



What should I do with a positive ANA or RF?

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Disclosures

- None

Why is this important?

- Patients are frequently tested for RF or ANA without specific indication
- Patients are referred to Rheumatology clinic on the basis of positive RF/ ANA
- Patients and primary care providers are often uncertain regarding the meaning of abnormal test

Learning objectives

- Understand the circumstances when ordering RF or ANA is helpful
- Learn how to interpret a positive RF or ANA
- Understand the indications for referral to Rheumatology in patients with positive RF or ANA

Laboratory Case 1

- You are asked to evaluate a 74 year old woman who was found to have a positive RF of 84 IU/ml by her orthopedic surgeon. The test was obtained to evaluate multiple chronic joint pains in her hands and knees. She is otherwise well and takes OTC naproxen for her pains. Her ESR is 24 mm/ hour
- Your examination reveals nodular osteoarthritis of the fingers, bilateral genu varus deformities of the knees with a small, cool effusion on the right.

Laboratory Case 3

- You conclude that she has generalized osteoarthritis, no signs of RA and that the positive RF is likely age related.
- Which one of the following tests could you order to further support your opinion?
 1. C-reactive protein
 2. CCP antibody
 3. Antinuclear antibody
 4. Serum protein electrophoresis

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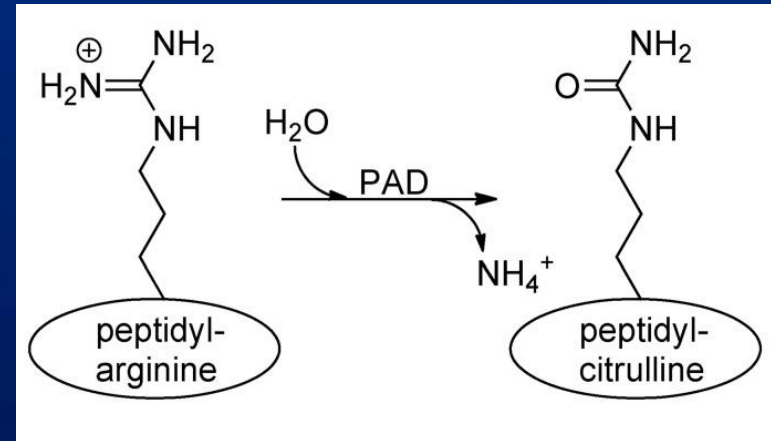
RA laboratory panel

	Anti-CCP	RF
Sensitivity	70%	72%
Specificity	95%	80%
Utility	<ul style="list-style-type: none">- Identifying early inflammatory arthritis patients at risk for erosive disease- Evaluating RF negative inflammatory arthritis patients- Evaluating a positive RF in a person who doesn't seem to have RA- In high titers uniquely specific for potentially erosive rheumatoid arthritis	<ul style="list-style-type: none">- Higher likelihood of detection and higher titers in established disease- High titers correlate with more severe disease
Impact of other factors	Smoking	Smoking, age

Cyclic citrullinated peptide and anti-CCP antibodies

What are they?

- Citrullinated peptides: can be found in physiological and inflammatory states
 - Tobacco increases their production in the lung
 - Gingivitis may increase their production in the gums



PAD = Peptidyl-arginine deiminase

- Antibodies to citrullinated peptides – specific for RA
May be present for years before the appearance of clinical RA
- Presence of CCP antibody is strongly linked to the HLA-DRB1 disease epitope

Anti-CCP Antibody in Early Arthritis

Identifying Early Arthritis Patients with a Poor Prognosis

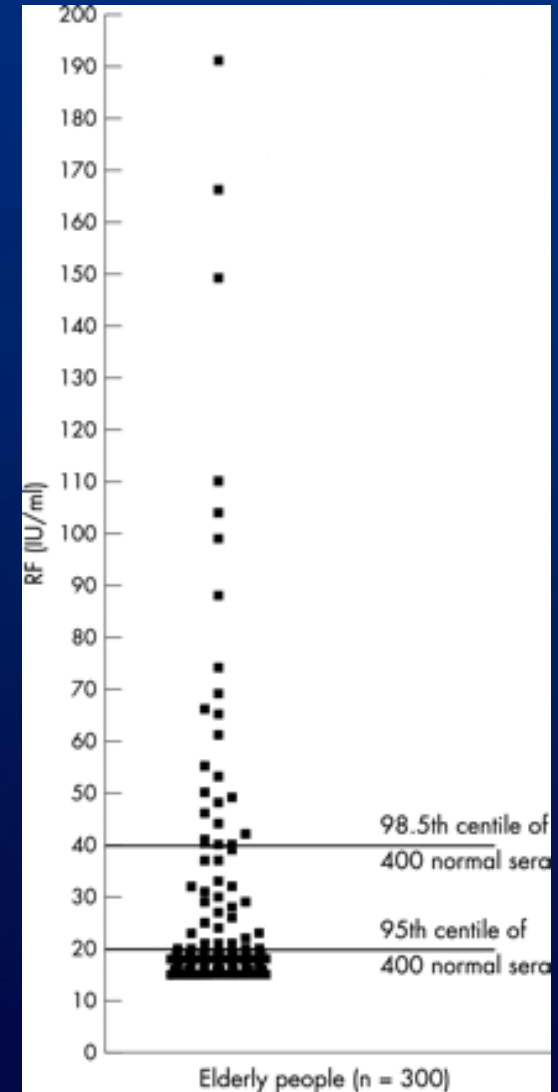
Patients Fulfilling ACR Criteria for RA			
	After 1 Year	After 2 Years	After 3 Years
Anti-CCP +	83%	90%	93%
Anti-CCP -	18%	24%	25%

