D.W. and L. Plotnick, Type 1 diabetes mellitus in pediatrics. Pediatr Rev, 2008. 29(11): p. 374-84; quiz 385.
[2] Chiang, J.L., et al., Type 1 diabetes through the life span: a position statement of the American Diabetes Association. Diabetes Care, 2014. 37(7): p. 2034-54.
[3] Knip, M., et al., Environmental triggers and determinants of type 1 diabetes. Diabetes, 2005. 54 Suppl 2: p. S125-36.
[4] Shapiro, A.M., C. Ricordi, and B. Hering, Edmonton's islet success has indeed been replicated elsewhere. Lancet, 2003. 362(9391): p. 1242.
[5] Hirshberg, B., et al., Benefits and risks of solitary islet transplantation for type 1 diabetes using steroid-sparing immunosuppression: the National Institutes of Health experience. Diabetes Care, 2003. 26(12): p. 3288-95.
[6] Robertson, R.P., Islet transplantation as a treatment for diabetes - a work in progress. N Engl J Med, 2004. 350(7): p. 694-705.
[7] Piemonti, L., et al., Alloantibody and autoantibody monitoring predicts islet transplantation outcome in human type 1 diabetes. Diabetes, 2013. 62(5): p. 1656-64.
[8] Lacy, P.E. and M. Kostianovsky, Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes, 1967. 16(1): p. 35-9.
[9] Kemp, C.B., et al., Transplantation of isolated pancreatic islets into the portal vein of diabetic rats. Nature, 1973. 244(5416): p. 447.
[10] Scharp, D.W., et al., Transplantation of islets of Langerhans in diabetic rhesus monkeys. Surgery, 1975. 77(1): p. 100-5.
[11] Tzakis, A.G., et al., Pancreatic islet transplantation after upper abdominal exenteration and liver replacement. Lancet, 1990. 336(8712): p. 402-5
[12] Shapiro, A.M., et al., Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med, 2000. 343(4): p. 230-8.
[13] Healy, D.G., et al., Heart transplant candidates: factors influencing waiting list mortality. Ir Med J, 2005. 98(10): p. 235-7.
[14] Tisato, V. and E. Cozzi, Xenotransplantation: an overview of the field. Methods Mol Biol, 2012. 885: p. 1-16.
[15] van der Windt, D.J., et al., Clinical islet xenotransplantation: how close are we? Diabetes, 2012. 61(12): p. 3046-55.
[16] Platt, J.L. and M. Cascalho, New and old technologies for organ replacement. Curr Opin Organ Transplant, 2013. 18(2): p. 179-85.
[17] Bailey, L.L., et al., Baboon-to-human cardiac xenotransplantation in a neonate. JAMA, 1985. 254(23): p. 3321-9.
[18] Michler, R.E., Xenotransplantation: risks, clinical potential, and future prospects. Emerg Infect Dis, 1996. 2(1): p. 64-70.
[19] Dooldeniya, M.D. and A.N. Warrens, Xenotransplantation: where are we today? J R Soc Med, 2003. 96(3): p. 111-7.
[20] Taylor, A.L., et al., Pathways of helper CD4 T cell allorecognition in generating alloantibody and CD8 T cell alloimmunity. Transplantation, 2007. 83(7): p. 931-7.
[21] Candinas, D. and D.H. Adams, Xenotransplantation: postponed by a millennium? QJM, 2000. 93(2): p. 63-6.

P.1911

Acute rejection patterns of outbred rats to heterotopic heart xenotransplantation.

Necdet Ozcay[1], Mehmet Haberal[1], Handan Ozdemir[2].
[1]General Surgery, Baskent University, Ankara, Turkey; [2]Pathology, Baskent University, Ankara, Turkey.

Introduction: Syngenic inbred mice and rats have long been using for xenotransplantation studies in different strain combinations. Genetically engineered big animals are also commonly use worldwide. In this study we aimed to investigate acute rejection patterns of outbred rats after xenotransplantation. For this purpose we used a heterotopic heart xenotransplantation model from mouse to rat and evaluated macroscopic and microscopic findings of acute rejection patterns of xenografts.
Materials and Method: Thirty male Balb/c mice, weighing from 30-40 g were used as donors and thirty male outbred Sprague-Dawley (SD) rats, weighing from 150-200 g were used as recipients. Donor operations were performed first. For recipient operation, vena cava and aorta were prepared. An end –to-side anastomosis between donor's pulmonary artery and the recipient's inferior vena cava and between donor aorta and recipient's abdominal aorta were performed with continuous 10/0 nylon sutures. Animals were allocated into five groups according to sacrificed day and each group had 6 animals: Animals in group I was followed one day and then sacrificed. Animals in group II,III, IV and V were followed up 2, 3, 4 and 5 days after transplantation, respectively. Operative findings were recorded. During scarification, the appearance and heartbeat strength of the grafts were evaluated and scored from 0 to 4. Histopathologic examinations of the grafts were also performed to determine acute rejection. The data of the groups are reported as the mean±SE.
Results: Thirty heterotopic heart xenotransplantation from mouse to rat were performed. All animals survived during the study period, animal survival rate was 100%. The survival rate of the xenografts was 95%; two grafts were lost due to a vascular thrombosis. The mean time to recover the donor's hearts was 4.8 ± 2.1 minutes The time of vascular reconstruction and total operative times were 25 ± 5.2 minutes and 50±5 minutes, respectively. Heartbeat strength were strong (4.5 ±0.5) and graft appearance were normal in group one animals. However, heartbeat scores of the xenografts were dramatically declined thereafter. Mean heartbeat scores were 2.5±0.5 animals in group II, 1.0±0.5 in group III and 0 in group IV and V animals. Histopatologicaly, there were inflammation patterns in all xenografts, The infiltrating of neutrophils and the formation of platelet and fibrin thrombi were seen in postoperative first day and gradually increased daily.
Conclusion: The study design allowed us to investigate macroscopically and microscopically to the grafts daily for five days. Our microscopic findings consistently indicated that the animals developed acute rejection immediately. As a consequence of acute rejection hertbeatings of the xenografts weakened day by day and stopped eventually. In the light of our findings we conclude that outbred rats have reliable acute xenograft rejection patterns microscopically and macroscopically in heterotopic heart xenotransplantation model.

P.1912

Surgical technique of harvesting two kidneys from a mouse for xenotransplantation studies

Necdet Ozcay, Mehmet Haberal.
General surgery, Baskent University, Ankara, Turkey.

