Rheumatology Labs for the NIH NP/PA

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NIAMS

Agenda

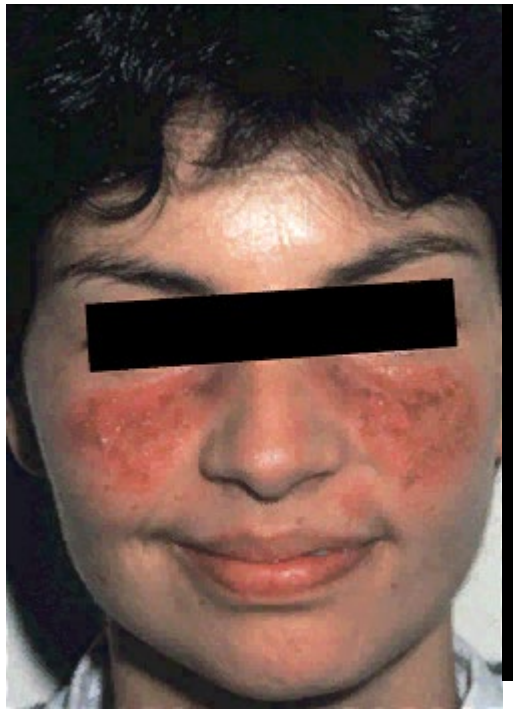
- Principles of testing in rheumatology
- Inflammatory markers
- Autoimmune serologies
- Common caveats at the NIH
- Case studies

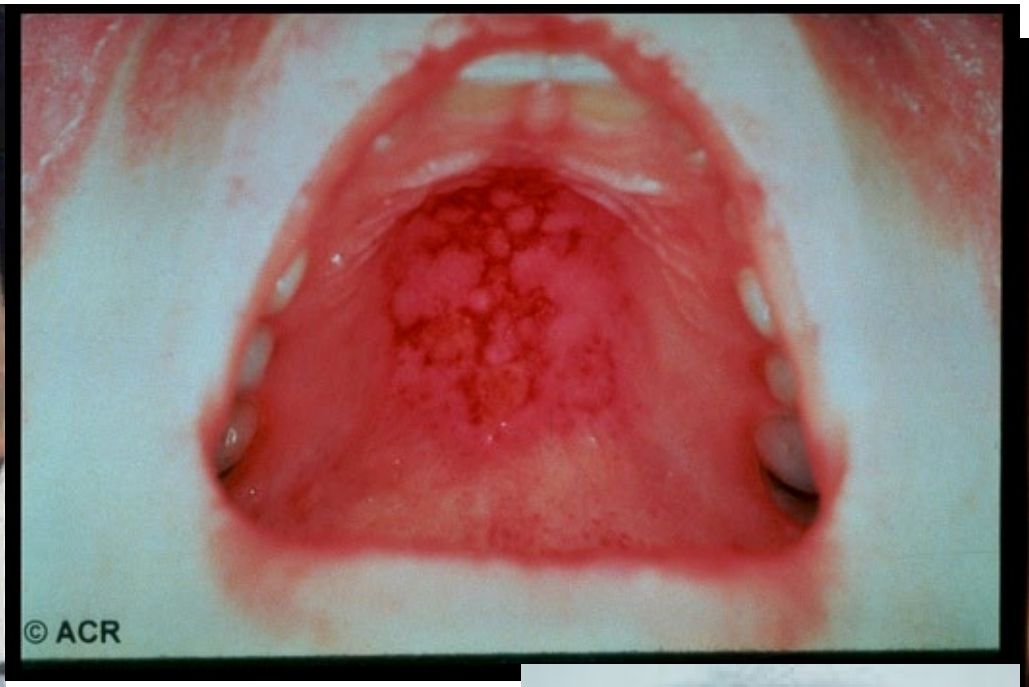
General principles

- Positive predictive value is key in ordering rheum labs- most of these tests are not great
- Family history is important- assess prior to testing
- No gold standards/hard and fast→clinical context
- Negative testing, including inflammatory markers, doesn’t eliminate rheumatic disease in the right context
- Positive testing, including inflammatory markers, doesn’t rule in rheumatic disease

Patient Context

- Energy level affected
- ADLs affected
- Markers of inflammation- blood counts, inflammatory markers
- Rashes
- Never say never

