HELP! What Do All These Rheumatology Lab Tests Mean??

Use and Interpretation of Common Rheumatologic Tests:

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Dec. 1, 2021
Mcgill Family Medicine Review Course

Disclosures:
Advisory boards, speaker, clinical trials.....

- Amgen
- Pfizer
- Novartis
- BMS
- Novartis
- Janssen
- UCB
- Roche
- Abbvie
- Merck
- Sanofi
- Lilly

❖None that affect this workshop

Objectives

By the end of this lecture, participants will be able to:

- Better understand the interpretation and significance of common Rheumatological lab tests
- Improve skills in appropriate ordering of rheumatological lab investigations depending on the clinical context
- Use this knowledge to enhance quality of patient referrals
- Apply these skills in daily practice in order to improve patient diagnosis and care

Laboratory Investigations in rheumatology: General Principles

- Guided by patient history and physical exam
- If no clue to diagnosis after history and exam, odds of making diagnosis with lab is poor
- Order tests that address most likely diagnosis
- "Arthritis panel" ie: shotgun approach - should not be used
- Choose subsequent tests to refine diagnosis, monitor disease progress, ensure safety of Tx

Commonly used Rheumatologic and Immunologic tests:

- Rheumatoid factor
- Anti-CCP
- ANA
- ENA
- Anti-DNA
- ANCA
- Antiphospholipid antibody
- Complement levels

- ESR
- CRP
- Uric Acid
- Synovial Fluid Analysis
- HLA B27
- Myositis panel
- Scleroderma panel

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Facture / Invoice No:

Order Date: October 29, 2010

Laboratory Test Billing *Quebec Resident*

Health Insurance #: CONCORDIA/ BLUE CROSS

Birth Date:

Payment Received? ☐

Montreal
Canada

Quebec

Code:	Description	Price:
30132	Creatinine	$2.84
90008	Liver Profile (MGH)	$19.43
20706	Antinuclear Antibody Screen	$104.28
40790	Rheumatoid Factor Quant	$30.81
20776	C3 C4 Complement(MGH)	$22.28
20710	Anti-Extractable Nuclear Antigen	$31.76
30276	IgE	$41.24
20361	Complete Blood Count	$5.69
20362	Manual Differential	$42.66
90003	Coag Profile	$10.43
20490	Sedimentation Rate	$5.69
30413	C-Reactive Protein	$11.85

CUSM/MUHC
Approuvé/Approved
29 OCT. 2010
Par
Per

Order Total: **$328.96**

Case

- **You see a 24 year old woman with a few months of polyarthralgias and fatigue, which started post partum**
  - Among other things, your differential diagnosis includes rheumatoid arthritis and systemic lupus erythematosus

How do you screen for RA and SLE?

- **Primarily by history and physical examination**
  - Increase your “pretest probability” by asking questions that support the diagnosis of inflammatory arthropathy or systemic rheumatic disease
  - Look for clues on physical examination

- Example: morning stiffness, swollen joints, rash, fatigue, other systemic symptoms……

What tests do you order?

- Routine tests may reveal helpful information
  - Anemia, low WBC, low platelets
  - Urinary abnormalities- blood, protein
  - Elevated creatinine
  - Thyroid
  - Abnormal chest X-ray

Should you order ESR or CRP?

- **Nonspecific tests**
- **Assess acute phase response in the blood**

ESR

- Increased by
  - Acute phase reactants
  - Paraproteins
  - Anemia (fewer cells, less repellent forces)
- ALSO
  - Age, gender, pregnancy, diabetes, renal failure, malignancy, infection

    tissue damage (ex: MI)