This test has the greatest utility for predicting the progression of early arthritis to RA

Rheumatoid Factor and Aging

- 300 healthy elderly subjects aged 78-88 years
- 17% had a RF greater than 20 IU/ml
- 8% had a RF greater than 40 IU/ml
- Immunosenescence

Palosuo Tet al. Ann Rheum Dis 2003; 62:261-263



Laboratory Case 2

- A 37 year old man comes in to establish ongoing primary care. He has generalized arthralgias as well as pain and swelling of both knees.
- His rheumatoid factor was 259 IU/ml.
- His examination is notable for active synovitis in bilateral knees. His skin, nails and scalp reveal no signs of psoriasis, but a tattoo on his shoulder. His cardiac examination is normal.

Laboratory Case 2

Which test would you order to further evaluate the positive rheumatoid factor?

1. ANA
2. HLA-B27
3. Knee x-rays
4. Hepatitis C serology

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What is a rheumatoid factor?

- RF = antibody against antibody
- RF is usually an IgM against Fc of IgG
- Elevated RF can emerge several years before the onset of RA
- Can be present in RA and other conditions

Kelmenson L., et al. Arth Rheum 2020; 72:251-61

Rheumatoid Factor (IgM anti-IgG)

Rheumatic Diseases

- Rheumatoid arthritis 50-90%
- Sjögren's syndrome 75-95%
- SLE 15-35%
- Systemic sclerosis 20-30%
- PM/DM 5-10%
- MCTD 50-60%

Other Conditions

- Mixed Cryoglobulinemia (types II and III) 40-100%
- Hepatitis C 54-76%
- IPF
- Endocarditis
- Chronic infections
- Advancing age
- Malignancy

Laboratory Case 3

- You inherit a 36 year old woman in your new continuity clinic who has multiple unexplained medical symptoms.
- Multiple tests and imaging studies had been performed and no satisfactory explanation for her problems of fatigue, headaches, dizziness and blurring of her vision.
- She also has fibromyalgia which has been addressed in the Fibromyalgia Treatment Program. She is still in pain.

Laboratory Case 3

- Her examination is normal
- She wants to discuss the issue of possible lupus.
- Multiple connective tissue cascades have been obtained over the past six years.
- Results have revealed mildly positive ANAs fluctuating in the range of 1:80 – 1:160. ENA assays have been negative, anti -dsDNA and C3/C4, ESR, CBCs have always been normal

Laboratory Case 3

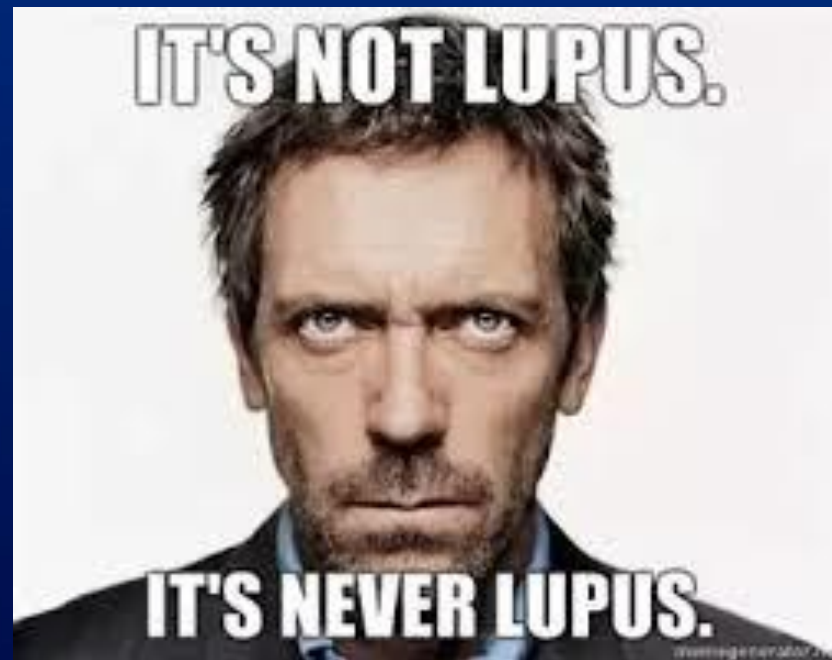
The best way to handle this situation would be to