Introduction: Traditionally two animals are used for each transplantaion model, one as a donor and the other as a recipient.This situation doubled the number of animals for transplantation studies. At the present time, developing microsurgical instruments and refine surgical techniques should allow us to reduce the number of animal use for transplantation research. In this study we aimed to harvest two kidneys from one donor to be able to minimize the animal need for transplantation studies. For this purpose we developed a kidney xenotransplantation model from mouse-to-rat, in which only one animal used as donor for two kidney recipients.
Surgical Technique: Balb/c mice, weighing from 25-30 g were used as donors and Sprague Dawley(SD) rats, weighing from 150-200 g were used as recipients. For donor operation, both kidneys were exposed first by moving the intestine laterally. The right and left ovarian and adrenal veins were ligated and transected. Both ureters were dissected free from the renal hilus to the bladder. For arterial exposure, aorta, celiac artery (CA), superior mesenteric artery (SMA),and both renal arteries were dissected. The lumbar branches of aorta and SMA were ligated and transected. After this point the grafts were perfused; A needle introduced into the aorta and the grafts were slowly perfused in situ with 3 ml heparinized saline solution. In order to share the aorta between two kidneys it was ligated proximally and distally as far as possible and transected from the middle where the area between left and right renal artery. For same reason IVC was ligated at the middle of the area between left and right renal veins first. Then the left renal vein was transected at its junction with the IVC and later IVC was transected at the level of right adrenal vein. In this way the left renal vein and left renal artery along with aortic patch were prepared for left kidney graft, the right renal vein along with a segment of IVC and right renal artery along with aortic patch were prepared for right kidney graft's vascular continuity. After transection of right and left ureters as distal as possible, both kidneys and their vascular supply, along with the both ureters were removed en bloc and stored separately in Ringer's lactate solution at 4°C. Two SD rats were prepared for xenotransplantation. One animal received left, and the other received right kidney graft. During xenotransplantation, recipient's abdominal aorta and IVC were used for anastomosis. Continuous 10/0 nylon sutures were used for end-to-side vascular anastomosis. The ureter anastomosis was performed with ureter to bladder insertion technique.
Conclusion: Although harvesting two kidneys from a mouse and mouse-to-rat kidney xenotransplantation is a highly challenging microsurgical procedure, we believe that every experienced surgeon could do that with some practice. This model allows us to reduce the number of animals in transplantation studies without compromising the graft quality. We strongly recommend our refine harvesting technique to researchers especially in terms of animal rights.

P.1925

Temperature change in the kidney graft during laparoscopic kidney transplant

Bulang He[1,2], Gabrielle Musk[3], Monika Zwierzchoniewska[1], Jack Mak[1], Lingjun Mou[1], Jeffrey M.Hamdorf[2], Luc Delriviere[1,2].
[1]Department of Surgery, Sir Charles Gairdner Hospital, Perth, Australia; [2]School of Surgery, The University of Western Australia, Perth, Australia; [3]Animal Care Service, The University of Western Australia, Perth, Australia.

Introduction: Recently, laparoscopic and robotic technique has been employed in kidney transplant. However, there is a concern whether the prolonged time for vessel anastomosis causes substantial damage to the kidney graft during the learning period for the surgeon. The aim of this study was to examine the efficiency of regional cooling on the kidney graft and its effect on core body temperature during laparoscopic kidney transplant.
Materials and Methods: Seven domestic large white pigs weighing 40-45 kg were used in this study. The orthotopic left auto transplantation was performed with the laparoscopic technique. The order of anastomoses was the renal artery, the renal vein and then the ureter. The kidney graft was continuously irrigated with cold normal saline (4 ºC) at 1200 mL/h during the anastomoses. The temperature of the kidney was measured with a 15 mm needle probe inserted into the parenchyma of the kidney. Core body temperature was monitored continuously. After transplantation of the left kidney, the right kidney was rendered dysfunctional by complete ligation of the ureter. The pigs recovered from surgery and were observed for 4 weeks. Blood samples were collected regularly for haematology and biochemistry analyses. The kidneys were retrieved after completion of the study for histopathology on day 28. Data was compared using a Student's t-test. $P < 0.05$ was considered significant.
Results and Discussion: There was a significant increase in the kidney graft temperature from 5 °C to 27.8 °C from the time point when the kidney was returned to the body to the completion of the renal artery anastomosis ($P < 0.05$). There was a minimal increase in the kidney temperature from 27.8 °C to 29.2 °C during anastomosis of the renal vein prior to kidney reperfusion. The body temperature remained stable between 36.1 °C and 36.4 °C ($P=0.82$). The peak creatinine level was 160.6 mmol/L on day 3 and it improved by day 7 (136 mmol/L).
Conclusion: Continuous irrigation of the kidney graft using cold normal saline has no adverse effect on core body temperature. Although the kidney graft temperature rapidly increased during the initial period of vessel anastomosis, it was able to maintain between 27.8 and 29.2 °C prior to reperfusion. The kidney graft function was satisfactory. when the kidney was transplanted by laparoscopic technique.

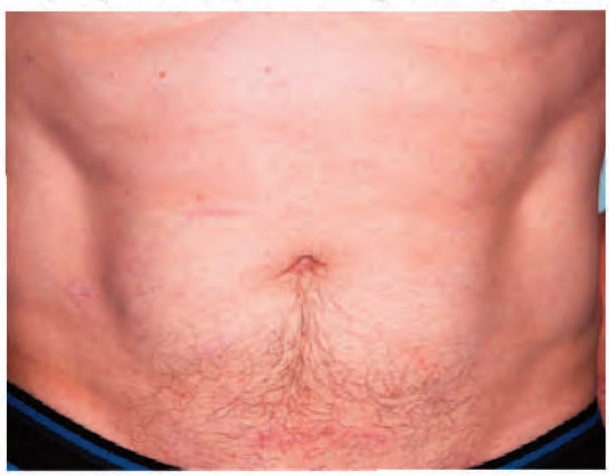
P.1926

Laparoscopic kidney transplant by extra peritoneal approach: Two years follow up

Bulang He[1,2], Lingjun Mou[1], Suda Swaminathan[3], Jeffrey M.Hamdorf[2], Luc Delriviere[1,2].
[1]Department of Surgery, Sir Charles Gairdner Hospital, Perth, Australia; [2]School of Surgery, The University of Western Australia, Perth, Australia; [3]Renal Medicine, Fiona Stanley Hospital, Perth, Australia.

Introduction: Recently, we have applied an innovative laparoscopic technique for kidney transplant via extra peritoneal approach. The aim of this study is to report its outcomes over two years follow up.
Materials and Methods: The study patient was a 49-year-old male and received the kidney that was transplanted by laparoscopic technique via extra peritoneal approach. The details of the technique were reported elsewhere in the past[1]. Briefly, a Pfannenstiel incision (7 cm) was made for delivery of the kidney graft to the right iliac fossa. The renal artery and renal vein was anastomosed to the side of external iliac artery and vein by laparoscopic technique. The control patient was a 66-year-old gentleman received the contralateral kidney that was transplanted by open surgery.
Results and Discussion: The surgery was successful for both patients. Both kidneys experienced delayed graft function but the kidneys started function on day 6 post transplant. The recovery of kidney graft function by laparoscopic transplantation is the same as the kidney from open transplant. The creatinine level is stable (109 vs 107 umol/L) over two years follow up. The incision is much less visible on study patient (Figure 1). There is no surgical complication over two years follow up.
Conclusion: This report has shown that the kidney graft function is satisfactory when transplanted by laparoscopic technique. The benefit for the patient is a smaller incision, less pain and better cosmetic appearance. There was no adverse effect seen from laparoscopic surgery.