Many labs not doing anymore

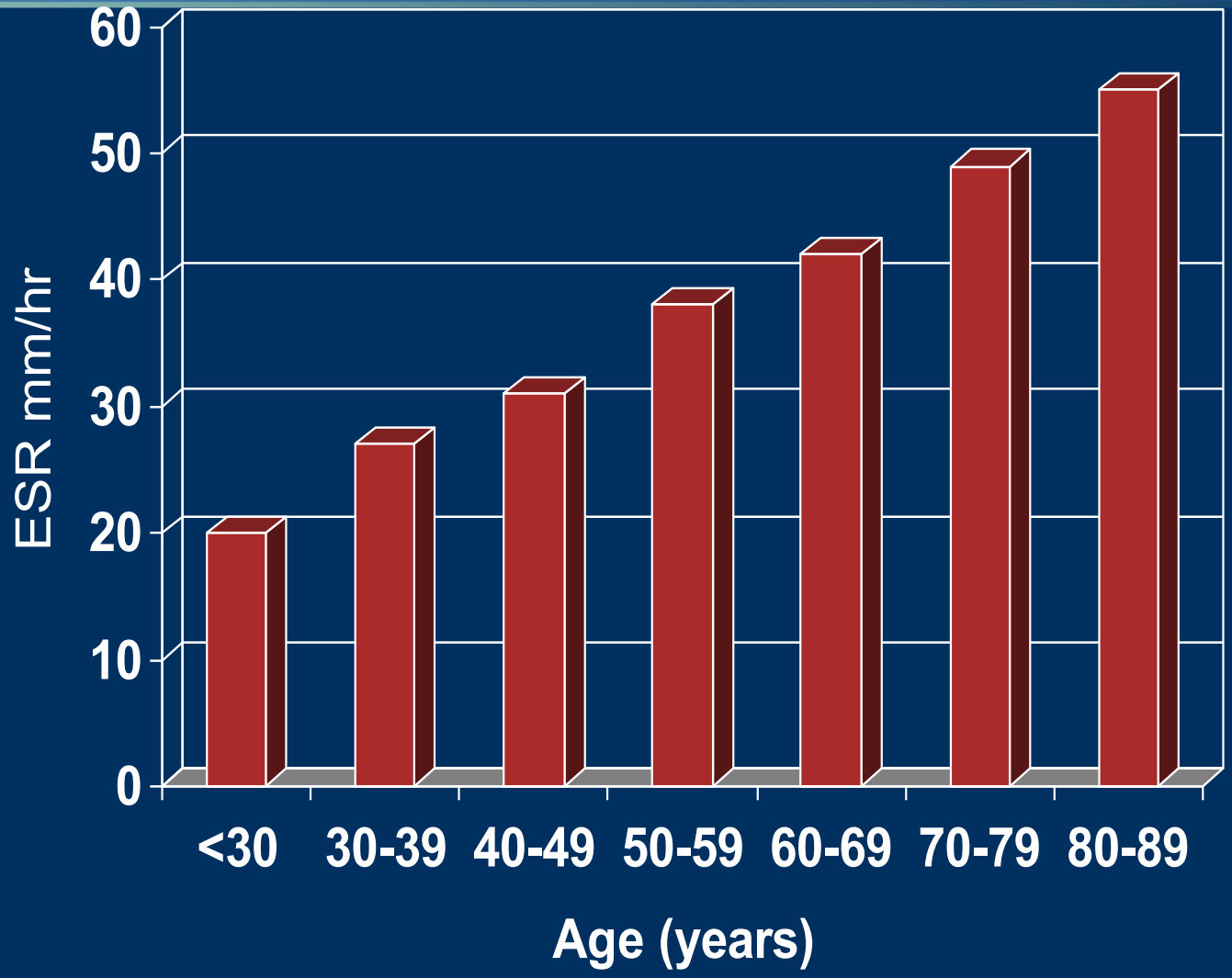
A good rule of thumb,…

- **For Men**
  - **Upper limit of normal of ESR = $\frac{\text{Age}}{2}$**
- **For Women**
  - **Upper limit of normal of ESR = $\frac{\text{Age} + 10}{2}$**

Thus, test is of limited value in the elderly population!

ESR & Age

M=Age/2
F=Age+10/2



CRP

- Direct measure of acute phase reactants
  - Less sensitive to irrelevant factors- age, anemia….
  - Responds more quickly
  - More expensive
  - Don’t always know how to interpret- may be elevated in metabolic syndrome, obesity

Extreme Elevation of CRP

Table 1.

Causes of markedly elevated CRP levels: *N = 839 patients.*

DIAGNOSIS	PATIENTS, N (%)
Infection	462 (55.1)
Inflammatory	45 (5.4)
Rheumatologic	63 (7.5)
Malignancy	43 (5.1)
Drug reaction	14 (1.7)
Multiple*	47 (5.6)
Uncertain	148 (17.6)
Other	17 (2.0)

CRP—C-reactive protein.

*Of the 47 patients who had multiple diagnoses, 46 also had infection.

Table 2.

Noninfectious diagnoses and associated peak CRP values

DIAGNOSIS	NO. OF PATIENTS	PEAK CRP LEVEL, mg/L
Rheumatologic	63	361.0
Solid tumour	25	325.0
Pericarditis	20	277.0
Inflammatory bowel disease	21	203.2
Hematologic malignancy	18	321.0
Drug reaction	14	321.0
COPD exacerbation	5	312.9

COPD—chronic obstructive pulmonary disease, CRP—C-reactive protein.

Can Fam Physician. 2017 Jun; 63(6): e316–e323.

Key Concepts

- ESR,CRP
  - Nonspecific indicators of inflammation
  - Not useful as screening tests for rheumatic diseases
  - Cannot differentiate one disease from another
  - Useful in PMR/GCA, and to monitor RA disease activity

Should you order a Rheumatoid Factor?

Rheumatoid factor

- The higher the level of RF the higher the likelihood of disease
- RF levels are not useful in monitoring RA
- RF should be ordered in patients with a high pretest probability of RA (or Sjogren's syndrome)
- RF is associated with nodules and vasculitis in RA patients
- RF is inexpensive

Rheumatoid Factor

- **Sensitivity 80% *in patients with RA***
- **Specificity ranges from 80-90%**

- **Prevalence of RA is 1%**

So, lots of positive RA are false positive!

Other conditions causing positive Rheumatoid Factor

- Other systemic rheumatic diseases-
  - Sjogren's, SLE, MCTD
- Infections- bacterial endocarditis
  - Hepatitis, TB, Syphilis, parasitic disease, viral
- Pulmonary diseases
- Malignancy

RF is positive in 80% of RA Cases : ie: Up to 20% are “Seronegative”

- So, RF is not diagnostic for RA on its own
- Testing is most useful when there is a moderate level of clinical suspicion for RA

Pretest probability	Post-test Probability RF +
1%	16%
25%	84%
90%	99%

In patients with established RA

- RF correlates with severe articular disease and extra-articular manifestations
- May have prognostic value

- Once test is positive, no value in re-testing
  - does not change with disease activity

What About anti-CCP ?

