Rheumatologic Tests

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Learning Objectives

- To become familiar with basic rheumatology tests concept
- To understand how ESR and CRP are measured
- To learn about different antibodies used in the diagnosis of rheumatic diseases
- To be able to interpret synovial fluid analysis

Key Points

- Diagnosis of most rheumatic diseases is clinical.
- Laboratory results alone are rarely sufficient to make a diagnosis in rheumatology.
- Therefore, it's important to establish a pre-test probability before ordering a laboratory test in rheumatology.

Test	disease present	Disease absent
+	a	b
-	c	d

Sensitivity= a/(a+c)

Specificity= d/(b+d)

Positive likelihood ratio= sensitivity/(1-specificity)

Negative likelihood ratio= (1-sensitivity)/specificity

Positive predictive value= a/(a+b)

Negative predictive value= d/(c+d)

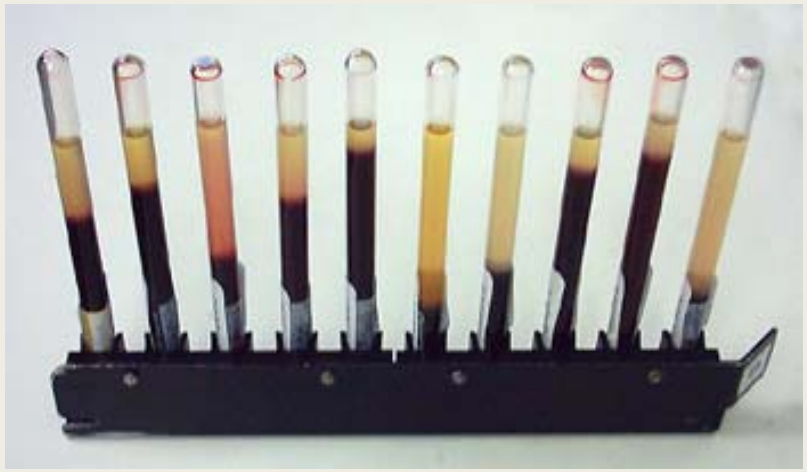
Prevalence = pre-test probability = (a+c)/(a+b+c+d)

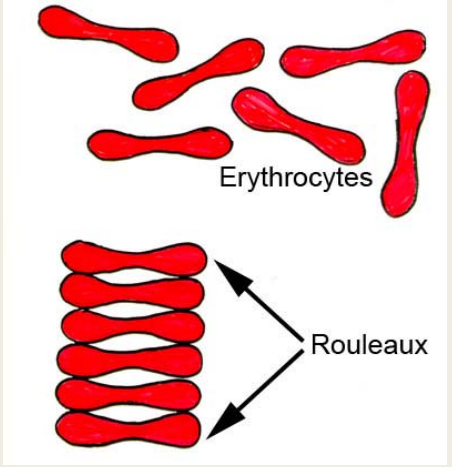
Acute phase reactants

- The measurement of serum acute phase reactant levels is useful because abnormalities generally reflect the presence and intensity of an inflammatory process.
- Not specific to any particular disease.
- Can NOT distinguish infection from other causes of acute and chronic inflammation.
- Most widely used indicators are erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels

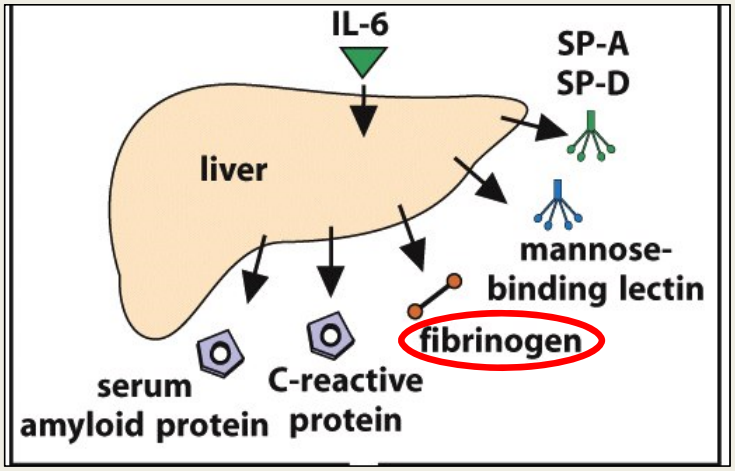
ESR

- Indirect measure of acute phase response
- **Slow** response to clinical change
- Normal state: RBCs are separated from each other by negative changes
- When charges are disrupted → stick to each other → sediment faster → ESR increases





ESR



Causes of increased ESR:

1. Increase in fibrinogen (i.e. inflammatory state)
2. Increase in immunoglobulins (i.e. auto-immune diseases, multiple myeloma)
3. Low albumin (i.e. nephrotic syndrome)

In general, ESR can be elevated in:

- Any systemic and localized inflammatory and infectious diseases
- Malignancy
- Tissue injury/ischemia
- Trauma

ESR

- *Falsely decreased ESR:*

1. Abnormal RBCs: polycythemia, spherocytosis, sickle cell disease
2. Leukocytosis
3. Heart failure
4. Hypofibrinogenemia
5. Cachexia
6. Technical factors (i.e. Clotting of the blood sample or delay in testing of greater than two hours)

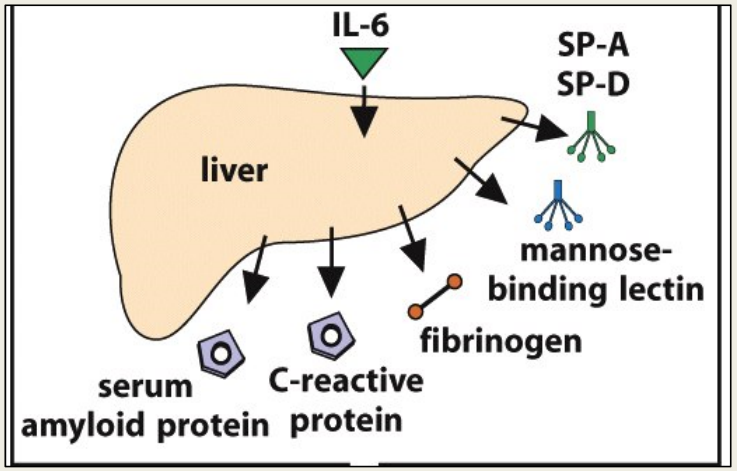
ESR

- *Falsely elevated ESR:*

1. Increased age and female sex
2. Anemia
3. Renal disease
4. Obesity
5. Technical factors (high room temperature)

   - *Men: Age/2*
   - *Women: (Age + 10)/2*

CRP



- Changes much more rapidly than ESR level
- Not affected by protein issues
- Thus it is a more precise measurement of inflammation
- Should not normally be elevated, although may be slightly elevated in obesity

  - *Men: Age/5*
  - *Women: (Age + 30)/5*

(Wener et al J Rheum 2000; 27:2351)

CRP and ESR pattern of response:

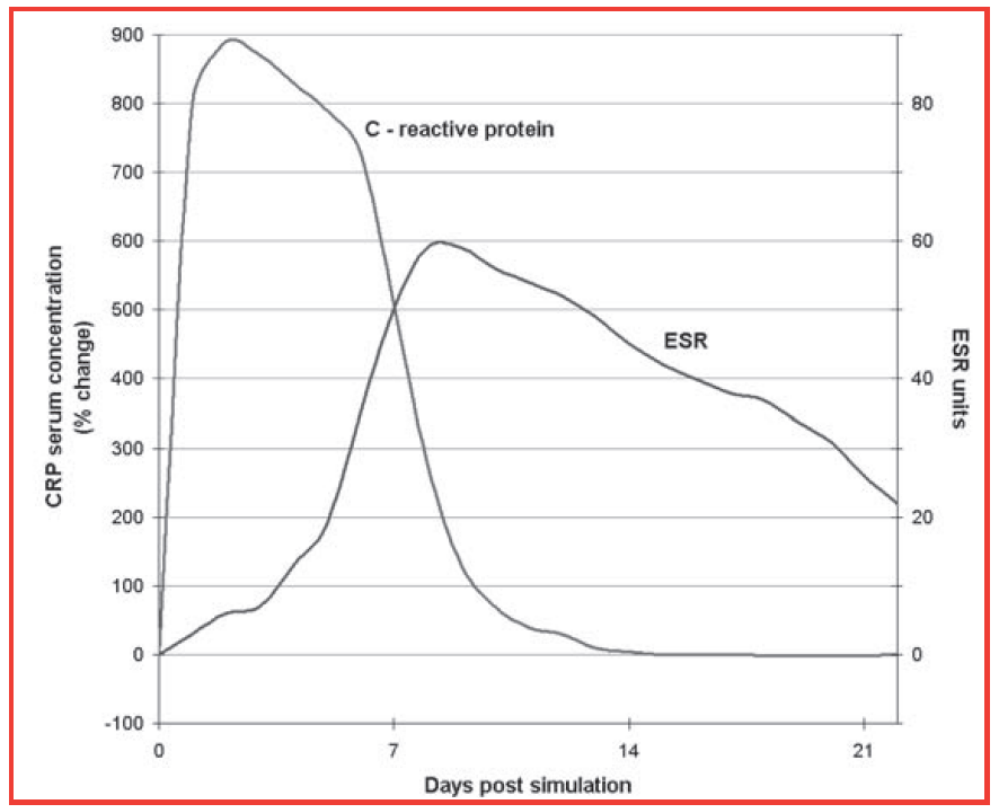


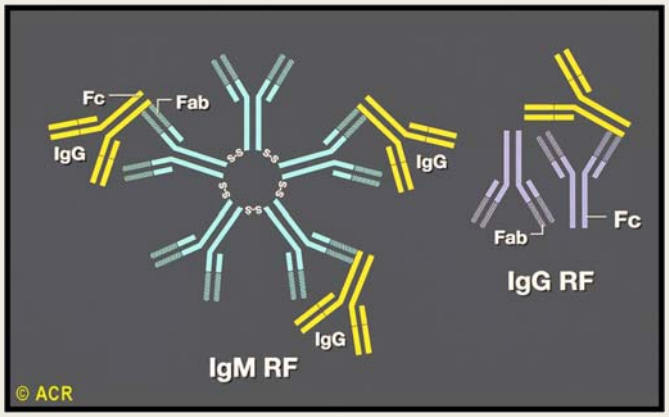
Table 3. The 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for rheumatoid arthritis

	Score
Target population (Who should be tested?): Patients who	
1) have at least 1 joint with definite clinical synovitis (swelling)*	
2) with the synovitis not better explained by another disease†	
Classification criteria for RA (score-based algorithm: add score of categories A–D; a score of ≥6/10 is needed for classification of a patient as having definite RA)‡	
A. Joint involvement§	
1 large joint¶	0
2–10 large joints	1
1–3 small joints (with or without involvement of large joints)#	2
4–10 small joints (with or without involvement of large joints)	3
>10 joints (at least 1 small joint)**	5
B. Serology (at least 1 test result is needed for classification)††	
Negative RF *and* negative ACPA	0
Low-positive RF *or* low-positive ACPA	2
High-positive RF *or* high-positive ACPA	3
C. Acute-phase reactants (at least 1 test result is needed for classification)‡‡	
Normal CRP *and* normal ESR	0
Abnormal CRP *or* abnormal ESR	1
D. Duration of symptoms§§	
<6 weeks	0
≥6 weeks	1

* A Score of 6 or more is considered to have RA

Rheumatoid factor (RF)

- RFs are antibodies directed against the Fc portion of immunoglobulin G (IgG)
- *RF is NOT* specific for RA
- Rheumatic Disease
  - *Primary Sjogren's Syndrome*
  - *Mixed Connective Tissue Disease*
  - *SLE*
  - *Cryoglobulinemia*
  - *Polyarticular JIA*
- Produced in many chronic inflammatory conditions
  - *Sub-acute bacterial endocarditis*
  - *Hepatitis B & C*
  - *TB*
  - *Chronic Bronchitis*
- (+) in 5-25% of people age > 60



Cyclic Citrullinated Antibody (CCP/ACPA)

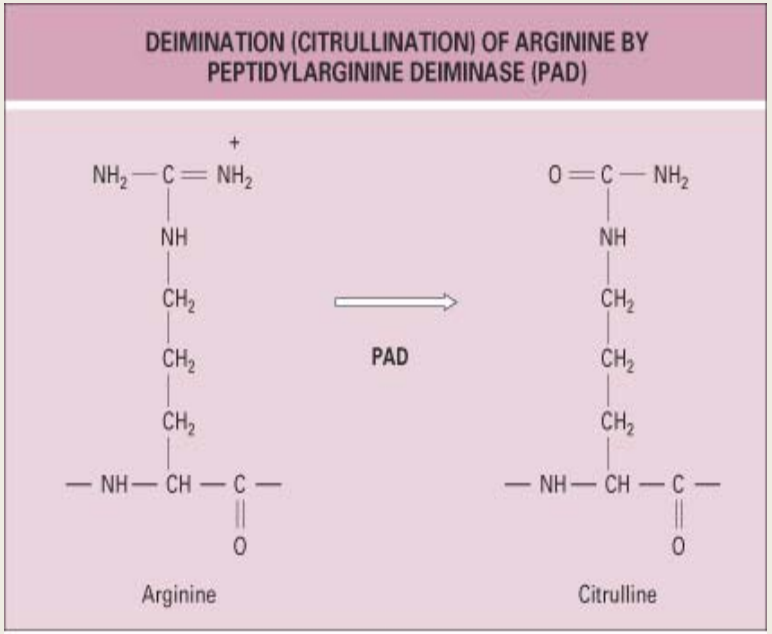
- Sensitivity of ACPA for detecting RA is 67%
- Specificity is 96 %

- CCP/ACPA is a poor prognostic factor in RA:

→ Positive patients with early RA are at increased risk of progressive joint damage and radiographic progression.

→ ? Has recently been linked to severity of ILD in patients with RA.

Ann Rheum Dis. 2014 Aug;73(8):1487-94. Epub 2013 May 28.



The ACR SLE classification criteria

- Serositis
- Oral ulcers
- Arthritis
- Photosensitivity
- Blood disorders
- Renal involvement
- Antinuclear antibodies
- Immunologic phenomena (eg, dsDNA; anti-Smith [Sm] antibodies; also anti-phospholipid antibodies)
- Neurologic disorder
- Malar rash
- Discoid rash

MNEMONIC: SOAP BRAIN MD

ANA test

- Useful screening test in symptomatic patients
- Immunofluorescence method
- Reported as a titer (i.e. 1/160, 1/320, etc….)
- **Non-specific but very sensitive (useful when negative)**
- Reasons to have a positive ANA
  - *Family member with AI disease*
  - *Age & Gender (females)*
  - *Drugs*
  - *Recent viral infection*

Clinical significance of a positive ANA test

- In a large multicenter study of healthy volunteers 20 to 60 years of age

  - ANA were detected in 32% of the sera at dilutions of 1:40
  - in 5% of the sera at dilutions of 1:160

→ Interpret ANA test carefully based on clinical presentation.

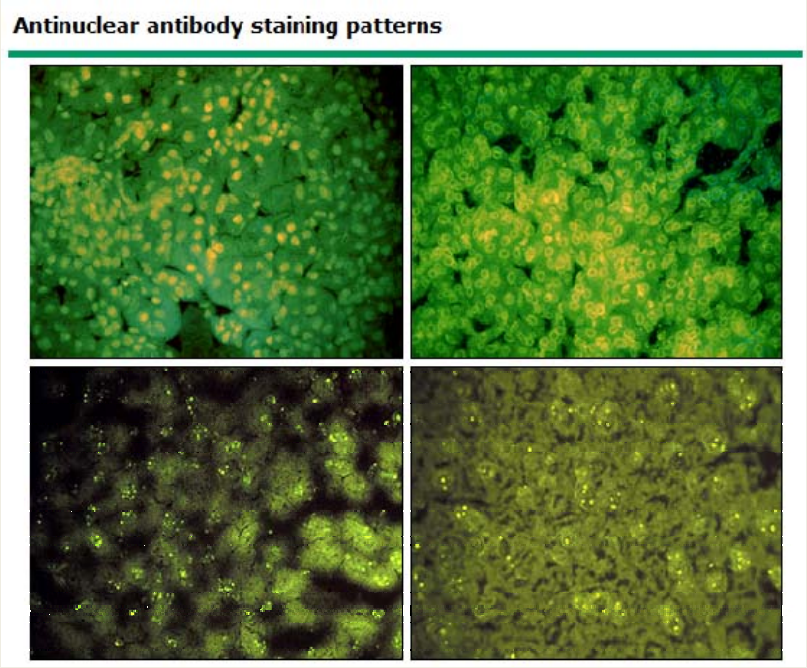
Arthritis Res Ther. 2008;10(6):R131. Epub 2008 Nov 11.