Figure 1. Surgical incision by laparoscopic kidney transplant



Post transplant: 12 months

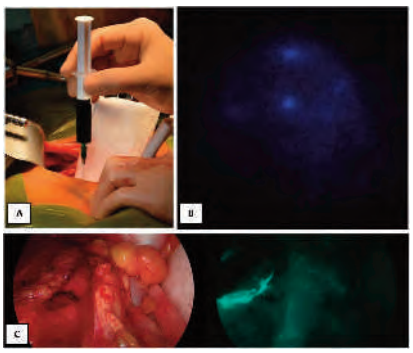
References:
[1] He B, et al. Laparoscopic kidney transplant by extra peritoneal approach: the safe transition from laboratory to clinic. Am J Transplant 2014; 14: 1931-1936.

P.1927

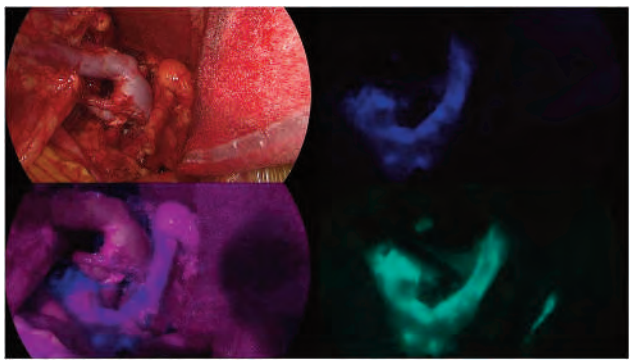
Real-time intraoperative fluorescent lymphography – a new technique for lymphatic sparing surgery

Giuseppe Ietto, Francesco Amico, Gabriele Soldini, Domenico Iovino, Marco Calussi, Andrea Romanzi, Giovanni Sammy Necchi, Matteo Urru, Matteo Tozzi, Giulio Carcano.
General and Transplant Surgery, University of Insubria, Varese, Italy.

Background: Many surgical procedures can produce persistent lymphorrea, lymphoceles and lymphedema after lymph nodes and lymph vessels damages. Appropriate visualization of the lymphatic system is challenging. Indocyanine green (ICG) is a well-known non-toxic dye for lymphatic flow evaluation. ICG fluorescent guided lymphography has emerged as a promising technique for intraoperative lymphatic mapping.
Objective: We aimed to develop a high spatial resolution real-time intraoperative imaging technique to avoid or early recognize deep lymphatic vessels damage.
Methods: We intraoperatively performed ICG fluorescence-guided lymphography during a kidney transplant. ICG was injected in the subcutaneous tissue of the patient's groin in the Scarpa's triangle (A). A dedicated laparoscopic high definition camera system was used



Results: Soon after ICG injection, lymphatic vessels were identified in the abdominal retroperitoneal compartment as fluorescent linear structures running side by side to the iliac vessels (B-C). Surgical dissection was therefore conducted avoiding iatrogenic damages to major lymphatic structures. Another ICG injection at the end of the procedure confirmed that the lymphatic vessels were intact without lymph spread.



Conclusions: Intraoperative lymphatic mapping with ICG fluorescence-sensitive camera system it's a safe and feasible procedure. ICG real-time fluorescent lymphography can be used to avoid or early recognize deep lymphatic vessels damage and reduce post-operative complications related to lymphatic system.

P.1928

Our experience with modified Lich-Gregoir technique in the prevention of urological complications post kidney transplant

Zi Qin Ng, Bulang He.
WA Liver & Kidney Transplant Service, Sir Charles Gairdner Hospital, Perth, Australia.

Aim: Various ureteroneocystostomy techniques for kidney transplant have been described with Lich-Gregoir (LG) technique being the technique of choice in most centres. However, even with multiple modifications attempted, urological complications especially urine leakage and ureteral stenosis remain prevalent and can be devastating. This study aims to report our experience by modifying Lich-Gregoir technique.
Method: From 26th January 2010 to 30th June 2014, 206 kidney transplants were performed. Mean age was 49.94 years (3 years-81 years). Of 206 recipients, 124 were deceased-donor, 69 were live-donor and 13 patients received kidney transplants using a small tumour excised kidney graft. Laparoscopic donor nephrectomy was routinely performed for live kidney donors. Four patients received the third and one received the fourth kidney transplant. Nine patients underwent dual-kidney transplant. All transplants were performed by conventional open surgery except one was done laparoscopically. Ureter-bladder anastomosis was conducted by using our own modified Lich-Gregoir technique with an additional stitch placed at proximal part of bladder muscular incision to the peri-ureteric tissue on each side. Urological complications were defined as urine leakage or ureteral stricture. The patients were followed-up from 19 to 72 months. Doppler ultrasound (DU) and renal nuclear scan were performed on day one post-operation and DU was repeated whenever the kidney graft function deteriorated.
Results: All kidney transplants were completed successfully. Six patients died during follow-up secondary to other medical co-morbidities. There were three graft losses; two of them were due to antibody-mediated rejection and one was due to dehiscence at the vascular anastomotic site. No urine leakage was observed. One case of ipsilateral dual-kidney transplant developed distal ureteral stricture in each of the graft secondary to a lymphocele. The stricture required surgical reconstruction. Seven cases were identified with mild to moderate hydronephrosis on DU; 4 were due to a lymphocele; 3 were secondary to urinary stones. 4 patients had renal pelvis prominence with satisfactory renal function and no intervention was required.
Conclusion: Our technique modification secures the entrance of ureter to the bladder, while it avoids potential over-tightening of the ureter at the entrance to the bladder by two separate sutures at each side.

P.1929

"Artery first, vein second" approach for vascular anastomosis in kidney transplantation

Zi Qin Ng, Bulang He.
WA Liver & Kidney Transplant Service, Sir Charles Gairdner Hospital, Perth, Australia.