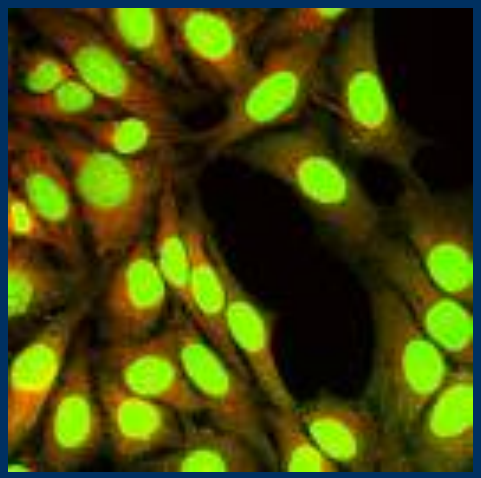
- Anti-cyclic citrullinated peptide antibody
- As sensitive & more specific than RF
- Appears earlier in disease
- A marker for poor prognosis, so can help guide treatment choices
- May be detected in healthy people years before onset of RA

Key Concepts

- **Rheumatoid Factor**
  - Rheumatoid factor is not diagnostic for rheumatoid arthritis
  - The test's utility is greatest when there is a moderate pre-test probability of disease
- **Anti CCP** now commercially available, more specific, and may help guide treatment

Should you order an ANA?

Antinuclear Antibodies



- A group of antibodies that bind to various nuclear (and some cytoplasmic) antigens

ANA

- Sensitivity >95% in patients with SLE
  - Almost all patients with SLE have positive ANA
- The ANA test is not specific for SLE
- ANA not synonymous with a diagnosis of Lupus
- As opposed to RA, ANA negative Lupus is rare

ACR Criteria For Lupus (ACR 1997)

A. **MUCOCUTANEOUS**
   1. Malar Rash
   2. Discoid Rash
   3. Photosensitivity
   4. Oral ulcers

B. **SYSTEMIC INFLAMMATORY**
   5. Arthritis
   6. Serositis – pleuritis or pericarditis
   7. Cerebritis – seizures or psychosis
   8. Nephritis – >0.5g proteinuria

C. **LABORATORY**
   9. Hematologic – Leucopenia, lymphopenia, thrombocytopenia or hemolytic anemia
   10. Immunologic – dsDNA, Sm, or Antiphospholipid antibodies
   11. ANA

4 out of 11 for diagnosis

Updated:
2019 Classification Criteria for SLE

Entry criterion

Antinuclear antibodies (ANA) at a titer of ≥1:80 on HEp-2 cells or an equivalent positive test (ever)

↓

If absent, do not classify as SLE
If present, apply additive criteria

↓

Additive criteria

Do not count a criterion if there is a more likely explanation than SLE.
Occurrence of a criterion on at least one occasion is sufficient.
SLE classification requires at least one clinical criterion and ≥10 points.
Criteria need not occur simultaneously.
Within each domain, only the highest weighted criterion is counted toward the total score§.

Clinical domains and criteria	Weight
Constitutional	
Fever	2
Hematologic	
Leukopenia	3
Thrombocytopenia	4
Autoimmune hemolysis	4
Neuropsychiatric	
Delirium	2
Psychosis	3
Seizure	5
Mucocutaneous	
Non-scarring alopecia	2
Oral ulcers	2
Subacute cutaneous OR discoid lupus	4
Acute cutaneous lupus	6
Serosal	
Pleural or pericardial effusion	5
Acute pericarditis	6
Musculoskeletal	
Joint involvement	6
Renal	
Proteinuria >0.5g/24h	4
Renal biopsy Class II or V lupus nephritis	8
Renal biopsy Class III or IV lupus nephritis	10

Immunology domains and criteria	Weight
Antiphospholipid antibodies	
Anti-cardiolipin antibodies OR	
Anti-β2GP1 antibodies OR	
Lupus anticoagulant	2
Complement proteins	
Low C3 OR low C4	3
Low C3 AND low C4	4
SLE-specific antibodies	
Anti-dsDNA antibody* OR	
Anti-Smith antibody	6

Total score:

↓

Classify as Systemic Lupus Erythematosus with a score of 10 or more if entry criterion fulfilled.

Aringer M, *et al*. *Ann Rheum Dis* 2019;**78**:1151–1159. doi:10.1136/annrheumdis-2018-214819

ANA

- **ANA 1:40** **Seen in almost 32%** of normals
- **ANA 1:80** **Seen in almost 13%**
- **ANA 1:160** **Seen in almost 5%**
- **ANA 1:320** **Seen in almost 3%**

- ***There is no set titer that can distinguish between those with and without SLE***
- ***Most people with positive ANA don't have disease***

Non-rheumatic conditions causing positive ANA

- **Normal individuals:**
  females > males, increasing age, relatives of patients, pregnancy
- **Hepatic diseases:**
  hepatitis
- **Pulmonary diseases:**
  idiopathic pulmonary fibrosis
- **Chronic infections**
- **Malignancies:**
  lymphoma, leukemia, melanoma, some solid tumors
- **Hematologic disorders:**
  ITP, autoimmune hemolytic anemia
- **Drug- induced** :
  procainamide, hydralazine, quinidine, tetracycline, TNF inhibitors
- **Miscellaneous autoimmune conditions:**
  autoimmune thyroid disease, type 1 diabetes mellitus, IBD, celiac, MS

ANA Patterns: Not specific though

PATTERN	NUCLEAR ANTIGEN	DISEASE Associations
Homogeneous	Histone/DNA	SLE, Drug induced SLE
Speckled	Saline-ENA's	MCTD, SLE, Sjogrens syndrome, poly/ dermatomyositis, infection and neoplasia
Nucleolar	RNA associated antigens	Scleroderma
Peripheral	DNA	SLE
Centromere	Centromere	Limited Scleroderma: CREST Syndrome

Once ANA screen is positive…consider more specific autoantibody tests

- Specific autoantibody tests possess diagnostic significance in the right clinical setting ex: anti-dsDNA, ENA, anti-Histones(drug induced LE)

- ENA (extractable nuclear antigen) panel includes
  - Anti Sm (Smith)
  - Anti RNP (Ribonucleoprotein)
  - Anti SS-A, SS-B (aka Ro and La)
  - Anti Scl-70
  - Anti Jo-1

More on Antibody Specificity….