1. Repeat another connective tissue cascade
2. Repeat another MRI to look for CNS changes of lupus or MS
3. Make an appointment with your doctor for a fluoxetine prescription
4. Don't repeat the ANA, discuss the operating characteristics of this test with your new patient and avoid additional testing
5. Refer her to Rheumatology for her third consultation

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Prevalence of positive ANA

- Large multicenter study
- Healthy volunteers 20-60 years
- ANA 1:40 32%
- ANA 1:80 13.3%
- ANA 1:160 5%
- ANA 1:320 3.3%

Tan EM, et al. Arthritis Rheum. 1997;40(9):1601

What are antinuclear antibodies?

- A heterogeneous group of antibodies directed to complexes of DNA-proteins and RNA-proteins within the nucleus.
- Low concentrations are found in normal individuals
- Solid phase essays (e.g. ELISA): automated, lower cost, higher false-negative rate
- The IIF test for ANA is the gold standard (more sensitive)
- ANA at a titer of $\geq 1:80$ on HEp-2 cells is considered positive/ abnormal, but low specificity

What are antinuclear antibodies?

- High antibody levels ($\geq 1:640$) have a high specificity for SLE and other CTD
- Changes in ANA titer are not helpful for monitoring disease activity
- Repeat determinations of ANA are not indicated
- In the presence of 0 or 1 clinical manifestations of SLE, a positive ANA is associated with very low posttest probability of SLE.
- Choosing wisely: no need for ANA testing if there is no clinical suspicion for a CTD

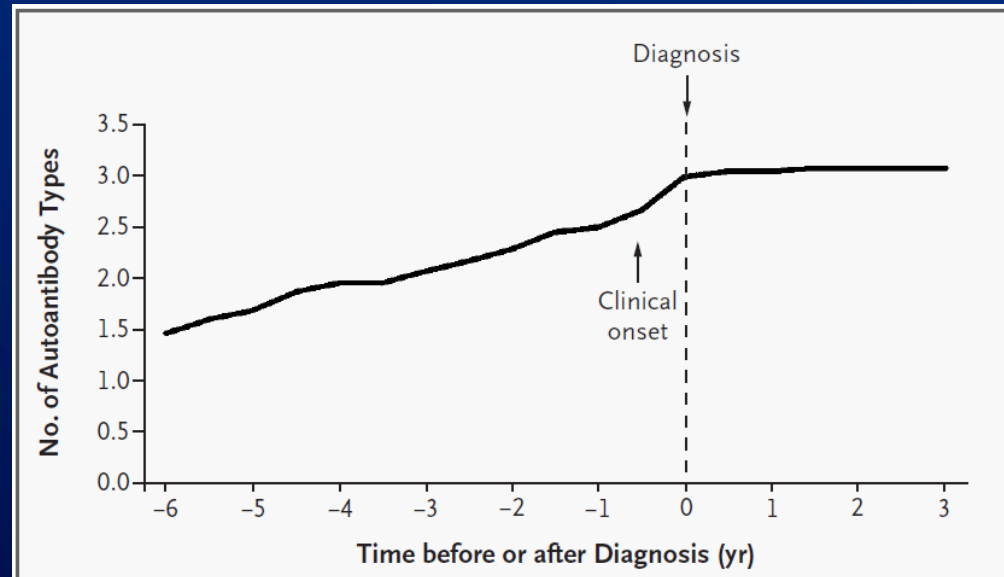
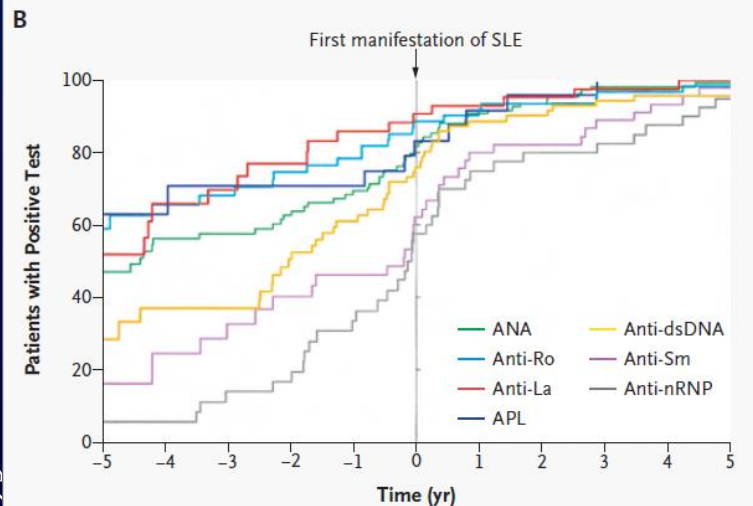
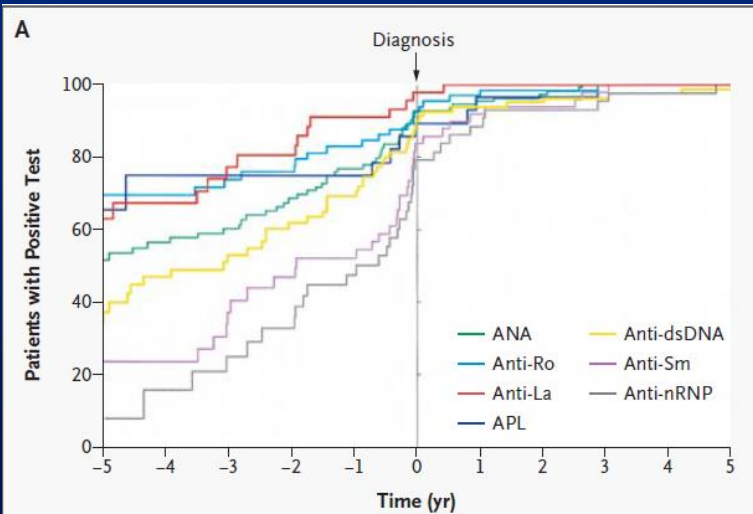
Detection Technology for ANA