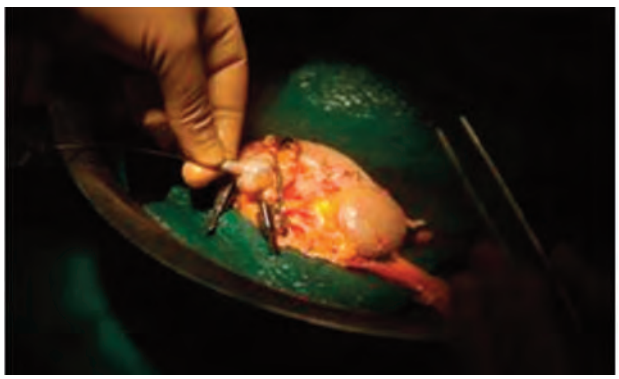
Introduction: The surgery for kidney transplantation has been well established since 1950's with the renal vein conventionally being anastomosed first followed by renal artery anastomosis. The aim of this study is to review the outcomes of alternate vascular anastomosis in the kidney transplantation in a series of 206 recipients.
Material and Method: From 26th January 2010 to 30th May 2014, 206 kidney transplants were performed. Of 206, 69 were from live donor, 124 were deceased donor and 13 patients received a small tumour excised kidney graft. Laparoscopic or retroperitoneoscopic donor nephrectomy was routinely performed for live kidney donors. The majority of patients had their first kidney transplant, whereas 33, 4 and 1 patients received second, third and fourth transplant respectively. There were 9 recipients who had dual kidney transplantation. All recipient surgeries were performed by open surgery except one by laparoscopic surgery via the extraperitoneal approach. In our practice, the renal artery was anastomosed first either to the external or to the internal iliac artery followed by renal vein anastomosis to external iliac vein. A modified Lich-Gregoir technique was utilized for ureteroneocystostomy. A routine colour doppler ultrasound (CDU) and renal nuclear scan (99mTc MAG3) were obtained on day one post-transplant. CDU was repeated when there was an evidence of graft function deterioration. The patients were followed-up ranging from 18 to 72 months.
Results: All recipients transplants were successfully performed. There were 4 cases that developed renal artery stenosis and required interventional angioplasty and stent placement. There was no cases of vessel thrombosis. There was no urine leakage, but one case developed ureteral stenosis. The was one graft loss due to dehiscence at the anastomotic site, in which the multiple renal arteries were reconstructed and anastomosed to the side of external iliac artery.
Conclusion: In conclusion, the alternative vascular anastomotic approach of "Artery first, Vein Second" achieved good results. The benefit is that the kidney graft is less flipped around during vessel anastomoses and it may be time saving for the procedure.

P.1930

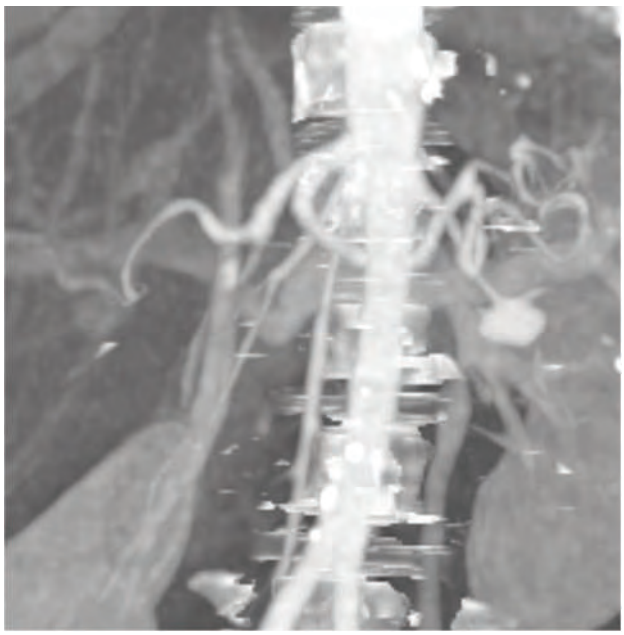
3D surgical planning in complex renal artery aneurysms: mid term results

Gabriele Soldini, Giuseppe Ietto, Marco Franchin, Domenico Iovino, Marco Calussi, Faranak Rostami Zarrabi, Matteo Tozzi, Giulio Carcano.
General and Transplant Surgery, University of Insubria, Varese, Italy.

Objectives: Operative repair of renal artery aneurysm (RAA) may be accomplished by different techniques; actually, endovascular stent-grafting or embolization procedures are an attractive alternative for the RAAs treatment. However, RAA beyond the renal artery bifurcation may require an open treatment with in vivo or ex vivo surgical repair. We report five cases of a complex RAA treated with back bench reconstruction with autologous tissue (gonadal vein) whereof two planned and performed through 3D virtual reconstruction and 3D print for surgical planning. We evaluated the benefit in terms of reduction of cold ischemia of the surgical planning in 3D .
Methods: From 2013 to 2015 five patients with complex renal artery aneurysms were treated with laparoscopic nephrectomy, ex vivo back bench reconstruction with multiple gonadic vein grafts and autotransplantation. The aneurysms ranged from 2.5 to 5.0 cm. In all cases, the aneurysm was resected ex vivo, leaving multiple branch arteries that anastomoses to the internal iliac artery and vein. Through 3D virtual reconstruction and 3D print of the kidney vessel it was possible to maximise the security of the laparoscopic nephrectomy and the to optimize the planning of the back bench reconstruction. The average follow-up time was 16 months (2-53).
Results: In all cases the artery aneurysms were successfully reconstructed. In only one case it was possible to perform anastomosis after aneurysmectomy and in all the other cases it was necessary to perform more than 4 anastomosis by microsurgery technique. An interesting aspect added in 2 cases is the use of the 3d virtual reconstruction and 3d print of the vessels of the kidney for planning the back bench reconstruction. In our cases, major advantages were a reliable configuration and anatomical relationship of the renal vessels, aneurysm and ureter anatomy virtual 3D reconstruction, based on the CT images in the DICOM format, and the 3D print allowed a 30% decrease of cold ischemia time during the back bench reconstruction phase (the decrease went from a mean of 6 hours in the first three cases to a mean of 4 hours in the last two cases).



The programming of the length of the gonadal vein to use, the number of anastomoses to be performed, the segments of the primary or secondary artery to replace over the arrangement and relations of each vessel with the anatomical structure involved (e.g. pelvis, ureter) represent a valuable aid.

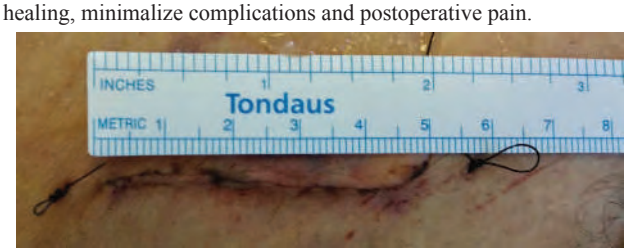


P.1931

Minimal invasive access in kidney transplantation - self experience

Marek Ostrowski, Anita Suwała, Jan Pawlus, Anna Prekwa, Jerzy Sieńko.
Department of General Surgery and Transplantation, Pomeranian Medical University, Szczecin, Poland.