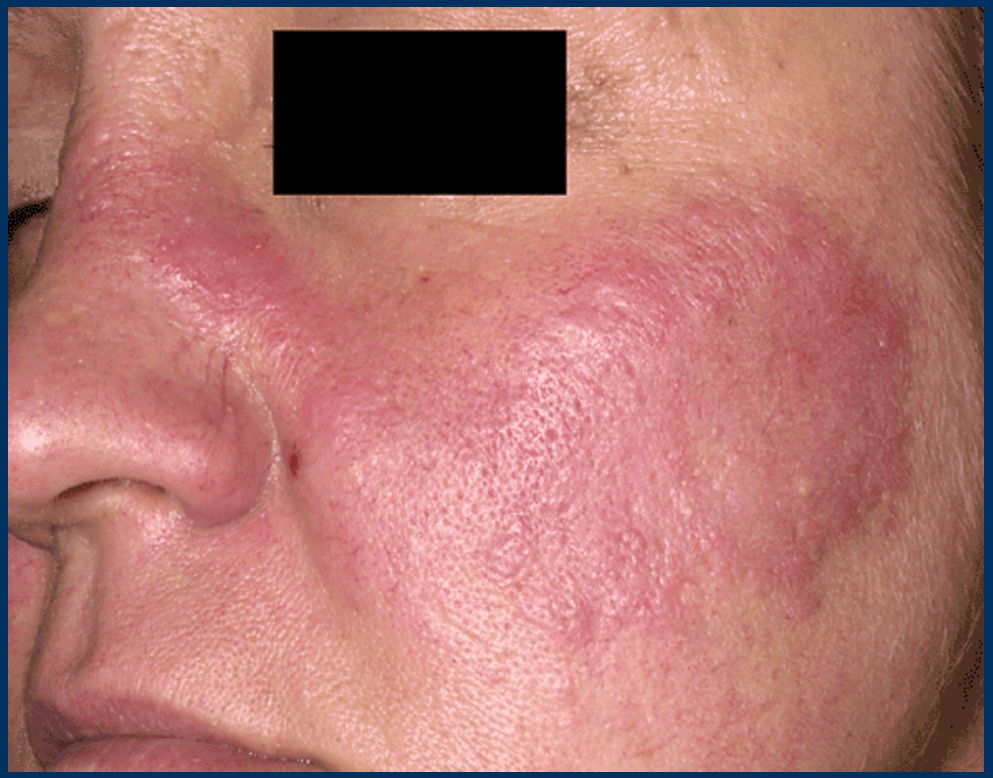
- **Anti-dsDNA**
  - Specific for SLE (60-70%)
  - *May fluctuate with disease activity*
- **Anti-Sm (Smith)**
  - Highly specific for SLE (but not sensitive)
- **Anti-RNP**
  - Nonspecific, seen in SLE but also is part of criteria for mixed connective tissue disease (MCTD)

More Antibody Specificities…

- **Anti-centromere antibody (ACA)**
  - **Associated with CREST syndrome**
- **Anti-topoisomerase I (Scl-70)**
  - **Associated with diffuse Scleroderma**
- **Anti-Ro (SS-A) and La (SS-B)**
  - **Associated with Sjogren's**
  - **Can be seen in SLE, SCLE**
  - **May be associated with neonatal heart block in babies of mothers with this antibody**
- **Anti-Jo-1 (anti-histidyl-tRNA synthetase)**
  - **Specific for myositis associated with interstitial lung disease, Raynaud's**

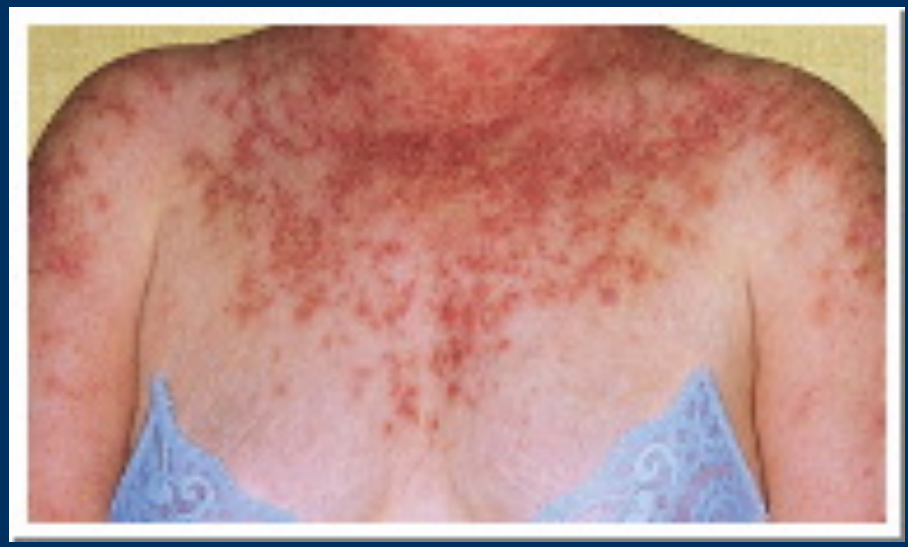
Malar Rash

- Fixed
- May be flat or raised
- Erythematous
- Over the malar eminences
- Sparing the nasolabial folds