- If you have a high pretest suspicion for a connective tissue disease:
 - A negative automated ANA requires a subsequent IIF ANA assay
 - A negative automated ANA requires a separate ENA assay
- In some rheumatic diseases there are specific ANAs that may lead to tissue damage:
 - Anti-DNA antibodies in lupus nephritis
 - Anti-SSA antibodies in neonatal lupus

Extractable nuclear antigen (ENA) panel

- Anti-Ro/SSA, anti-La/SSB, Sm, RNP, Scl-70 and Jo1

How do anti-nuclear antibodies behave in patients with early SLE?



ANA/SSA/SSB → aDNA → Sm/RNP

Arbuckle et al. NEJM 2003;349:1526

Thinking about pretest probability

Pick the scenario where a positive ANA would support your diagnosis of lupus

1. Patient with fatigue, migraine headaches and chronic widespread pain
2. Patient with fatigue, treated Hashimoto's thyroiditis, and marital discord
3. Patient with fatigue, fevers of 39°C, anemia, lymphopenia, thrombocytopenia and oral ulcers
4. Patient with fatigue, morbid obesity and an elevated CRP
5. Patient with fatigue, chronic low back pain and inability to work

ANA testing is NOT a screening test!

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ANA testing is NOT a screening test!

Other SLE testing. Clarify what you order for diagnosis versus monitoring!

	Diagnosis	Monitoring
Complement		+++
Anti-DNA	+	++
LAC APLA	+++	

Laboratory Case 4

- You are seeing a 43 year old woman who has developed four extremity Raynaud's phenomenon over the past two years. It is not bothersome enough for her to warrant vasodilator therapy. She takes no medications and does not smoke.
- Her examination is notable for a healthy appearing woman. She has a normal cardiovascular examination. Her skin and joints are normal except for mild capillary prominence in the nail folds.

Laboratory Case 4

The one best set of tests to order for her evaluation would be

1. Connective tissue cascade and cold agglutinin assay
2. Connective tissue cascade and antiphospholipid antibody assay
3. Connective tissue cascade and rheumatoid factor
4. Connective tissue cascade and urine metanephrines
5. Connective tissue cascade and anti-centromere antibody

Laboratory Case 4

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3. Connective tissue cascade and rheumatoid factor
4. Connective tissue cascade and urine metanephrines
5. **Connective tissue cascade and anti-centromere antibody**

Adding to the Confusion

- Some antinuclear antibodies are not detected by the current ANA/ENA assay:
 - Anti-centromere, RNA 3 polymerase antibodies
 - Many new “myositis specific” antibodies

Summary

- History and physical examination are still paramount in making rheumatologic diagnoses
 - Laboratory tests are rarely diagnostic in Rheumatology
- Laboratory testing is costly and should be performed based on the clinical diagnosis
 - Be careful conservators of limited health care resources
- Avoid creating unnecessary patient anxiety by ordering tests which may reveal results you can't explain or act on therapeutically

Helpful resources

- <https://www.acponline.org/featured-products/mksap-18>
- <https://www.rheumatology.org/Practice-Quality/Clinical-Support/Clinical-Practice-Guidelines>
- <https://www.rheumatology.org/Learning-Center/Medication-Guides>
- <https://www.uptodate.com/contents/search>



Questions & Discussion