Introduction: There has been great progress in development of minimally invasive surgery (MIS), during the last years[1]. MIS is popular technique both in surgery as well as in transplantation[2]. Many conventional surgical procedures were replaced by laparoscopic kidney transplantation or robotic kidney transplantation, especially for obese patients. Oyen et all described in 2006 the first experience in MIKT (minimally invasive technique for kidney transplantation)[2]. The incision is minimal (7-9 cm) and not larger than the one required for laparoscopic kidney transplantation. Using MIKT leads to better cosmetic effect, faster healing, minimalize complications and postoperative pain[2]. This technique is using in Department of General Surgery and Transplantation since 2014.
We retrospectively analized this technique and report our results here.
Patients and Methods: Since 2014 117 recipients were underwent kidney transplantation (57 women, 60 man). In 41 patients (35%) we used MIKT, in 76 - conventional technique (contol group). Incisions are restricted to 5 - 8 cm with minimal tissue trauma and minimal dissection. Extraperitoneally iliac vessels are dissected, renal vein is anastomosed to external iliac vein (end to side) and renal artery to external iliac artery (end to side). After revascularization the kidney is not moved from retroperitoneal pouch. Implantation of the ureter to the bladder is with minimal dissection too.
We analyzed body mass index, warm ischemia time, DGF/IGF, opioids usage and complication (lymphocele, wound infection).
Results: No minimally invasive techniques for kidney transplantation procedures were converted to the conventional technique. Incisions on the skin in MIKT were smaller than in control group (5-7 cm vs. 9-16 cm). Warm ischemia time was shorter in MIKT group than in control group (median - 22 min <18-38'> vs. 24 min <12-47'>). Moreover, usage opioid drugs during postoperative period were less in MIKT group. We did not observe any statistical significance between MIKT and control groups. In MIKT group were patients with lower BMI than in control group (median - 23,5 vs. 26,4). In MIKT group we observed complications, such as: lymphocele – 3 recipients, wound infection 2 recipients and 1 graft loss.
Conclusion: Minimally invasive technique for kidney transplantation is safe procedure for recipients, leads to better cosmetic effect, faster healing, minimalize complications and postoperative pain.



References:
[1] O. Øyen, T. Scholz, A. Hartmann, P. Pfeffera: Minimally Invasive Kidney Transplantation: The First Experience. Transplantation Proceedings Volume 38, Issue 9, November 2006, Pages 2798–2802
[2] S.C. Park, S.D. Kim, J.I. Kim, I.S. Moon:Minimal Skin Incision in Living Kidney Transplantation. Transplantation Proceedings Volume 40, Issue 7, September 2008, Pages 2347–2348
[3] Ole Øyen,Ph.D., Bjørn Lien, Pål-Dag Line, Per Pfeffer: Minimally Invasive Renal Auto-Transplantation: The First Report Journal of Surgical Research Volume 164, Issue 1, November 2010, Pages e181–e184

P.1932

End-to-side versus end-to-end uretero-ureteral anastomosis in kidney transplant recipients with disused atrophic bladder

Volkan Turunç[1,2], Aşkın Eroğlu[2], Babek Tabandeh[1], Hamad Dheir[2].
[1]Department of General Surgery, Bahcesehir University, Faculty of Medicine, Istanbul, Turkey; [2]Organ Transplantation Center, Goztepe Medical Park Hospital, Istanbul, Turkey.

Introduction: Extravesical Lich-Gregoir ureteroneocystostomy (UC) is the most widely used method for urinary reconstruction during kidney transplantation due to technical ease and lower complication rates. Sometimes it is difficult to perform UC in cases with short donor ureter and disused atrophic bladder. Pyelo-ureteral anastomosis (PUA) and uretero-ureteral anastomosis (UUA) may be preferred to UC for these patients. A limited number of studies about different techniques of PUA and UUA have been reported in the literature[1]-[3], but to our knowledge, there is no study comparing the efficacy of end-to-side and end-to-end UUA.
The aim of this study was to compare the results of end-to-side UUA with end-to-end UUA performed by our transplant team for urinary tract reconstruction in kidney transplantation.
Materials and Methods: We retrospectively reviewed the charts of 833 kidney transplant recipients operated in our transplant center between July 2010 and November 2014. We identified patients who underwent end-to-side or end-to-end UUA for urinary pathway reconstruction during kidney transplantation due to disused atrophic bladder. Group I consisted of 16 patients who underwent end-to-side UUA and Group II consisted of 20 patients who underwent end-to-end UUA. The two groups were compared in terms of efficacy, safety and graft function.
Results and Discussion: The two groups were similar in regards to mean age at transplantation, duration of dialysis and duration of hospital stay (p>0,05).
Since first 2 patients in end-to-end UUA group who underwent native ureteral ligation without nephrectomy developed hydronephrosis in their native kidneys, requiring nephrectomy in the post-transplant period, we performed native nephrectomy in all remaining patients of Group II. No native ureteral ligation and no native nephrectomies were performed in Group I. That's why, mean operative time was significantly longer in Group II compared to Group I (p=0,000). As we began performing end-to-side UUA as a relatively new technique compared to end-to-end UUA in order to eliminate the need for native nephrectomy and shorten the operative time of transplantation, post-transplant follow-up period of Group II was significantly longer than Group I (p=0,000), but all the patients in both groups had at least 1 year follow-up.
Although only 1 case of urine leakage occurred in Group I (6%) while 2 cases of urinary obstruction (10%), 2 cases of hydronephrosis (10%) and 2 cases of bleeding (10%) occurred in Group II, that was not statistically significant most probably due to the limited number of patients involved in the study (p=0,07).
The both groups were similar in regards to graft function and post-transplant urinary infection episodes, and no graft loss occurred.
Conclusion: End-to-side UUA without native ureteral ligation is a safe surgical technique for urinary tract reconstruction during kidney transplantation in patients with disused atrophic bladder due to its low complication rates and and significantly shorter operative time than end-to-end UUA.
References:
[1] Kockelbergh RC, Millar RJ, Walker RG, et al. Pyeloureterostomy in the management of renal allograft ureteral complications: an alternative technique. *J Urol* 1993; 149: 366-8.
[2] Nie Z, Zhang K, Huo W, et al. Comparison of urological complications with primary ureteroureterostomy versus conventional ureteroneocystostomy. *Clin Transplant* 2010; 24: 615-9.
[3] Sasaki H, Sato Y, Matsuhashi E, et al. Urinary tract reconstruction using uretero-ureteral end-to-side anastomosis in kidney transplant recipients. *Transplant Proc* 2015; 47: 359-62.

P.1933

Vena cava resection without venous bypass for liver transplant (LT) does not increase morbidity but does decrease operative time

Rolando Rebolledo[1], Josefina Saez[1], Pablo Achurra[1], Carlos Benitez[2], Francisco Barrera[2], Marco Arrese[2], Rodrigo Wolf[2], Blanca Norero[2], Alejandro Soza[2], Jorge Martinez[1], Nicolás Jarufe[1], Juan Francisco Guerra[1].
[1]Departamento de Cirugía Digestiva, Pontificia Universidad Católica de Chile, Santiago, Chile; [2]Departamento de Gastroenterología, Pontificia Universidad Católica de Chile, Santiago, Chile.