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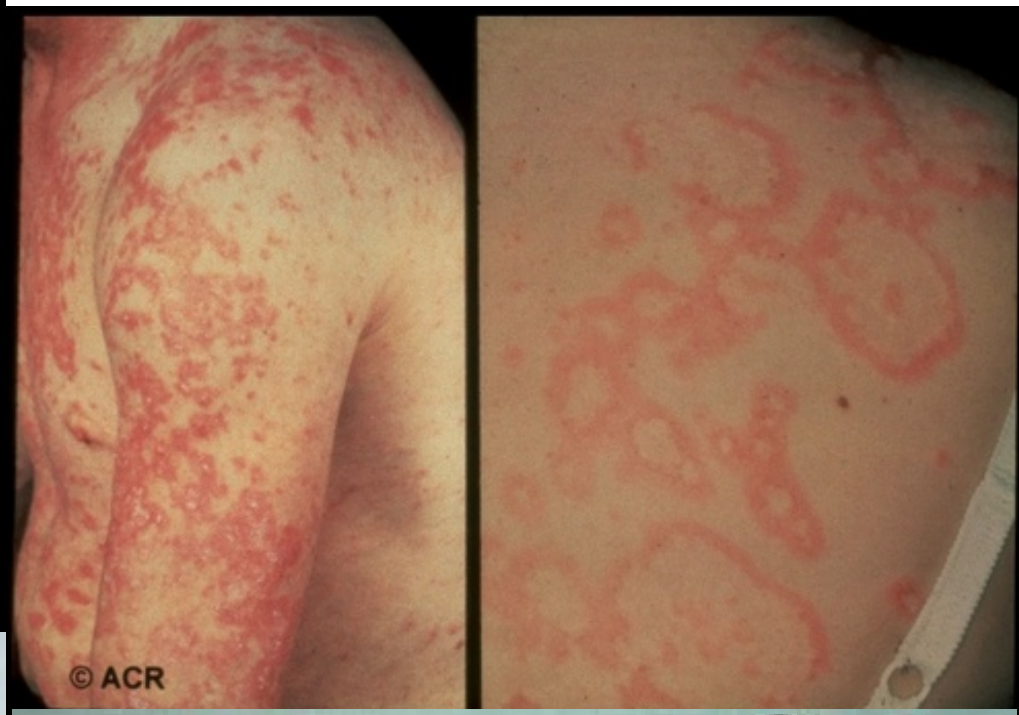
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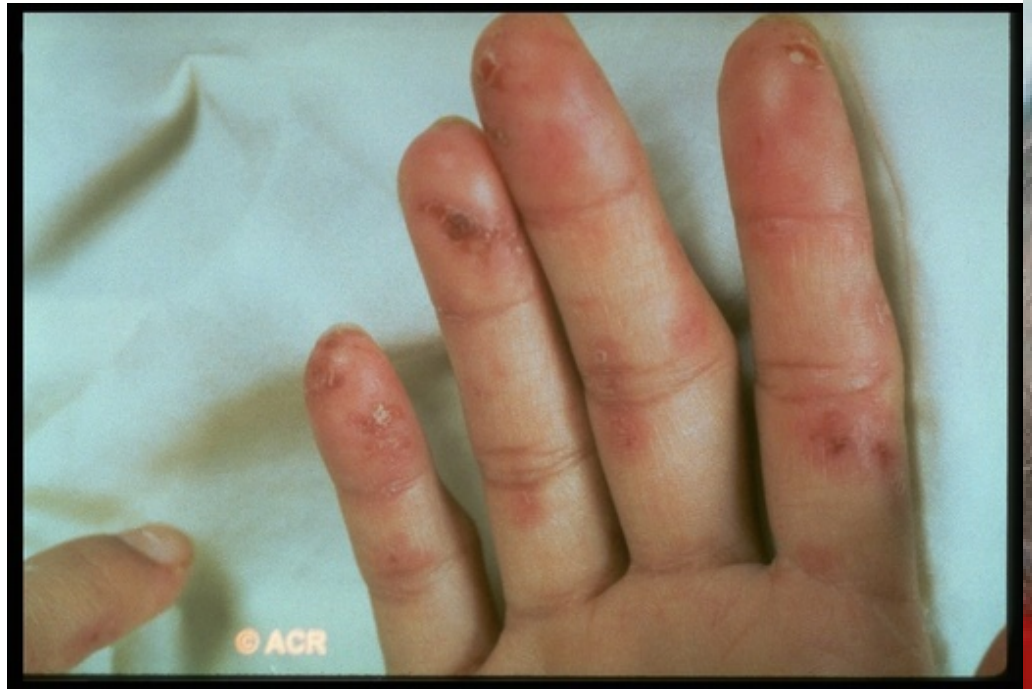
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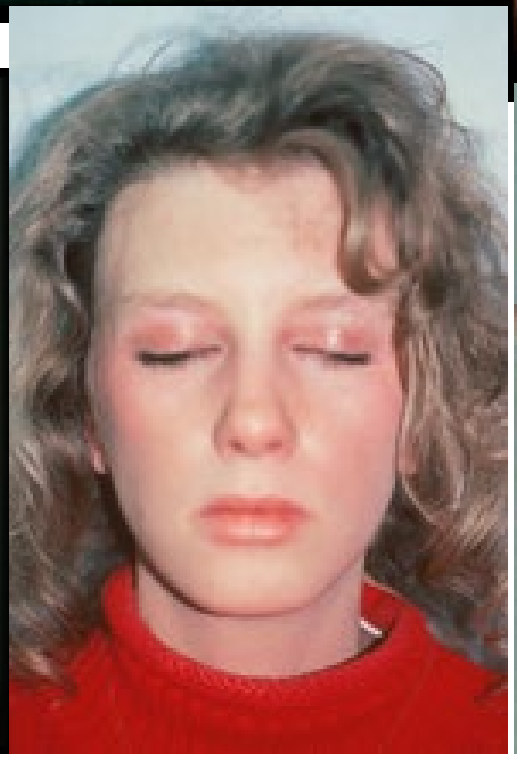
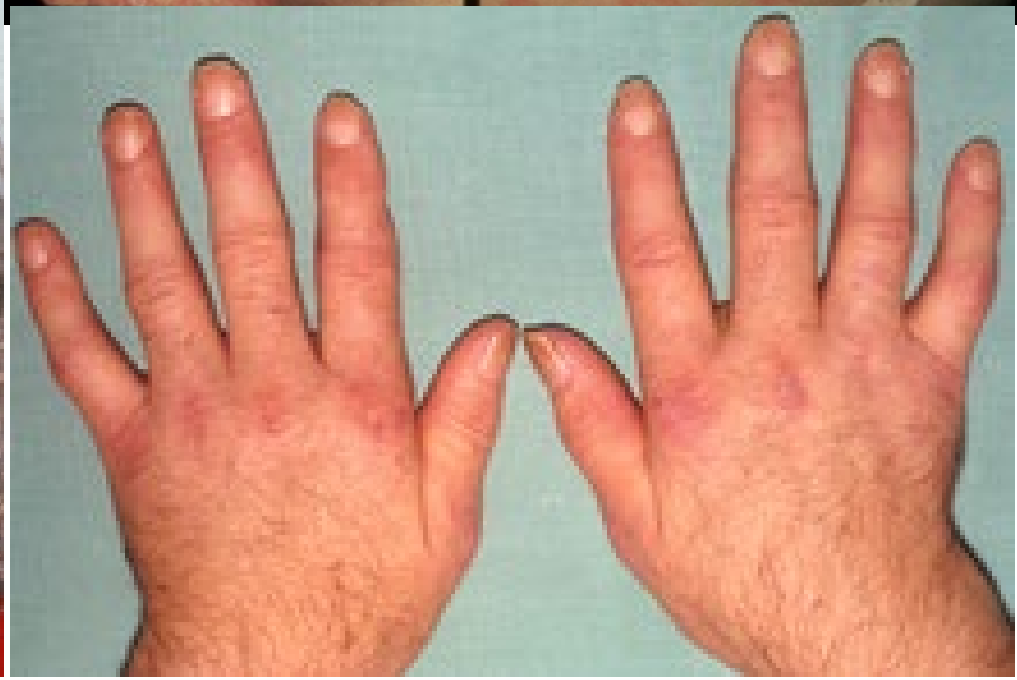