Interpretation of ANA test

- 99% of patients with SLE have positive ANA.
- Only 15% of people with a positive ANA have SLE
- Serial ANAs have NO clinical value in monitoring SLE

Pattern of ANA

- **Peripheral = Rim** = SLE
- **Homogeneous = Diffuse** = RA, SLE, drug-induced lupus
- **Speckled** = Scleroderma, Sjogren's SLE
- **Nucleolar** = Scleroderma, Raynaud's
- **Anti-centromere** = CREST, Raynaud's



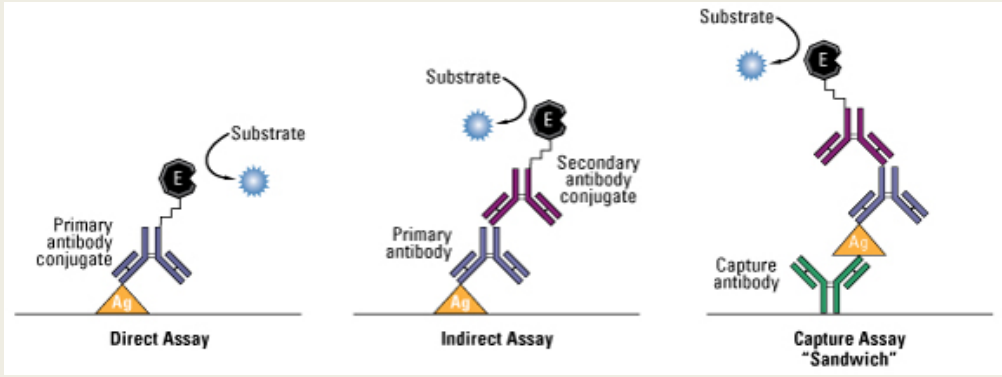
What to order next?

- A titer of 1/40 or 1/80 is in most cases clinical insignificant.
- Becomes suspicious when >1/160
- Positive ANA can antedate symptoms by many years.
- High titers should be followed by specific nuclear antibodies

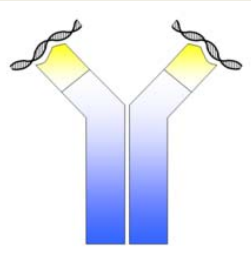
  → ENA panel.

Specific Nuclear Antigens (Extractable Nuclear Antibody (ENA) Panel)

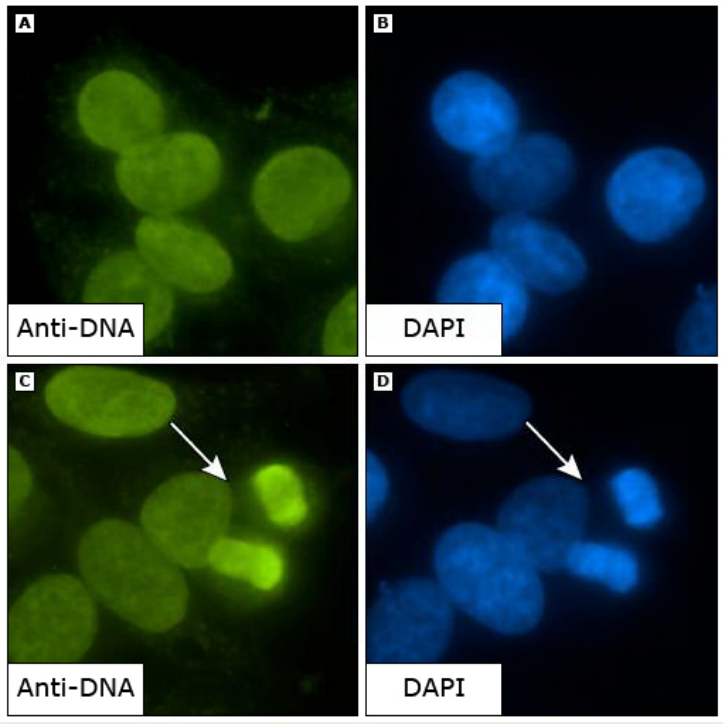
- Measured by immunoassays such as ELISA (image below)
- Quantitative
- Can help determine the type of connective tissue disease



Double-Stranded DNA (dsDNA or anti-native DNA)



- 95% Specific for SLE
- 50-70% Sensitive for SLE
- There is a strong association between the level of anti-dsDNA antibodies and glomerulonephritis
- Is the one antibody that may be used as an activity marker in SLE nephritis



Smith (Sm) antibody

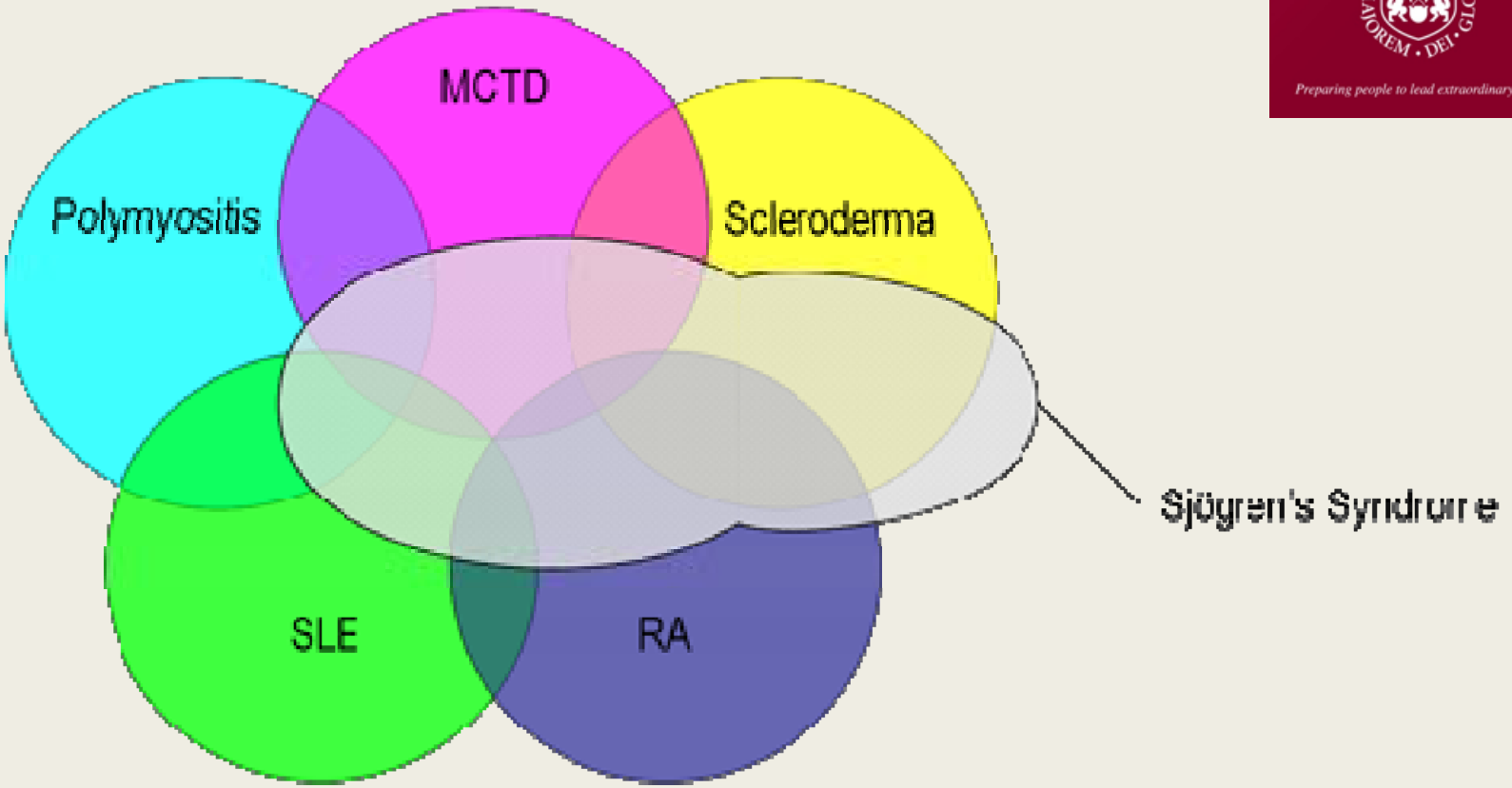
- Found almost exclusively in SLE patients
- Anti-Sm antibodies bind to one or more of a series of Sm proteins
- Specificity can be considered 100% for SLE
- Poor sensitivity (only 10-40%), so most SLE patients will be negative