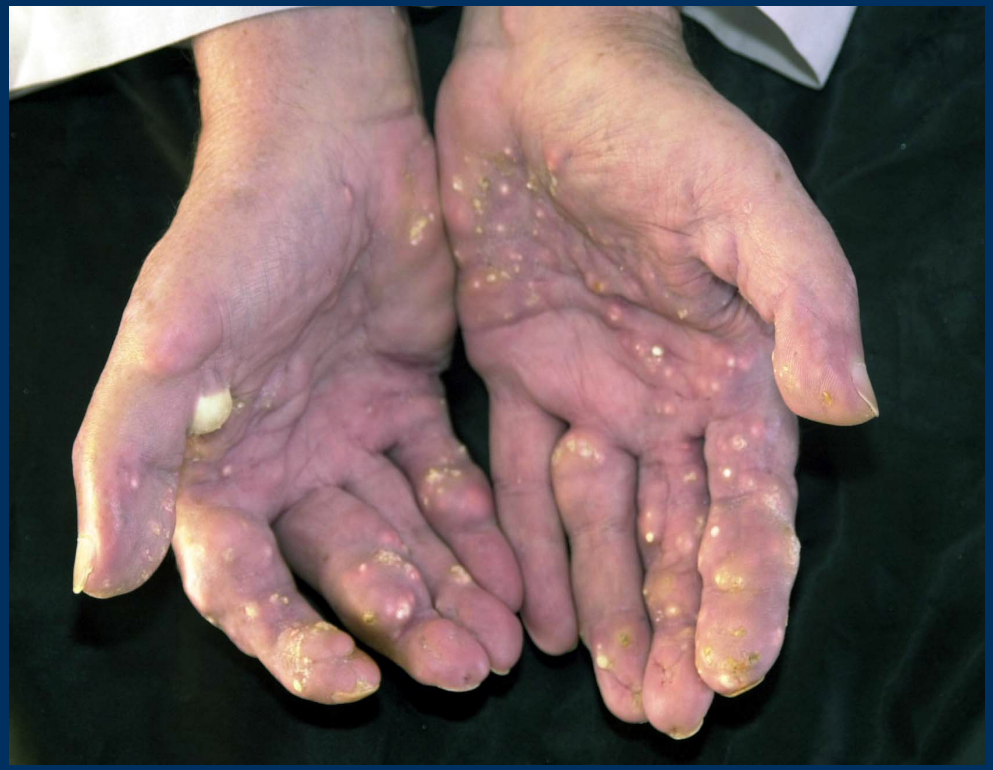


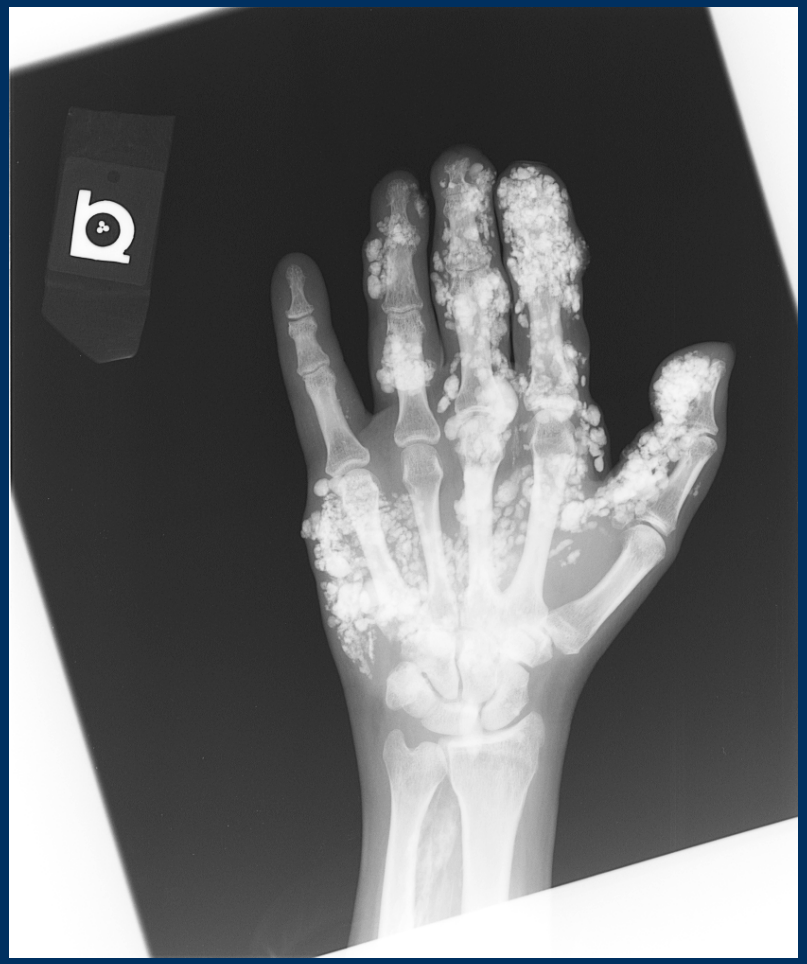
Photosensitivity in SLE

- Erythematous rash in sun exposed areas
- May look like sunburn
- Sun exposure may cause new DLE or other rashes.
- May flare internal disease with arthralgias and fatigue.