Inflammatory markers

- ESR, CRP most commonly used but many other positive and negative acute phase reactants
- Ferritin
- LDH
- Albumin

Acute phase reactants; ESR vs. CRP

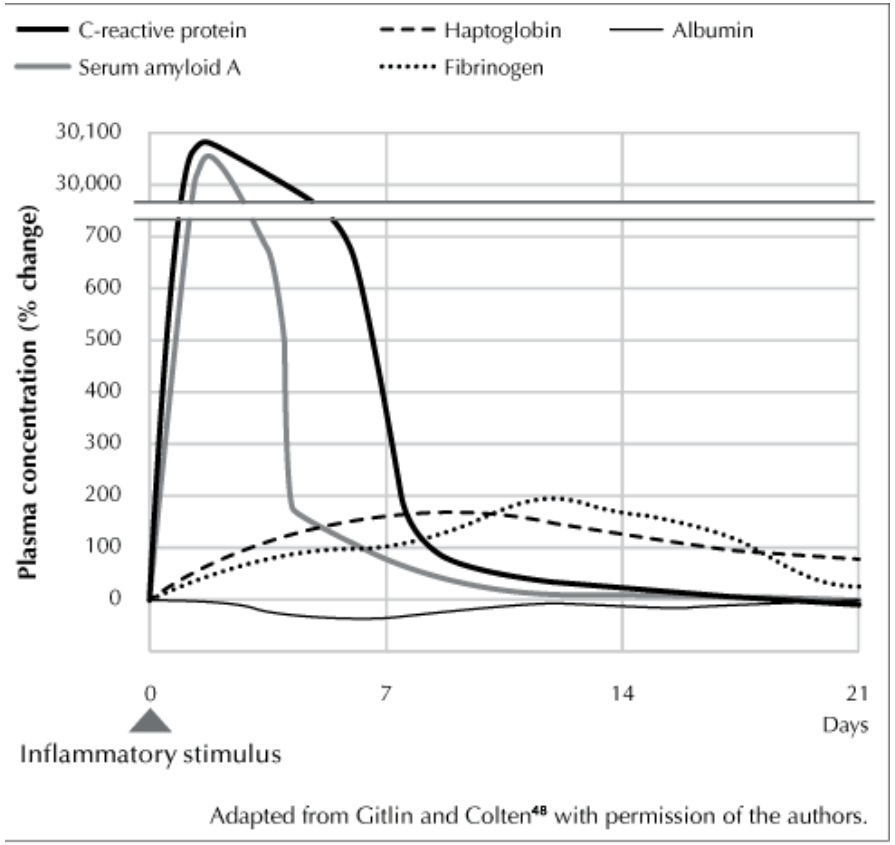
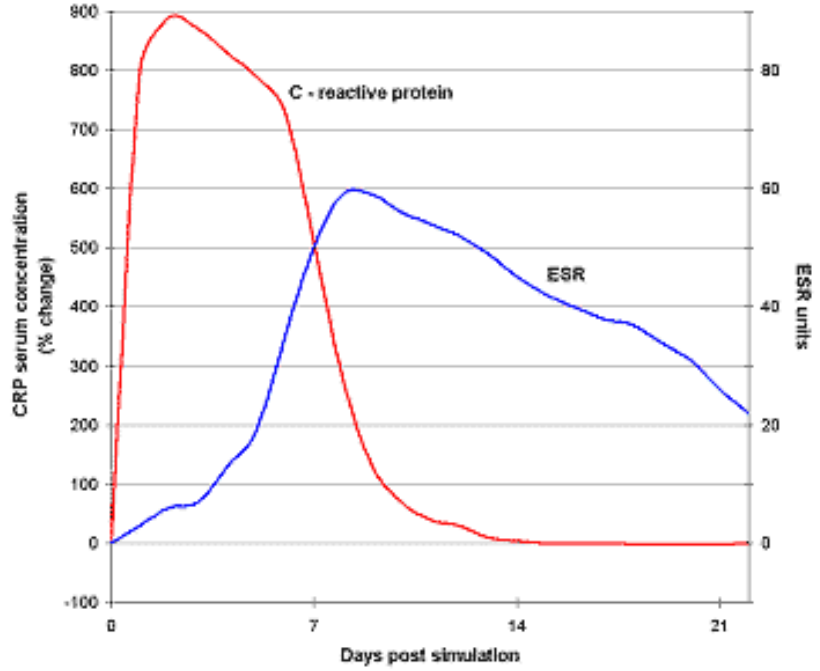


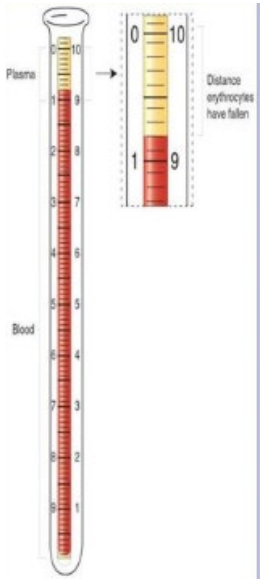
Figure 1. Characteristic pattern of inflammatory biomarkers in tissue damage.