Ribonuclear Protein (RNP)

- Anti-U1 RNP antibodies react with one or more of three proteins (70-kD, A, and C) that are specifically present in the U1 snRNP complex
- Found in 40-60% of SLE patients but not specific
- In high titers, they are useful in diagnosing mixed connective tissue disease (MCTD)

SS-A (Ro) & SS-B (La)

- Can be seen in SLE & Sjogren's disease (dry eyes and dry mouth)
- SSA can be associated with subacute cutaneous lupus & photosensitivity
- SS-A and SS-B antibodies are also associated with neonatal lupus & congenital heart block


MCTD
Polymyositis
Scleroderma
Sjögren's Syndrome
SLE
RA

Antiphospholipid antibodies (APL)

Types of APL antibodies

- Anticardiolipin antibodies (aCL); immunoglobulin G (IgG) and/or IgM
- Anti-beta2-GP I antibodies; IgG and/or IgM
- Lupus anticoagulant (LAC)

Diagnostic criteria (Sydney criteria)

- APS is present in patients who meet at least **one** of the following clinical criteria **and** at least one of the following laboratory criteria

1. Clinical criteria – One or more of the following is present:

- **Vascular thrombosis** – One or more episodes of venous, arterial, or small vessel thrombosis in any tissue or organ, with unequivocal imaging or histologic evidence of thrombosis.
- **Pregnancy morbidity** – One or more unexplained deaths of a morphologically normal fetus at ≥10 weeks gestation, **or** one or more premature births of a morphologically normal neonate before 34 weeks gestation because of eclampsia, preeclampsia, or placental insufficiency, **or** three or more consecutive spontaneous pregnancy losses at <10 weeks gestation, unexplained by chromosomal abnormalities or by maternal anatomic or hormonal causes.

2. **Laboratory criteria** – The presence of one or more of the following antiphospholipid antibodies (aPL) on two or more occasions at least 12 weeks apart:

- Immunoglobulin G (IgG) and/or IgM anticardiolipin antibodies (aCL) in moderate or high titer (>40 GPL or MPL units, respectively, or a titer >99th percentile
- IgG and/or IgM anti-beta2-glycoprotein (GP) I >40 GPL or MPL units, respectively, or a titer >99th percentile
- Lupus anticoagulant (LA) detected

Protein Targets for Antiphospholipid Antibodies

- Beta 2 Glycoprotein I (B2GPI)
- Prothrombin
- Other Coagulation Cascade Proteins
- Protein C or S
- Annexin A5
- Oxidized LDL
- Tissue Plasminogen Activator (tPA)
- Some Complement Factors

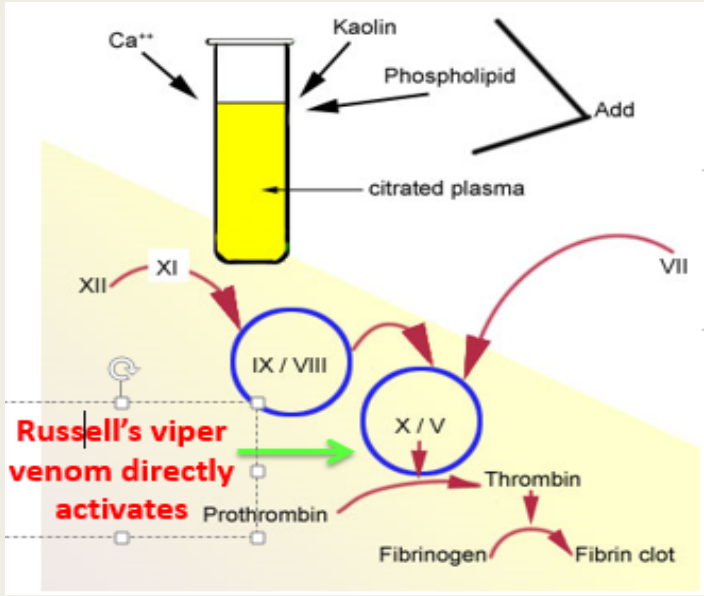
Beta 2 Glycoprotein
(NOT Beta 2 microglobulin!!)

- A major inhibitor of the intrinsic activation pathway of the coagulation cascade

- Antibodies to this protein tip the scales toward thrombosis

Lupus Anticoagulant (LAC)

- Term is a paradox
  - *in vitro: prolongs the aPTT*
  - *in vivo: thrombosis*
- When aPTT is prolonged, a mixing study is done
- When mixing study does not correct aPTT, confirmatory test is done-DRVVT
  - *Sprinkle in more phospholipids to overcome LAC*
  - *Ratio of 1st time to 2nd time > 1.2 considered positive*



aPTT Test: how long it takes you to clot (intrinsic pathway)

ELISA Studies

Anticardiolipin (aCL)

- IgG & IgM
- Sensitive, but less specificity
  - *Associated with infections (syphilis, TB, HIV, Hepatitis, etc)*
  - *Transient low titers*
- Newer assays may be more specific

Anti-Beta2 Glycoprotein I

- IgG & IgM
- Relatively specific
- Sensitivity is about 40-70%

FOR BOTH: Would like titers to be at least > 40

Expected effects of anticoagulant drugs on commonly used coagulation tests

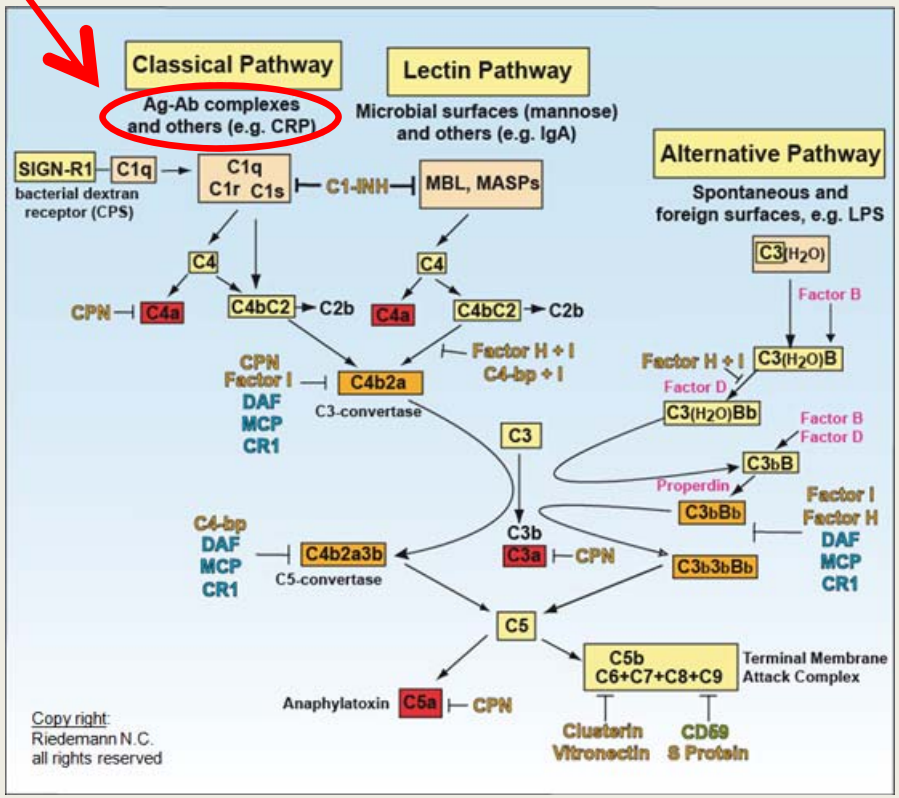
Drug class	Drug	Brand name(s)	PT	aPTT	Anti-factor Xa activity
Vitamin K antagonists	Warfarin	Coumadin, Jantoven	↑	↑/–*	–
	Acenocoumarol	Sintrom	↑	↑/–*	–
Heparins	Unfractionated heparin		–¶	↑	↑
	LMW heparins Enoxaparin Dalteparin Nadroparin	 Lovenox Fragmin Fraxiparine	–	↑/–	↑
	Fondaparinux	Arixtra	–	↑/–	↑
Direct thrombin inhibitors	Argatroban	Acova	↑	↑	–
	Dabigatran	Pradaxa	↑/–	↑	–
Direct factor Xa inhibitors	Rivaroxaban	Xarelto	↑/–	↑/–	↑/–Δ
	Apixaban	Eliquis	↑/–	↑/–	↑/–Δ