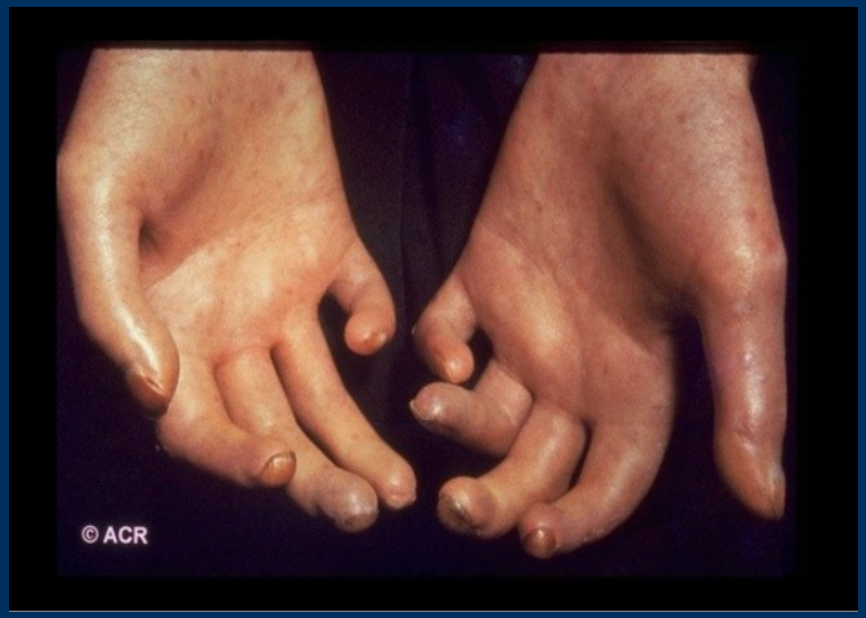


Calcinosis in CREST Syndrome



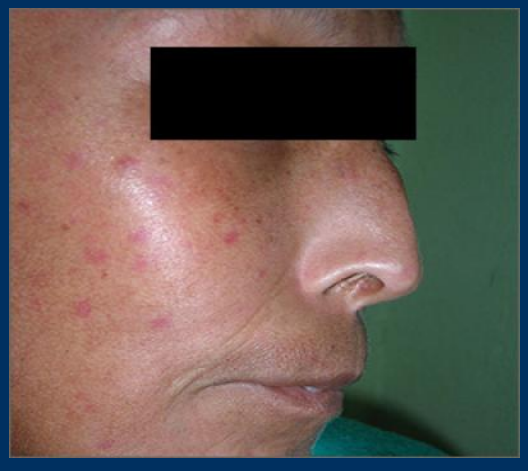


Scleroderma Features



Sclerodactly

Digital Ulcers



Telangiectasias

Dermatomyositis

Gottron's Papules

Heliotrope Rash

Drug induced lupus: Anti histone antibodies

- Seen with
  - Procainamide
  - Sulfasalazine
  - Hydralazine
  - Minocycline
  - Isoniazid
  - Anti-TNF agents
- Must fulfill ACR criteria for lupus
  - renal and hematologic involvement rare
- ANA and Anti Histone positive
- Usually resolves on stopping the drug

Choosing Wisely: ANA

Don't order ANA as a screening test in patients without specific signs or symptoms of systemic lupus erythematosus (SLE) or another connective tissue disease (CTD).

ANA testing should not be used to screen subjects without specific symptoms (e.g., photosensitivity, malar rash, symmetrical polyarthritis, etc.) or without a clinical evaluation that may lead to a presumptive diagnosis of SLE or other CTD, since ANA reactivity is present in many non-rheumatic conditions and even in "healthy" control subjects (up to 20%). In a patient with low pre-test probability for ANA-associated rheumatic disease, positive ANA results can be misleading and may precipitate further unnecessary testing, erroneous diagnosis or even inappropriate therapy.

Sources:

BC Guidelines. Antinuclear antibody (ANA) testing protocol [Internet]. 2013 Jun [cited 2017 May 5].

Key Concepts

- **ANA**
  - Not recommended as a routine screening test
  - Greatest utility for diagnosis of lupus with moderate pre-test probability
  - Virtually rules out SLE when negative
  - If ANA strongly positive, use more specific Ab testing (in the correct clinical context) to help you refine the diagnosis

What other immune tests might be helpful to order?

APL Antibodies: Antiphospholipid syndrome

Simplified antiphospholipid syndrome classification criteria

Clinical criteria

- blood clots within arteries, veins, or small blood vessels
- adverse outcomes during pregnancies, such as three or more spontaneous abortions before 10th week of pregnancy, unexplained fetal deaths[1] at or beyond 10th week of pregnancy, or premature births before 34th week of pregnancy due to severe preeclampsia[2] or eclampsia[3]

Laboratory criteria (antiphospholipid antibody tests)

- positive lupus anticoagulant test
- positive anticardiolipin antibody (aCL) IgG or IgM
- positive anti-Beta-2-glycoprotein-I antibody (aβ_2GPI) IgG or IgM

1. Spontaneous death of fetus due to any cause which leads to pregnancy loss.
2. A disorder characterized by new-onset high blood pressure that develops after the 20th week of pregnancy that leads to damage in various organs, most commonly protein leakage from kidneys. Severity depends on the degree of damage to organs or level of blood pressure.
3. Development of seizures in women with preeclampsia that cannot be explained by any other cause.