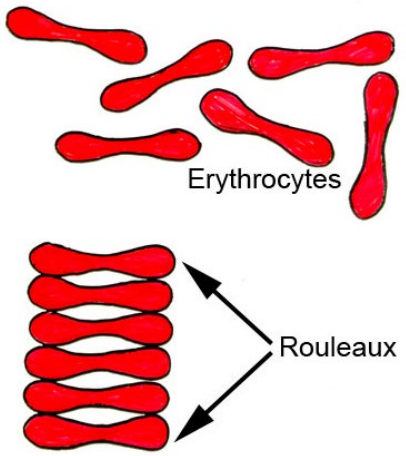


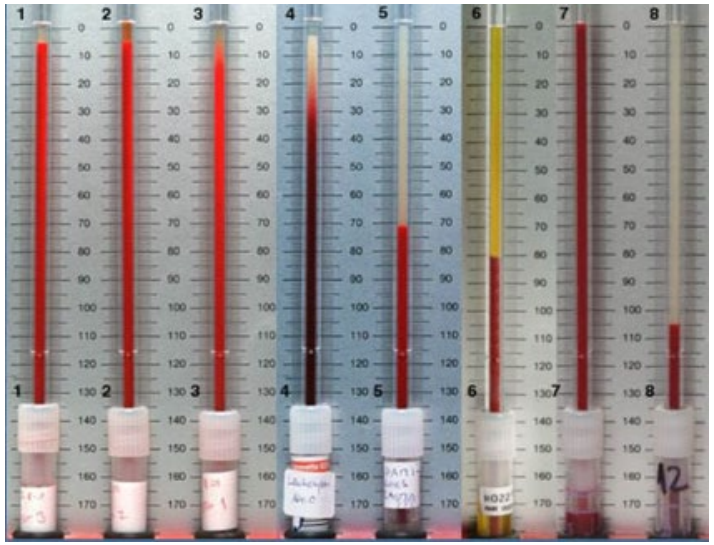
Gitlin JD, Colten HR: Molecular biology of the acute-phase plasma proteins. In Pick E, Landy M, editors: Lymphokines, vol 14, San Diego, 1987, Academic Press, pp 123–153.

ESR technique

- Drawn with Westergreen tube to 200 mm mark (but EDTA acceptable also)
- After 60 min, measure distance erythrocytes have fallen (acute phase proteins)
- Various factors increase ESR-inflammation, immune globulins; technique-tilted, ambient temperature too high
- Factors that decrease ESR-morphology; technique-room too cold, time >2 hours to measurement
- Normal ESR=age/2 (+5 in women)