NB:
with all antiphospholipid tests, the tests should be positive & high titer on 2 occasions greater than 12 weeks apart to rule out transient elevations

Complement Assays: C3 & C4

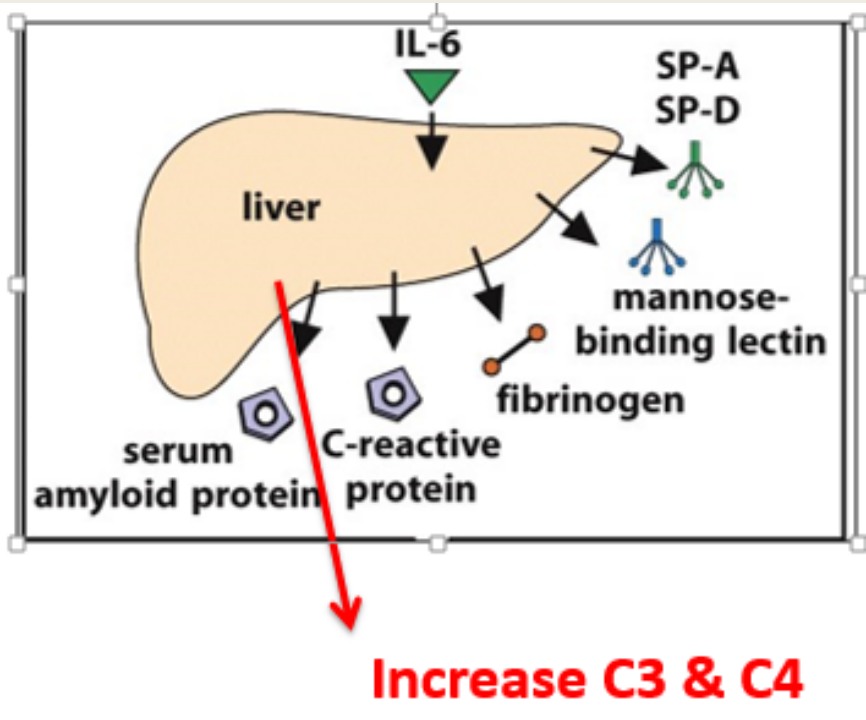
- Immune Complex disease, like SLE, consumes complement
- Low values may indicate disease activity
- Used as markers of disease activity

Immune Complex Disease, like SLE or post-strep glomerulonephritis



Complement Assays: C3 & C4

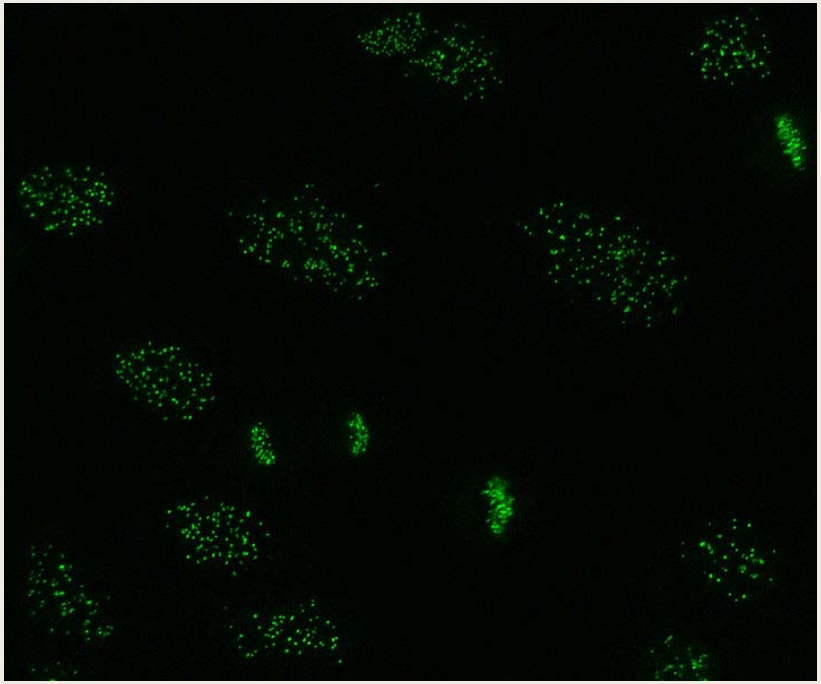
- Consider that C3 & C4 are made by the liver
- May be decreased in severe liver failure
- May be increased in acute inflammation or other non-immune complex chronic inflammation
- Therefore, may be falsely elevated in an SLE patient with acute infection, etc....