Tektonidou MG, *et al*. *Ann Rheum Dis* 2019;**78**:1296–1304. doi:10.1136/annrheumdis-2019-215213

Serum complements

- Not an antibody test, but useful for monitoring disease activity in SLE

- Low C3, C4
  - Reflect consumption of complement
  - Usually caused by presence of immune complexes in SLE, particularly nephritis

  -Seen in some forms of vasculitis

  - Complement deficiency states may predispose to SLE

ANCA (Anti-Neutrophil Cytoplasmic Antibodies)

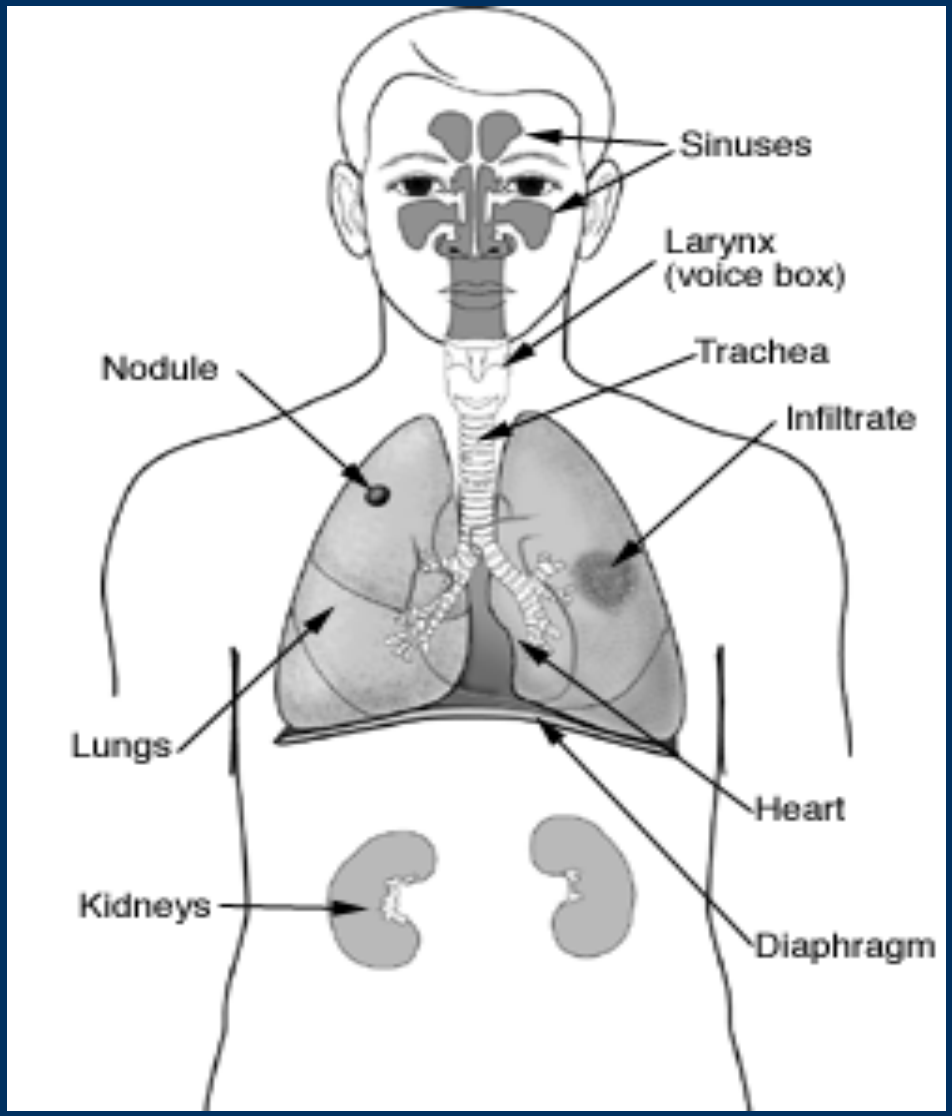
- **ANCA :The major target antigens are**

  proteinase 3 (PR3) for cANCA
  myeloperoxidase (MPO) for pANCA

ANCA

- **ANCA most strongly associated with necrotizing vasculitis**
  - **C-ANCA (PR3)→ GPA (Wegener's granulomatosis)**
  - **P-ANCA (MPO)→ EGPA/ MPA (Microscopic polyangiitis Churg-Strauss vasculitis)**
    - » May also be positive in Crohn's, UC, Hepatitis, Primary biliary cirrhosis, Primary sclerosing cholangitis

GPA (Wegener's Granulomatosis)



Key Concepts

- **ANCA**
  - **ANCA alone is not diagnostic for vasculitis, but +ve PR3 correlates with GPA, and MPO with MPA**
  - **If not PR3 or MPO positive, consider diagnosis other than systemic vasculitis**

And if this isn't complicarted enough…A few more tests that you are unlikely to order - Leave These to Us!

- Scleroderma Panel
- Myositis Panel

Systemic sclerosis: Scleroderma

Limited cutaneous (includes CREST)

- Skin fibrosis starts distally
- Scelrodactyly
- Usually also involves face
- Delay in the internal manifestations
- Calcinosis and telangiectasias

Diffuse cutaneous (can have CREST)

- Rapid progression of skin thickening involving proximal and distal extremities
- Trunk and face
- TENDON RUBS
- Early visceral involvement

Overlap syndrome

- Associated with
  - Polymyositis/Dermatomyositis
  - SLE
  - MCTD

Scleroderma sine scleroderma

- No detectable skin thickening
- Classic internal organ disease
- Accounts for 10% of scleroderma pts.