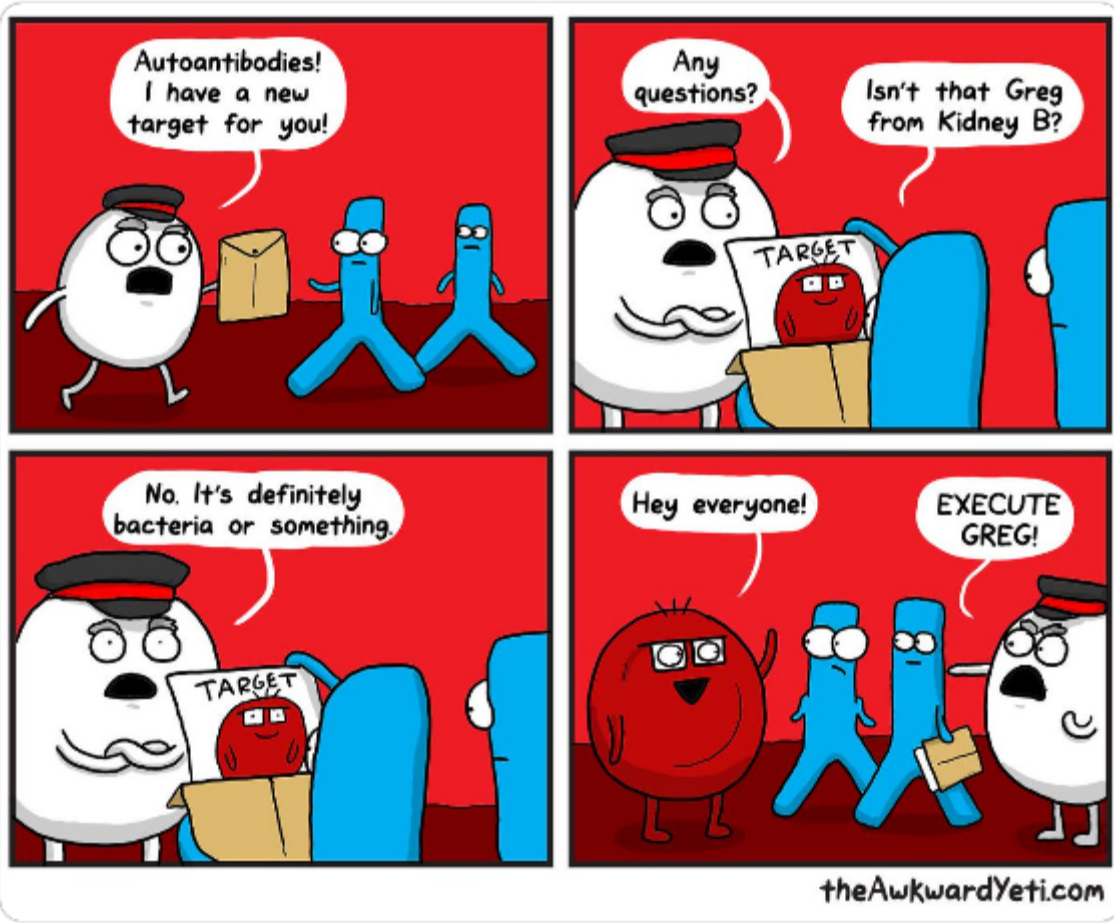
CRP

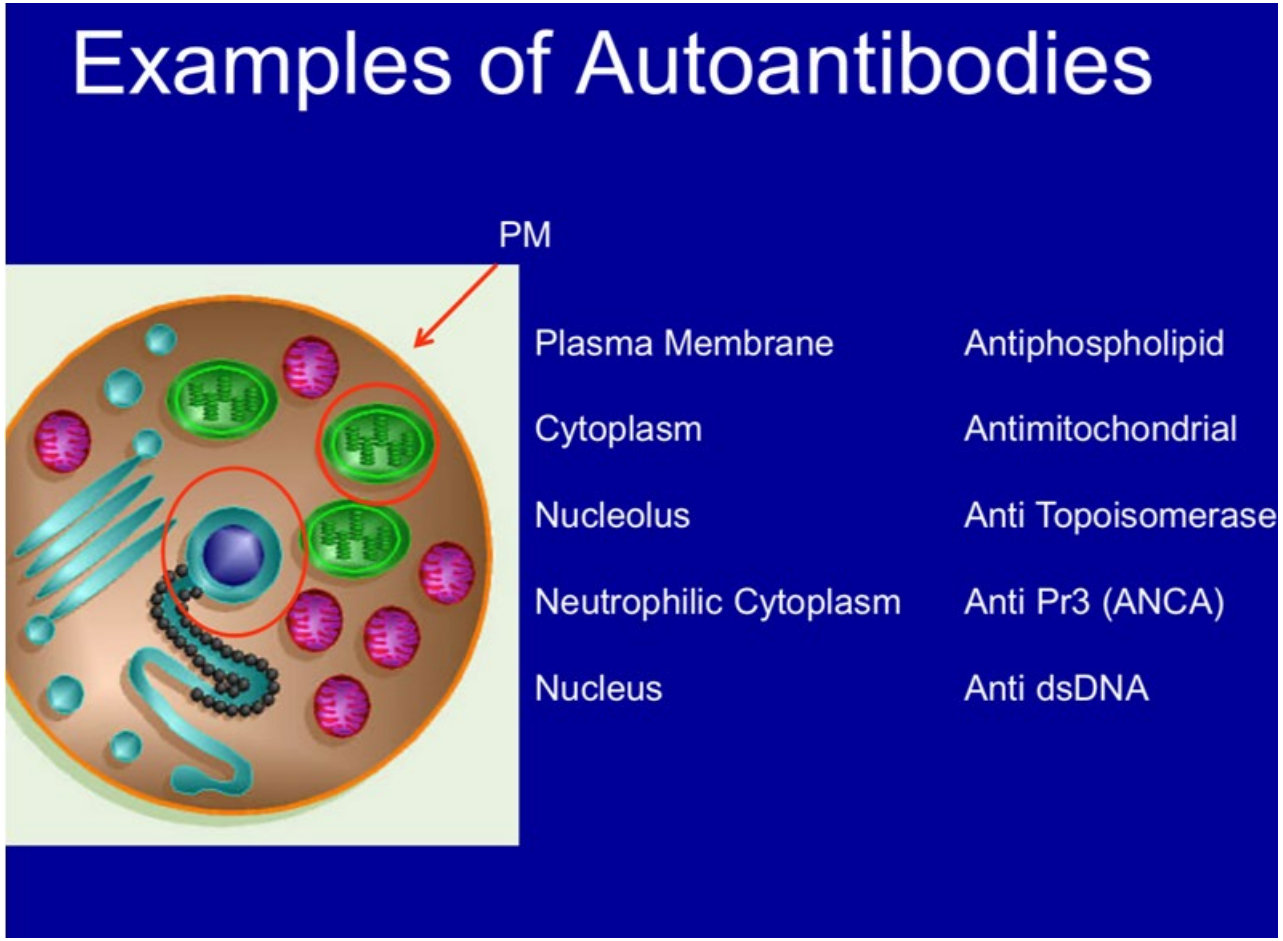
- Measured directly via ELISA
- More rapid on/off as compared with ESR
- Not affected by as many underlying conditions as ESR
- Obesity, NAFLD, metabolic syndrome can cause mild chronic elevation
- Attention to unit of measurement- differs by lab and means tenfold difference (i.e. 13 mg/dL vs. 130 mg/L)

Autoimmune serologies

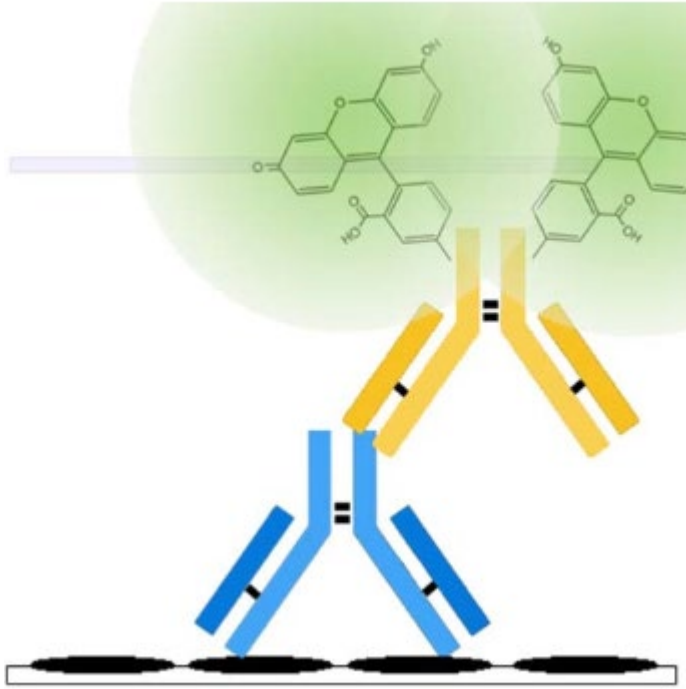
- Measure antibodies to self-antigens (components of cells)

#autoimmunediseases





Anti-nuclear antibody



- Preferred method is immunofluorescence to

Hep-2 cells (human laryngeal epithelioma cancer cell line)