Introduction: Vena cava reconstruction options in LT are controversial. Original Cava reconstruction included venous bypass and Cava resection (CR). Currently, Cava sparing (CS) techniques (Piggy-Back and cavocavostomy) have developed and demonstrated some potential benefits. Our aim is to compare CR without venous bypass and CS techniques.
Methods: An historical cohort from Mar/1994 until May/2015 was analyzed. Variables as: age, gender, MELD, cause of liver failure (LF), complications, surgical outcomes and mortality were included. These variables were compared between Cava reconstruction technique groups.
Results: 240 transplants were performed in our institution. 194 procedures (80,8%) were included at this study. Rest of cases were excluded due to missing data. CR: 41 (21,1%) and CS: 153 (78,9%) cases. There was no difference in MELD, cause of LF, age, gender, kidney failure, vascular, biliary nor cardiovascular complications between CR and CS groups. 30 -day survival was similar between groups CR: 97,6%, CP: 89,8% (p = 0,345). Operative time (CR: 271,5 ± 67,6 min vs CS: 436,3 ± 138,0 min) and warm ischemia time (CR : 29,9 ± 7,0 min vs CS: 44,9 ± 24,4 min) were significantly higher in CS group.
Conclusion: Clinical results of CR without bypass are comparable to CS techniques. CR is a safe and faster technique, associated to better operative and ischemic times. Our findings suggest that this technique is a valid option to be considered for vena cava reconstruction in LT.

P.1950

HLA haplotype frequencies of Hong Kong

Jenny Ho[1], Yuk-Sing Chan[1], Ivan W.H.Tang[1], Meng-Biao Guo[2], Wan-Ling Yang[2], Cheuk-Kwong Lee[3], Janette S.Y.Kwok[1].
[1]Division of Transplantation and Immunogenetics, Department of Pathology & Clinical Biochemistry, Queen Mary Hospital, Pokfulam, Hong Kong; [2]Department of Paediatrics and Adolescent Medicine, The University of Hong Kong, Pokfulam, Hong Kong; [3]Blood Transfusion Service, Hong Kong Red Cross, Kowloon, Hong Kong.

Background: Hong Kong Bone Marrow Donor Registry (HKBMDR) managed volunteer donor registry to facilitate the unrelated hematopoietic stem cell transplantation as the curative treatment for blood malignancies and many other disorders for patients in Hong Kong. Better outcome requires optimal matching between donor and recipient at the HLA-A, -B, -C, and -DRB1 loci (i.e. an 8/8 high resolution HLA match). Since the level of polymorphism of HLA is extremely high polymorphic and allelic variation is population-specific, optimally HLA-matched unrelated donors and cord-blood units are not always available for patients, even within large registries[1],[2]. In the current study, regional HLA haplotype frequencies were calculated and the likelihood of identifying HLA match donors was also estimated.
Study Design and Methods: Seven thousand and five hundred voluntary donors at the HKBMDR recruited between January 2013 and June 2015 were included in this study. All donors are of Chinese origin, HLA-A, -B, -C and -DRB1 genotypes were obtained by sequencing specific oligonucleotide probes methods supplemented with sequence specific primer or sequence based typing methods to resolve the ambiguity. Alleles were determined according to IMGT/HLA Database release 3.18.0. HLA haplotype frequencies were calculated using the Markov Chain Monte Carlo algorithm PHASE[3]. Matching probabilities for 8/8 and 7/8 HLA Match were determined using these haplotype frequencies via the matching models assuming the genotypes are in Hardy-Weinberg equilibrium[4],[5].
Results: The distribution of haplotype of Hong Kong Chinese is found to be more heterogeneous than Western countries. Over five thousand A-C-B-DRB1 haplotypes were estimated from these donors (n=7,500). As of 2015, 100,000 donors in are available in HKBMDR. The matching probability is 45% at 8/8 HLA Match level and 75% at the 7/8 Match level which is significantly less than those of White European in the American's NMDP registry (75% at 8/8 match and 97% at 7/8 match). To enhance the matching probability, the donor registry should be doubled to 200,000 to achieve a likelihood of match to 55% at 8/8 match and 85% at 7/8 match (Figure 1). When taking the number of retired and non-contact donors into account (about 2,000 per year), it is estimated that around 20,000 new donors are required annually to meet the target.

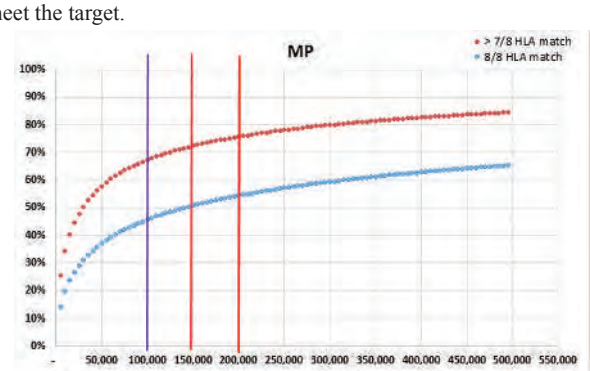


Figure 1 – Likelihood of finding an 8/8 HLA Match or > 7/8 HLA Match by different donor registry size in HKBMDR. Matching Probability (MP)

Conclusions: Due to the heterogeneity of the HLA typing of Hong Kong Chinese, the donor pool of the HKBMDR should be further expanded in order to enhance the likelihood of match. Our data provide vital information for defining donor recruitment target and planning for extra resources in order to support the cost in donor recruitment and HLA typing. Establishment of this cost effective donor registry could facilitate prompt curative treatment to benefit our local patients.

References:

[1] Beatty PG, Mori M, Milford E, et al. (1995) Impact of racial genetic polymorphism on the probability of finding an HLA matched donor. Transplantation 60:778-83.
[2] Kollman C, Maiers M, Gragert L, et al. (2007) Estimation of HLA-A, -B, -DRB1 haplotype frequencies using mixed resolution data from a national registry with selective retyping of volunteers. Hum Immunol 68:950-8.
[3] Stephens M, Smith NJ, Donnelly P. (2001) A New Statistical Method for Haplotype Reconstruction from Population Data. Am. J. Hum. Genet. 68:978-989.
[4] Beatty PG, Boucher KM, Mori M, Milford EL. (2000) Probability of finding HLA mismatched related or unrelated marrow or cord blood donors. Hum Immunol 61:834-40.
[5] Mori M, Graves M, Milford EL, Beatty PG. (1996) Computer program to predict likelihood of finding and HLA-matched donor: methodology, validation, and application. Biol Blood Marrow Transplant 2:134-44.

P.1951

Survey of questionnaire for transplant and dialysis patients on preparation for disaster

Masae Ikeda[1], Daisuke Ishii[1], Hiroyuki Ogura[1], Yuto Miyasaka[2], Fumino Noguchi,[3], Miki Tanaka[3], Masatsugu Iwamura[1], Kazunari Yoshida[1].
[1]Urology, Kitasato University, School of Medicine, Sagamihara, Japan; [2]Pharmaceutical, Kitasato University Hospital, Sagamihara, Japan; [3]Nursing, Kitasato University Hospital, Sagamihara, Japan.