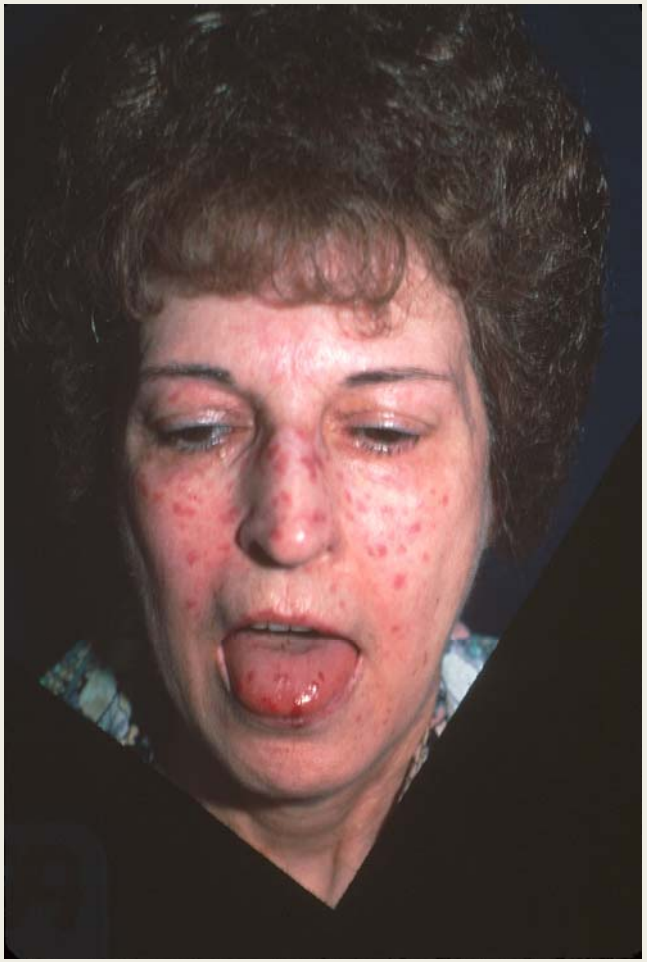


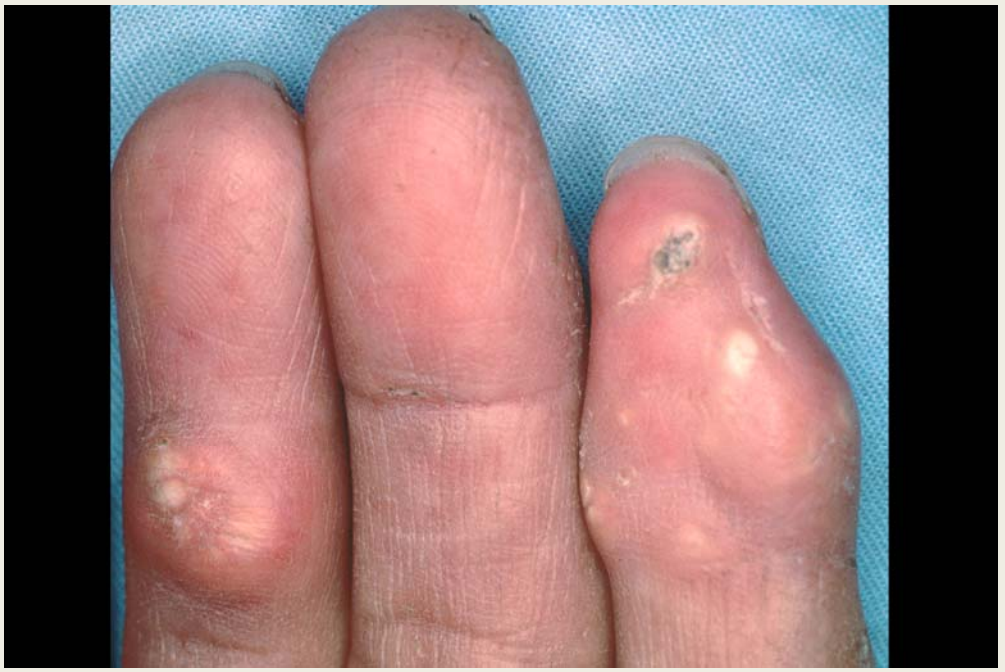
Scleroderma Antibodies

Centromere antibodies

- **Targets of antibodies** – Centromere proteins A, B, and C.
- Almost exclusively noted in patients with limited cutaneous scleroderma-CREST variant
- Associated with calcinosis
- Should prompt surveillance for pulmonary hypertension

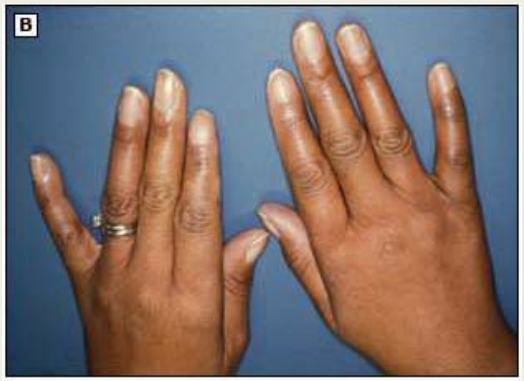


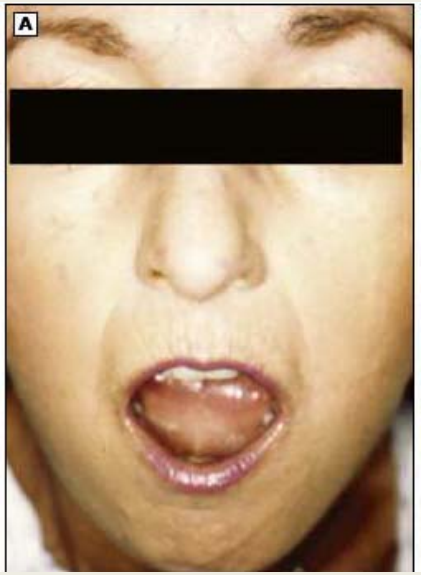


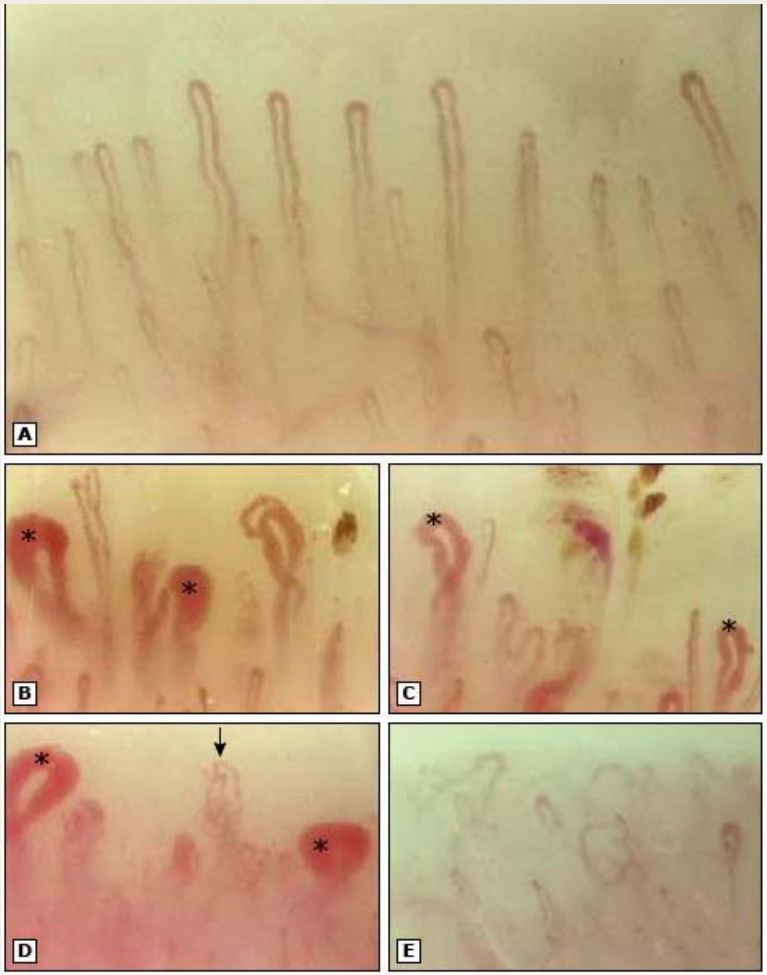


Anti-Topoisomerase I (Scl-70)

- Found in about 20% of systemic sclerosis patients
- May increase the risk for severe interstitial lung disease.






A
B
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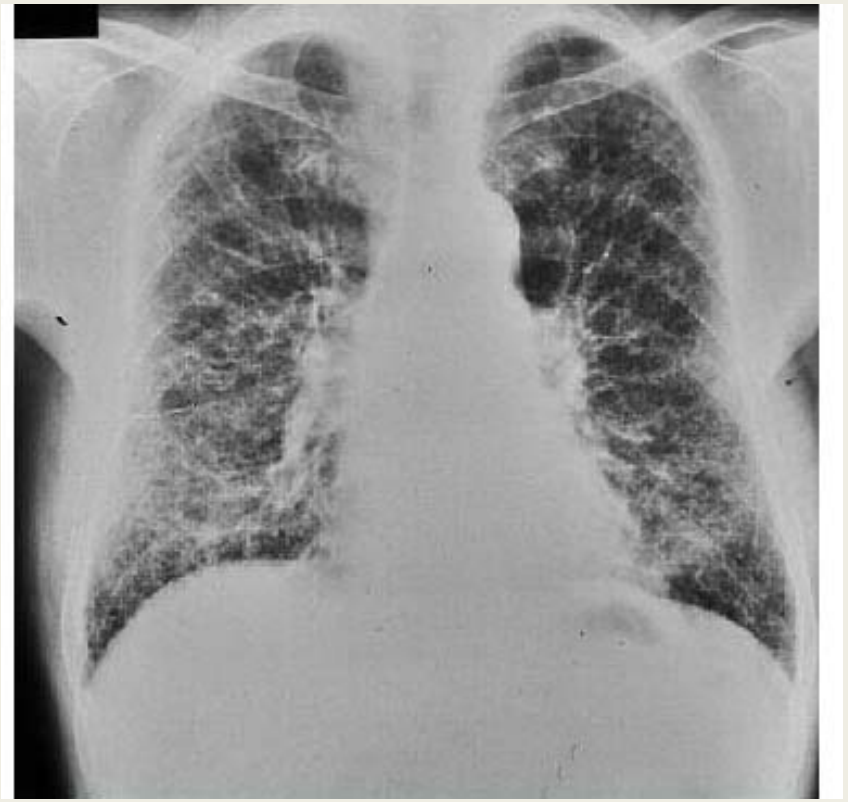


Anti-RNA Polymerase III

- Another scleroderma-associated antibody
- Found in patients with dcSSc
- Associated with rapidly progressive skin involvement as well as an increased risk for scleroderma renal crisis.
- These patients may also be at increase risk for concomitant cancer.