- At NIH, ELISA rather than IF is performed- value of 6 or

higher is more consistent with high titers

- IF is available by Mayo send out- titers 1:160 or higher can be considered as significant depending on context but usually higher is more specific (i.e., 1:640 and higher)

- Higher titer doesn't mean more severe disease, just increases PPV

ANA

- Positive does not equal lupus
- Not specific
- Positive in first degree relatives of patients with SLE
- Positivity precedes clinical disease by 7-10 years in SLE and other
- Not to be used for disease activity monitoring or “trended”

Arbuckle MR, McClain MT, Rubertone MV, et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N Engl J Med*. 2003;349(16):1526-1533

Autoantibody–Disease Associations: SLE and Drug-Induced Lupus

Antigen	SLE	Drug-Induced LE
Native DNA	40%	No
Denatured DNA	70%	75%-80%
Histones	70%	>95%
SM Antigen	30%	No
Nuclear RNP	30%	No
Ribosomal RNP	10%	
SS-A/Ro	35%	No
SS-B/La	15%	No

Drug induced lupus- common offenders

	High	Moderate	Low	Very Low
Antiarrhythmics	Procainamide	Quinidine		Disopyramide, propafenone, amiodarone
Antihypertensives	Hydralazine		Methyldopa, captopril, acebutolol	Enalapril, lisinopril, clonidine, atenolol, labetalol, pindolol, minoxidil, prazosin
Antipsychotics			Chlorpromazine	Phenelzine, chlorprothixene, lithium
Antibiotics			Isoniazid, minocycline	Nalidixic acid, sulfamethoxazole, quinine
Anticonvulsants			Carbamazepine	Clobazam, phenytoin, trimethadione, primidone, ethosuximide, valproic acid
Antithyroid			Propylthiouracil	
Diuretics				Chlorthalidone, hydrochlorothiazide
Biologics			TNF-α inhibitors	IFN-α
Miscellaneous				Statins, levodopa, aminoglutethimide, timolol drops, ticlodipine

Hogan J et al. Drug Induced Glomerular Disease. 2015. Clin J Am Soc Nephrol 10: 1300–1310.

Rheumatoid factor

- IgM antibody directed against IgG

(Fc portion)

- Not specific for RA- can be found in up to 20% of older unaffected persons
- Also found in infections, other rheumatic diseases
- Present in 1-5% of healthy individuals

Rheumatoid Factor in Rheumatic Diseases

Disease	Incidence
Rheumatoid Arthritis	80%
Juvenile Chronic Arthritis	20%
Ankylosing Spondylitis	< 15%
Reiter's Syndrome	Negative
Psoriatic Arthritis	Negative
Systemic Lupus Erythematosus	40%
Sjögren's Syndrome	90%
Cryoglobulinemia	> 90%

RF

- Measured by ELISA at NIH
- Usually 60 IU/mL or greater (NIH >13 is positive)
- High titers 200 or more- can see up to 4-5,000
- +prognostic value with higher titers in RA
- As with ANA, presence precedes clinical diagnosis by >10 years
- When found in very high titers- consider Sjogren's in your differential

Anti-ccp

- Antibodies to cyclic citrullinated protein (ACPA)
- Most specific test for RA (>90%)
- Prognostic value
- Order both RF and anti-ccp if suspecting RA
- Cross over in some other autoimmune diseases- SLE, MCTD/overlap

Anti-dsDNA

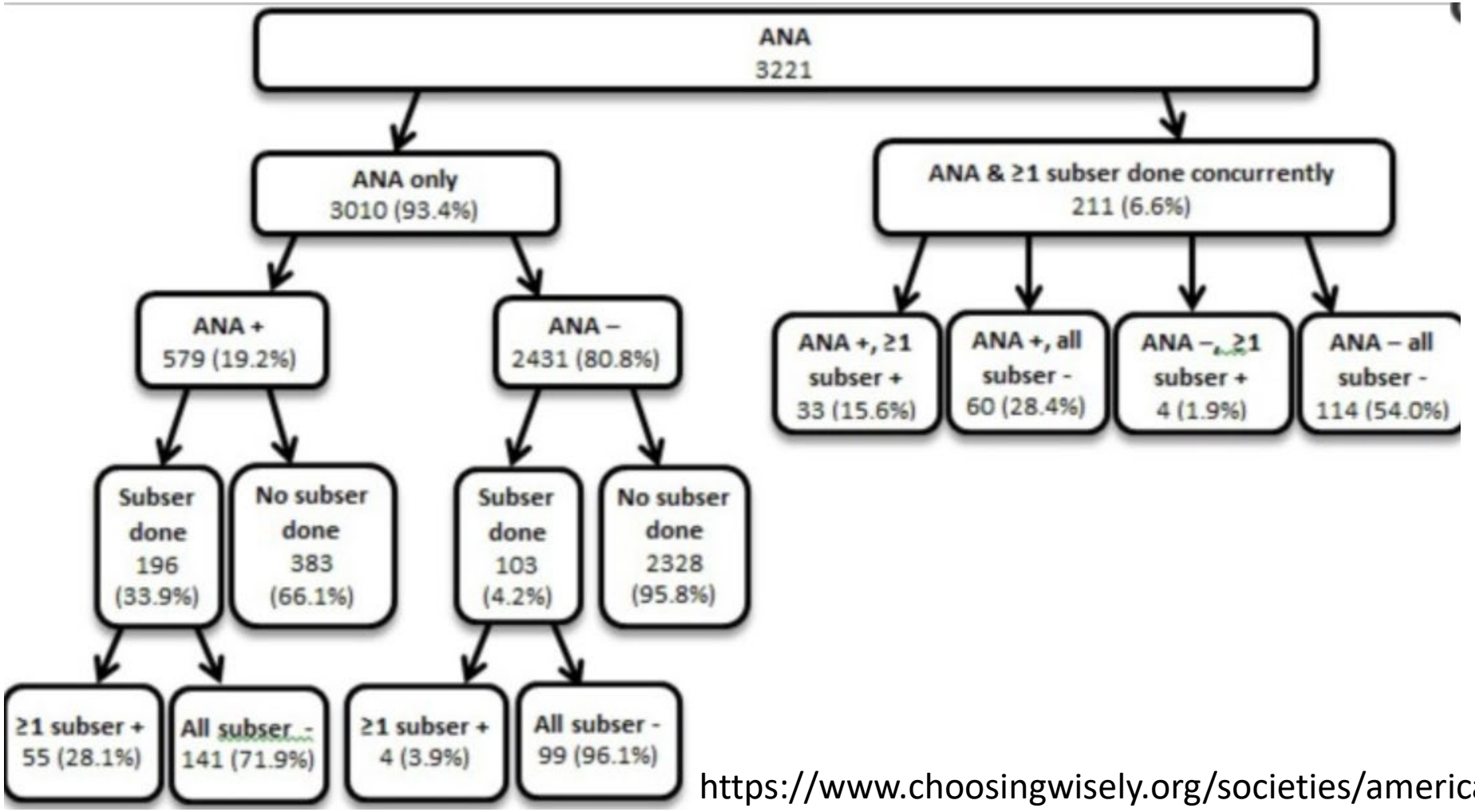
- Highly specific for SLE but sensitivity is poor
- Correlates with disease activity for patients who make them
- Association with active glomerulonephritis
- Anti-ssDNA- don’t order these, not useful or well validated