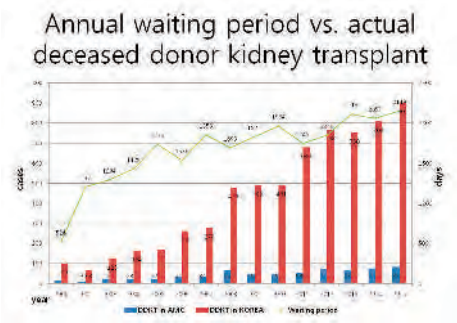
Introduction: We have frequent earthquakes in Japan. In the mega-earthquake and tsunami in 2011 in northern area of Japan, many patients suffered from their treatment. This scary experience recognized them the importance of preparation for disaster. We perform survey of questionnaire for renal transplant patient and dialysis patient about daily management of medication and other preparation for disaster.
Purpose: The purpose of this survey is to know the patients' awareness of daily management of their medication and preparation for disaster, and this results lead to better self-management of therapy.
Method and Object: This survey was performed for 75 hemodialysis patients (Group A) and for 71 renal transplant patients (Group B). The questionnaire consists of 4 easy yes/no questions.
• Question 1: Who manage your medication?
• Question 2: How do you store your medication?
• Question 3: Do you store your medication in fixed place?
• Question 4: Do you prepare for disaster?
We asked question about medication to the patients in Group B.
Result: The average age of Group A was 48 (39-87) year old, and 52 (29-81) for Group B. Group A was 26%, Group B was 35%, and many HD patients store their medication in vinyl bag. The most patients store their medication in the fixed place in both Group A and B.
Only 29.3% patients prepare for disaster, but 65.9% in transplant patients.
Discussion: Ministry of Health, Labour and Welfare study recommends patients the use of so-called "medicine notebook ". We know the internet system of medication registration, but the effectiveness of this system in terms of management of medication is unknown. We need to make the document to lead to awareness for disaster and importance of self-management by assistance of pharmacists in near future.
References:
[1] Ministry of Health, Labour and Welfare from http://www.mhlw.go.jp/bunya/seikatsuhogo/saigaikyujo5.html

P.1952

An analysis of waiting period of deceased donor kidney transplant - Korean single center analysis

Sarome Lee[1], Duck-Jong Han[2], Young-Hoon Kim[2], Hea-Seon Ha[1], Jung-Ja Hong[1], In-Ok Kim[1].
[1]Organ Transplant Center, Asan Medical Center, Seoul, Korea; [2]Department of Surgery, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea.

Background: Kidney transplantation is the best renal replacement treatment for an end-stage renal disease patient. However the shortage of donated organ remains as an unsolved problem. In Korea "Oragns Transplant Act" was developed in February 1999. Deceased organ donation (DDKT) began to be activated by the Korean Network for Organ Sharing (KONOS) under the control of government.
Material and Method: We have analyzed our 663(the largest single center number convering 10% of whole cases in Korea) kidney transplant recipients who were operated at Asan Medical Center (AMC) between January 2001 and December 2015 through retrospective analysis,
Results: There were 663 deceased donor kidney transplantation recipients between January 2001 and December 2015, and their average waiting period was 1,829 days. The waiting time from registration to actual transplantation were 1,302 days in the period of 2001~2005 (13.6%), 1,775 days in 2006~2010 (33.8%), and 1,999 days in 2011~2015 (52.6%). The recipients of the negative PRA group (72.1%) waited 1,765 days, while 1,932 days in the group of PRA 21~50% (9.4%), 2,013 days in PRA 51~80% (8%), and 2,395 days in PRA 81~100% (6.5%). The higher PRA had a longer waiting period. The waiting period categorized by age group is as follows: the recipients below 11 (1.7%) with 4 advantage points by KONOS had to wait for 1,060 days, the recipients in the age group between 12 and 18 (2.1%) with 3 advantage points wait for 1,317 days, the recipients in the age group of 19 to 60 (90%) wait for 1,851 days, and the recipients who were more than 61 (6.2%) wait for 1,889 days. Simultaneous pancreas and kidney transplantation group (15.2%) waited for 1,249 days. The waiting period by blood type is as follows: 1,793 days for A+ type (34.2%), 1,746 days for B+ type (29.4%), 2143 days for O+ type (23.8%), and 1,523 days for AB+ type (12.5%). In zero-antigen mismatching group's (12.1%) average waiting time was 1,249 days, and re-transplant group (10.1%) had to wait 2,008 days. Hepatitis B virus carrier group (6.2%) had to wait 1,517 days and hepatitis C virus carrier group (1.7%) waited 1,602 days which were shorter than the average. The average waiting period of male recipients (51.3%) was 1,802 days while female (48.7%) was 1,857 days
Discussion and Conclusion: The waiting period apparently becomes longer as time passes in spite of the increasing number of deceased donors. It is supposed that the number of DDKT patients on the waiting list is rapidly growing, which overwhelm the actual deceased donors. AMC plans to provide the wait-listed DDKT patients with definite and quantified information based on this analysis, which will also be helpful for the management of those listed for DDKT patients.



P.1953

The acquiring skill of the adolescents and young adults to get along with the difficulties in their social activities after transplantations

Yasuko Suzuki.
School of Medicine, Shinshu University, Matsumoto, Japan.

Backgounds: Many survivors of pediatric cancer or other severe conditions experienced transplantations in their terms of severe intensive treatments. The adolescents and young adults (AYA) experienced transplantations are leading various social lives while dealing with late effects of severe intensive treatments including various types of transplantations. The AYA have learned various skill to confront with difficulties through long-termed treatment periods, and the skill will be conjugate in the other stages after AYA.
Purpose: The purpose of this research is to identify the process to acquire the skill to get along with difficulties that the adolescents and young adults with chronic conditions have confrounted with their own lives after severe intensive treatment including transplantations.
Methods: Data was collected in the semi-structured interviews for 10 AYA experienced transplantations (5 college students and 5 members of society). These data was analyzed qualitatively and induced themes from experiences of the AYA as participants. Before research conducted, the proposal had been examined and consented by institutional review board. Then adolescents and young adults who had experienced transplantations announced own experience and conditions openly were recruited for the participation in this research through the representatives or members of several self-support groups of the AYA with chronic conditions. They had also been informed about purpose and methods, ethical consideration of this research processes before consenting.
Results: 6 themes emerged from the data as follows: 1) AYA experienced transplantations can grope for the way of advanced solution to problems beyond established and conventional idea. 2) AYA experienced transplantations can make a decision in accordance with own efficacy and capacity. 3) AYA experienced transplantations can try to explain own opinion for friends to understand them. 4) AYA experienced transplantations can recognize the defferenced and common points with people around themselves through the social activities after transplantations. 5) AYA experienced transplantations can get along with stressors and fifficulties in the social activities with friends. 6) AYA experienced transplantations can establish own identities with the recognition of own capacity.
Conclusion: There emerged 6 themes for acquiring of the skill to get along with the difficulties in AYA with experience of the transplantations. They should have acquired the reasonable skill with the recognition of efficacy and capacity. If someone hesitate to keep the connection with friends throughout the treatment, they should be able to resume the connection to friends after own intensive treatments, such as transplantations. These themes emerged in this research should suggest to be also needed for AYA to transit to the adult. Further theoretical sampling should be needed to identify the skill for AYA with the experiences of transplantations to acquire after taking of the social activities.