Arthritis Res Ther. 2011;13(6):R211. Epub 2011 Dec 22.

Myositis Antibodies

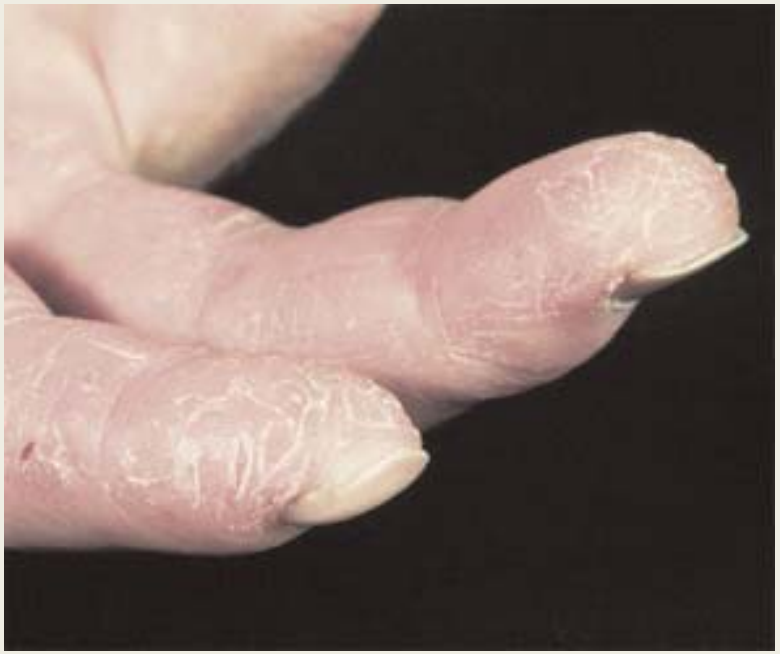
Myositis panel

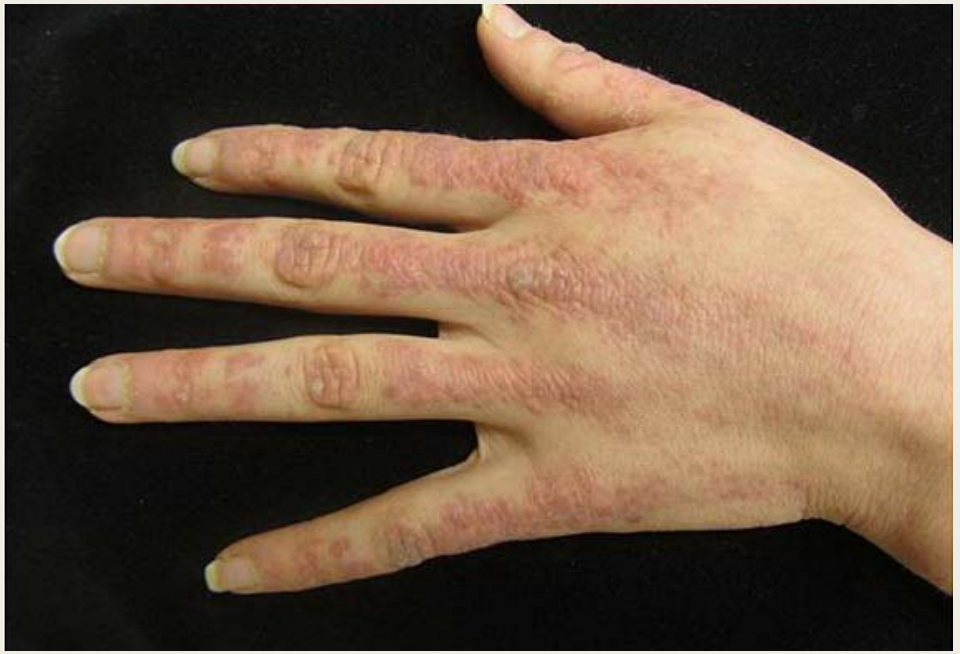
Autoantibody	Clinical Features
SRP	Severe necrotizing myopathy; Predominantly Polymyositis
Mi-2	Usually “classic dermatomyositis” in adults (sometimes children): “shawl sign”, gottron’s papules/sign, heliotrope rash
PM-Scl	Overlap features of myositis & SSc (or either disease alone); mechanic’s hands
p155/140	• Cancer-associated myositis in adults • >20% frequency seen in Juvenile DM cohorts • Severe, cutaneous disease in both adult & juvenile DM
HMGcoA Reductase	Statin associated autoimmune myopathy. Can be primary Necrotizing myopathy on histology Resistant to immunosuppressive therapy
ETC…	

Anti-tRNA Synthetase Antibodies

- Associated with myositis
- Fever, Raynaud's
- Mechanic's Hands
- Polyarthritis
- Interstitial lung disease

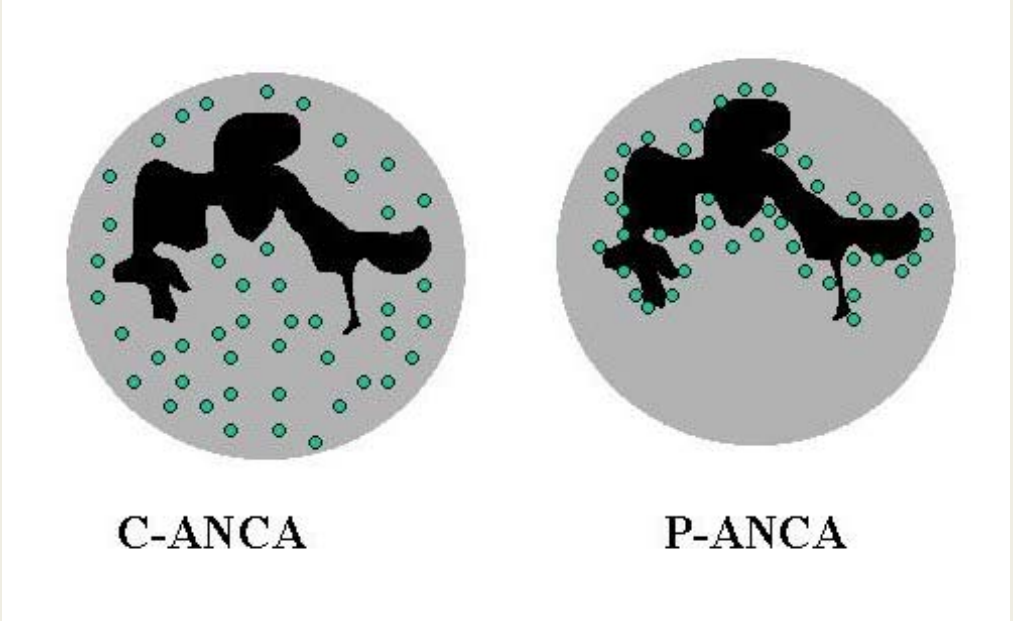
Auto-aby	Auto-antigen	Prevalence in inflammatory myositis (%)
Jo-1	Histidyl	20-30
PL-7	Threonyl	<5
PL-12	Alanyl	<5
OJ	Isoleucyl	<5
EJ	Glycyl	<5
KS	Asparaginyl	<1