Subserologies

- Refers to other autoantibodies within the nucleus (anti-ENA umbrella)
- Anti-Sm- highly specific for SLE
- Anti-RNP- seen in SLE, MCTD→ association with PAH
- Anti-SSA (Ro)/SSB (La)- highly specific for Sjogren's

Choosing Wisely: Subserologies

ACR recommendation- don't order sub-serologies unless ANA is positive



https://www.choosingwisely.org/societies/american-college-of-rheumatology/

ANCAs

- Anti-neutrophil cytoplasmic antibody (or monocyte)
- Small vessel vasculitides or AAV- ANCA associated vasculitis (GPA, EGPA, drug induced AAV)
- Can be c-ANCA or p-ANCA depending on staining pattern on immunofluorescence
  - c=cytoplasmic while p=perinuclear

  c-ANCA attacks the proteinase-3 (PR-3)

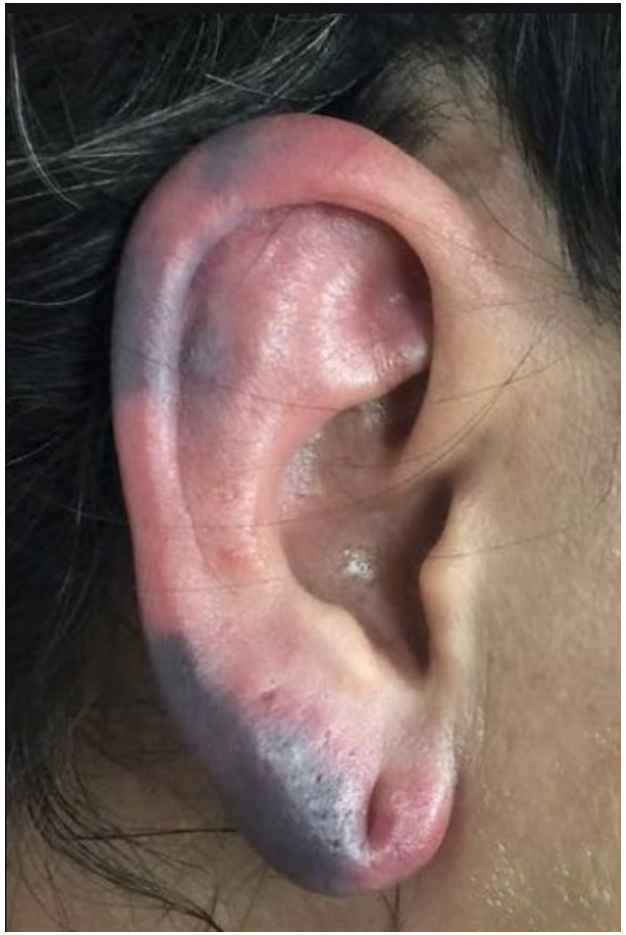
  P-ANCA attacks the myeloperoxidase (MPO)

ANCAs

- At NIH- can order anti-MPO (myeloperoxidase), anti-PR3 (proteinase-3) (ELISA)
- ANCAs (immunofluorescence) are send outs through Mayo
- Drug induced: hydralazine, propylthiouracil, levimasole (cocaine use)
- 60-80% of patients with UC have ANCAs, also seen in other autoimmune diseases without vasculitis

Drug induced ANCAs- physical exam clues

Figure 2. | Digital cutaneous vasculitis of a patient with levamisole-associated, ANCA-associated vasculitis. Reprinted from Joan Von Feldt and Robert Michelleti, with permission.