P.1954

Analyses of relationship between obstetric complications and preterm delivery in Japanese recipients received kidney transplant

Yuki Yoshikawa[1,4], Junji Uchida[2], Chiharu Akazawa[3], Nobuhiko Suganuma[4].
[1]Faculty of Nursing, Setsunan University, Hirakata, Japan; [2]Department of Urology, Osaka City University Graduate School of Medicine, Osaka, Japan; [3]Faculty of Nursing, Osaka Medical College, Takatsuki, Japan; [4] Department of Human Health Science, Kyoto University Graduate School of Medicine, Kyoto, Japan.

Introduction: Nowadays more than 1,600 kidney transplants are performed annually in Japan, and the frequency of pregnancies among the kidney-transplanted patients has been increasing due to progressive improvements for managing these recipients. However it has been reported that preterm deliveries resulting in low birth weight occurred frequently in the recipients.
Objectives: To investigate the factors associated with the preterm deliveries in the recipients received kidney transplantation, a relationship between obstetric complications and perinatal outcome were analyzed.
Materials and Methods: Sixty-five childbirths in 49 kidney-transplanted recipients were subjected to the present study. The clinical data were collected through medical records. Student's t-test was applied to evaluate the relationship between obstetric complications during pregnancies and gestational weeks at delivery, birth weight or other factors such as number of children, age at transplant, age at childbirth, delivery form, cesarean section rate and breastfeeding rate.
Results: Compared to general population, the recipients showed the higher rates of preterm delivery (62.7%), low birth weight (61.9%) and obstetric complications (60.0%) that consisted of pregnancy induced hypertension (PIH) as a main disease (87.0%). However there were no statistically significant differences between PIH occurrence and birth weight, gestational weeks at delivery or the other factors.

Table 1. Outcome of Pregnancies and Childbirths in Japanese Recipients Received Kidney Transplant

Characteristics of study population	n=19
Age at transplant (yr)	27.9±4.7 (16-37)
Transplant-pregnancy interval (mo)	29.4±24.2 (6-120)
Transplant-first childbirth interval (mo)	61.1±48.6 (15-264)
Number of children	1.4±.7 (1-4)
Outcome of childbirth of kidney-transplanted recipients	**n=65**
Age at childbirth (yr)	33.3±3.8 (24-41)
Birth weight (gr)	2280±639 (609-3236)
Gestational weeks at delivery	35.1±3.4 (27-40)
Low birth weight (< 2500gr)	61.9%
Preterm delivery (< 37wk)	62.7%
Obstetric complication	60.0%
pregnancy induced hypertension	52.3%
others	7.7%
Cesarean section	67.2%
Breastfeeding	89.6%

Table 2. Relation between pregnancy induced hypertension and birth weight or gestation at delivery

	PIH (+)	PIH (-)	P
birth weight (gr)	1827±629 (n=24)	2075±290 (n=15)	NS
gestation at delivery (wk)	33.7±3.7 (n=23)	33.7±1.8 (n=12)	NS

PIH; pregnancy induced hypertension, NS; not significant

Discussion: Some clinical studies have suggested that maternal comorbidities and complications during gestation would induce preterm delivery and low newborn weight in the kidney-transplanted recipients. Especially PIH that is somewhat related to renal function seems to affect the problems throughout pregnancy and delivery. However our present study indicated no relationship between PIH occurrence and gestational age at delivery or newborn weight though the recipients indicated the higher rates of preterm delivery, low birth weight and obstetric complications.
Conclusion: Although there was no direct proof concerning to the relation between preterm delivery and obstetric complications in the kidney-transplanted recipients, prudent perinatal care for them to prevent preterm and low-weight-newborn delivery.
This work was supported by JSPS Kakenhi Grant Number 25870952
References:
[1] Lisa A. Report from the National Transplantation Pregnancy Registry (NTPR): Outcomes of Pregnancy after Transplantation. Clinical Transplants. 2010; 65-85.
[2] Cornelia B. A Comparison of the Outcome of Pregnancies After Liver and Kidney Transplantation. Transplantation. 2013; 95: 222-227.
[3] N. A. Deshpande. Pregnancy Outcome in Kidney Transplant Recipients: A Systematic Review and Meta-Analysis. American Journal of Transplantation. 2011; 11: 2388-2404.

P.1955

"Preventing": A new application for smartphones to assess and improve immunosupressant's adherence in renal transplant recipients

Jorge Morales-Barría[1], Felipe Morales[2], Bernardita Celis[3], Pedro Johansson[4], Cristian Metcalfe[5].
[1]Centro de Trasplante, Clinica Las Condes, Santiago, Chile; [2]Fjord, Design and Innovation, Stockolm, Sweden; [3]Universidad San Sebastian, Santiago, Chile; [4]TNA, Tecnoasistencia, Santiago, Chile; [5]MRC, Comunicación y Diseño Ltda., Santiago, Chile.

Introduction: Long-term success of organ transplantation is severily affected by non- adherence (NA) to immunosuppressive (IS) treatment. NA is observed even in health care systems providing free cost medications. NA has been attributed to social, psychological, economic, therapy related factors, and health care system deficiencies. Paradoxically, NA incidence in the transplantation setting moves in the range of 24 to 70% but it is rarely suspected in the routinely patient physician relationship. Short-term measures have been adopted to detect NA and stimulate a recipient adherence behavior (patient education, counseling, mentors, pill ingestion monitored control, Ingestible Sensor System, applications, reminders, etc.), but results of these initiatives have not shown definitive good long-term results yet being some of them very expensive or difficult to implement.
Materials and Methods: This report describes "Preventing" a Smartphone, Tablet, and PC new application, created for the diagnosis and eventually prevention of immunosuppressive NA.The "Preventing" application is designed to allow a long-term connection between the patient and the transplant team, remitting the patient with doubts and uncertainties to his/her health care staff. Active patient participation by completing questioners related to physical parameters, (i.e. blood pressure, weight, renal function blood test results and immunosuppressant prescriptions and drug levels) is essential to prolong temporal commitment and compliance. The patient is assisted by the physician or the coordinator nurse to assure accuracy in reporting more specific laboratory data. All information obtained through different questionnaires, including detailed demographic aspects, physician appointments, IS drug prescription and intake, relevant laboratory tests and notifications and interventions will be collected in an institutional PC (back office) to be used by the staff, shared by the patient and eventually used for clinical research. A permanent score of Commitment and Attachment will be shown as a patient's guide of his/her compliance to prescriptions.
Conclusion: "Preventing" application's feasibility to use will be evaluated in a prospective randomised study performed in kidney transplant recipients. In the same study the impact on adherence will be appraised through the Morisky's Scale.