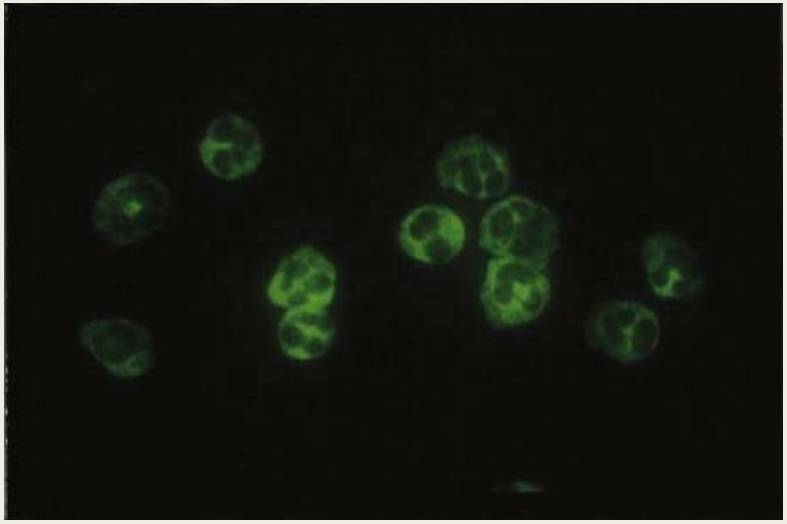
ANCA- Associated Vasculitides

Anti-neutrophilic cytoplasmic antibodies



C-ANCA & Proteinase-3 antibody (PR-3)

- Cytoplasmic-ANCA Immunofluorescence
- 90% specific for granulomatous polyangiitis (GPA) necrotizing vasculitis
- Confirm C-ANCA with PR-3 antibody ELISA



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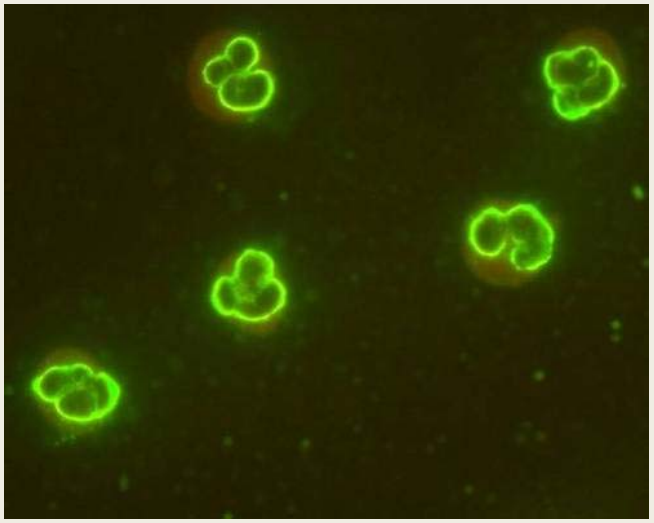
P-ANCA & Myeloperoxidase Antibody (MPO)

- Perinuclear-ANCA Immunofluorescence
- If associated with (+) MPO ELISA, then usually associated with microscopic polyangiitis (MPA) or Churg-Strauss vasculitis
- p-ANCA Can also be associated with inflammatory bowel disease or liver disease
  - *Usually ANCA is "atypical" in this case,*

  directed at different neutrophil proteins



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Drugs triggering positive ANCAs

- Esp. cocaine and Levamisole (used to cut cocaine).
- Can be PR3-ANCA, MPO-ANCA or atypical ANCA.
- Typically resolves after the offending drug is discontinued.

Spondylo-arthropathies

HLA-B27

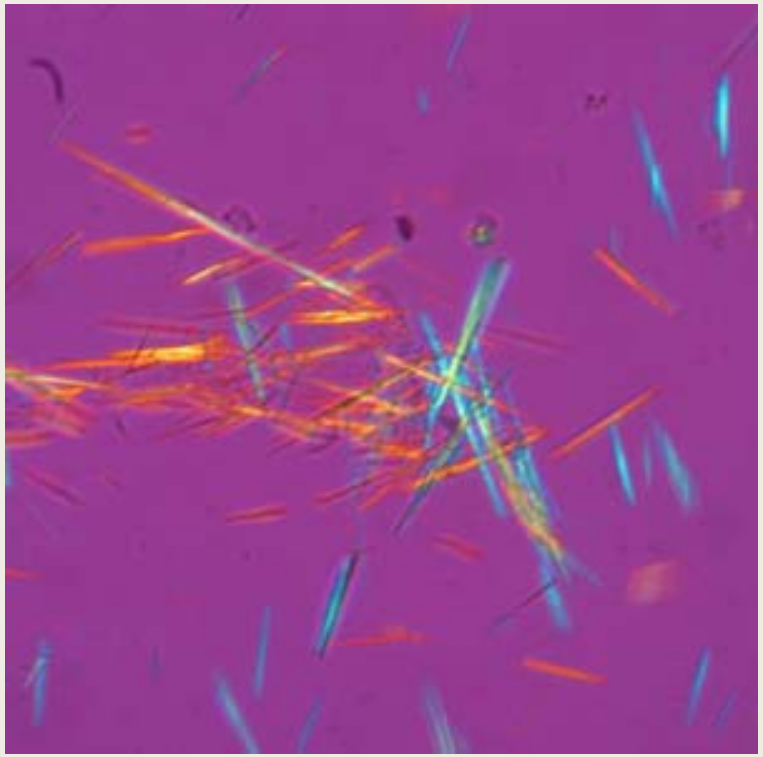
- Exact role of HLA-B27 in spondylo-arthropathies is unknown
- Greater prevalence in “axial spondylo-arthropathies” than peripheral only
- 90% in Ankylosing Spondylitis (AS)
- Usually not needed in classic AS
- Helpful in atypical presentations
- Present in 6-10% in normal population

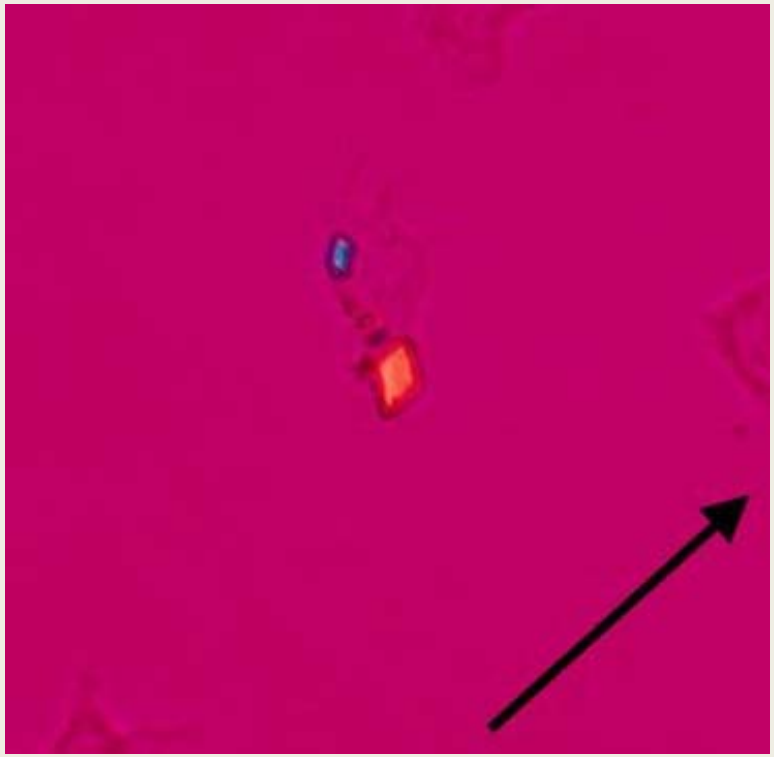
Synovial Fluid Analysis

General Principles

- Even a small amount of fluid is helpful!
- Prioritize Tests
  - *Gram Stain & Culture*
  - *Crystals (if only a drop, call rheum to go look with you!)*
  - *Cell count with differential*

Measure	Normal	Noninflammatory	Inflammatory	Septic	Hemorrhagic
Volume, mL (knee)	<3.5	Often >3.5	Often >3.5	Often >3.5	Usually >3.5
Clarity	Transparent	Transparent	Translucent-opaque	Opaque	Bloody
Color	Clear	Yellow	Yellow to opalescent	Yellow to green	Red
Viscosity	High	High	Low	Variable	Variable
White blood cell, per mm^3	<200	0 to 2000	>2000	>2000	200 to 2000
Polymorphonuclear leukocytes, percent	<25	<25	≥50	≥75	50 to 75
Culture	Negative	Negative	Negative	Often positive	Negative





Thank you!

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