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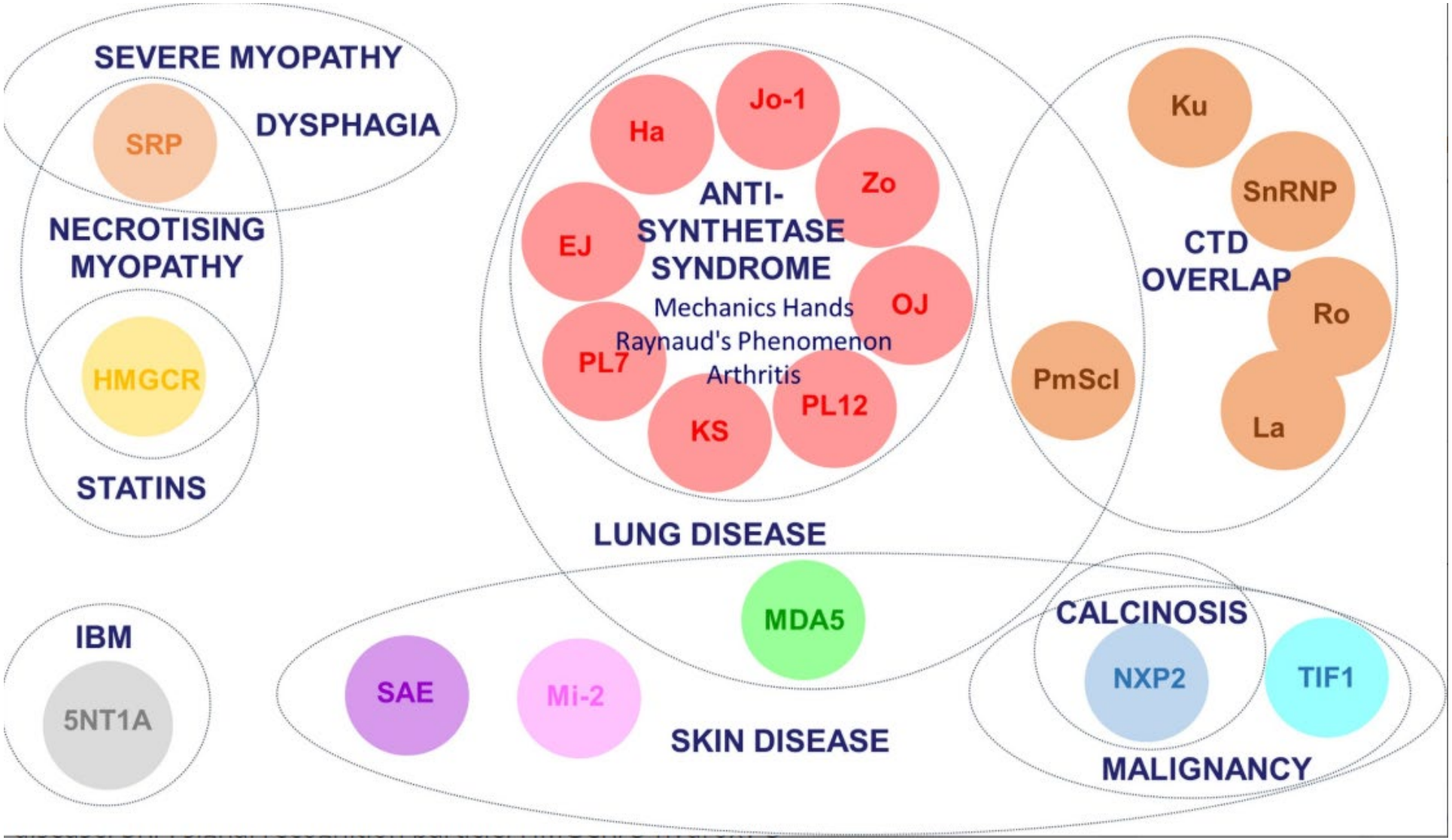
Myositis specific antibodies

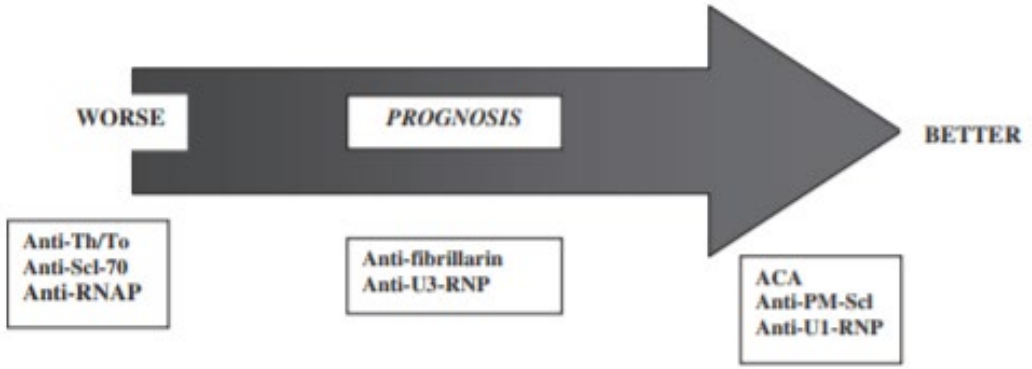


Betteridge Z, McHugh N. Myositis-specific autoantibodies: an important tool to support diagnosis of myositis. *J Intern Med*. 2016;280(1):8-23.

Scleroderma specific antibodies

- Anti-Scl-70, anti-centromere available within clinical center lab, ELISA
- Values >1.0 positive
- Each associated with a distinct clinical syndrome
- Sub-serologies
- Anti-Scl-70--→ diffuse scleroderma, ILD
- Anti-centromere --→limited disease (formerly CREST) PAH

Figure 1



Prognosis and systemic sclerosis-associated autoantibodies.

Ho KT, Reveille JD. The clinical relevance of autoantibodies in scleroderma. *Arthritis Res Ther*. 2003;5(2):80-93.

Antiphospholipid autoantibodies (aPL)

- Lupus Anticoagulant (LAC)
- Anti-cardiolipin (aCL) IgG
- Beta-2 glycoprotein

- Positive if two + tests separated by 12 weeks- distinct from syndrome which means associated thrombotic event

- Anticoagulation interferes with LAC

- Consider in patient with recurrent pregnancy loss (>3 1st trimester SAB), unprovoked venous/arterial thrombosis, thrombocytopenia

Caveats: NIH

-IViG and serologies

-Post BMT, SCT autoimmunity

-Study drugs, phase 1 trials

-Protocol driven care

Thank you!

References

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- Arbuckle MR, McClain MT, Rubertone MV, et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N Engl J Med*. 2003;349(16):1526-1533

- van der Molen RG, Hamann D, Jacobs JF, van der Meer A, de Jong J, Kramer C, Strengers PF, van der Meer JW. Anti-SSA antibodies are present in immunoglobulin preparations. Transfusion. 2015 Apr;55(4):832-7.

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