Scleroderma Classification Criteria

ACR/EULAR Criteria for the Classification of Systemic Scleroderma[a]		
Category	**Subitems**	**Score**
Skin thickening[b] of the fingers of both hands extending proximal to MCP joints (single criterion sufficient for classification of SSc)	n/a	9
Other skin thickening[b,c]	Puffy fingers	2
	Sclerodactyly of the fingers (distal to MCP, proximal to PIP joints)	4
Fingertip lesions[c]	Ulcers on digital tips	2
	Pitting scars	3
Telangiectasia	n/a	2
Abnormal nailfold capillaries	n/a	2
Pulmonary arterial hypertension and/or interstitial lung disease	n/a	2
Raynaud phenomenon	n/a	3
Scleroderma-related antibodies (any ACA, anti-Scl-70, or anti-RNA polymerase III antibodies)	n/a	3
Total score[d]		

Criteria antibodies for SSc include the following[4]:

- Anti-Scl-70 (also known as antitopoisomerase 1)
- ACA
- Anti-RNA polymerase III

Myositis "Panel": Myositis Antibodies: complicated!

Prevalence and clinical association of myositis autoantibodies

Autoantibodies	Prevalence (%)	Disease association	Clinical association/significance
Aminoacyl tRNA synthetases			
Jo-1	15–30	PM, DM	Anti-synthetase syndrome (myositis, ILD, polyarthritis, Raynaud's phenomenon, mechanic's hands)
PL-7	<5	PM, DM	Anti-synthetase syndrome
PL-12	<5	PM, DM, CADM, ILD	Anti-synthetase syndrome, ILD, CADM
EJ	<5	PM, DM	Anti-synthetase syndrome
OJ	<5	PM, DM	Anti-synthetase syndrome, ILD
KS	<1	PM, DM, ILD	ILD
ZO	Rare		Myositis
YRS (HA)	Rare		Myositis
SRP	5	PM	Myositis (necrotizing)
Mi2	10	DM	DM with typical skin lesions and mild myositis
MDA5/CADM140	15–20	CADM/ADM	CADM, rapidly progressive ILD, severe skin manifestations
TIF1γ/α	10–15	DM,	Malignancy-associated DM
MJ/NXP2	1–5	DM	Adult and juvenile DM with severe skin disease
SAE	1	DM	DM

Modified from [30]

PM polymyositis, *DM* dermatomyositis, *ILD* interstitial lung disease, *CADM* clinically amyopathic dermatomyositis, *ADM* amyopathic dermatomyositis

Clin Rev Allergy Immunol. 2017 Feb; 52(1): 1–19.
doi: 10.1007/s12016-015-8510-y

Back to a Few More Practical Tests….

- HLA- B27
- Uric acid

HLA-B27

- **Sensitivity 90-95% *for patients with ankylosing spondylitis***
- **Present in 8% of general population**
- **Incidence of disease is 0.5-1.0%**

- **HLA B27 of limited value in diagnosing usual patient with back pain seen in primary care office**

HLA-B27

- May be of help in patient with inflammatory sounding back pain but no sacroiliitis visible on plain Xray
- Not required to confirm a clinical and radiologic diagnosis of ankylosing spondylitis
- But may be important to help diagnose patients in the “clinical” arm of the new ASAS criteria for spondyloarthropathy

HLA B-27

- ASSOC. WITH SPONDYLOARTHROPATHIES:
  - A.S. : 95% SENS
  - REACTIVE: 80-85% SENS
  - PSORIATIC: 50-60% SENS
  - IBD: 50% SENS

Choosing Wisely: HLA- B27

Don't order an HLA-B27 unless spondyloarthritis is suspected based on specific signs or symptoms.

HLA-B27 testing is not useful as a single diagnostic test in a patient with low back pain without further spondyloarthropathy (SpA) signs or symptoms (e.g., inflammatory back pain ⩾3 months duration with age of onset <45 years, peripheral synovitis, enthesitis, dactylitis, psoriasis or uveitis) because the diagnosis of spondyloarthropathy in these patients is of low probability. If HLA-B27 is used, at least two SpA signs or symptoms, or the presence of positive imaging findings, need to be present to classify a patient as having axial SpA. There is no clinical utility to ordering an HLA-B27 in the absence of positive imaging or the minimally required SpA signs or symptoms.

Uric Acid

- An isolated gout attack may have normal uric acid
- Best time to measure uric acid is 2 weeks after an attack
- When treating, target value to stop flares is <360 um/L
- If > 540um/L risk of gout rises exponentially!

Summary: Key Concepts

- Use clinical picture to guide ordering
  - Tests don't usually make the diagnosis!
- Watch out for false positives
- Pretest probability matters
- Avoid using a shotgun "rheumatology panel" to screen for rheumatic disease
- But, ordering a few tests that you are comfortable interpreting will enhance your quality of referral and get the Rheumatologist's attention!

A few questions to wrap up.....
Have you been listening?

Which of the following tests is most specific for Rheumatoid Arthritis?

1. RA
2. CRP
3. Anti-CCP
4. ANA
5. Anti-Smith

A 27 y.o. man has LBP, prolonged morning stiffness, limited lumbar movements 2 episodes of uveitis, and bilateral sacroiliitis on x-Ray.

- Will you order an HLA B27 test to confirm a diagnosis of Ankylosing Spondylitis so that the patient can be appropriately treated?

1. YES
2. No

A 47 y.o. man presents with a 5 day history of a monoarthritis of the knee NYD. A joint aspiration is planned, but in the meantime which tests should you order?

1. RA
2. Anti-CCP
3. CRP
4. ANA
5. Uric Acid
6. 1 and 5
7. 1 and 3
8. 3 and 5
9. 1,3, and 5

A 37 yo woman presents with 6 months of general malaise, fatigue. dry eyes and mouth, morning stiffness, and arthralgias hands, feet and knees. All of these initial tests are appropriate except:

1. ANA
2. RA
3. ENA
4. ANCA
5. CBC
6. TSH
7. Creatinine
8. CRP
9. Urinalysis

Thank You !