UNDERSTANDING THE ALPHABET SOUP OF RHEUMATOLOGY LABS

Benjamin J Smith, DMSc, PA-C, DFAAPA
Florida State University College of Medicine
School of Physician Assistant Practice

Disclosures

Member, National Commission on Certification of Physician Assistants Board of Directors

Objectives

After completing this session, participants will be able to:

- Select appropriate laboratory tests when evaluating patients with symptoms suggesting rheumatic conditions.
- Interpret the results of laboratory tests used to diagnose and manage common rheumatic diseases.
- Evaluate the appropriate clinical applications for laboratory tests used to diagnose and manage common rheumatologic disorders.
- Explain to patients with rheumatic conditions the relevance of specific laboratory results.

Question 1

Which of the following laboratory tests can be used to measure rheumatic disease activity?

A. Antinuclear Antibody (ANA)
B. Rheumatoid Factor (RF)
C. Anti -Cyclic Citrullinated Peptide (CCP)
D. C-Reactive Protein

Question 2

Which of the following ANA results is most likely not to be a false positive result?

A. ≥1:2560, homogenous
B. 1:160, speckled
C. 1:80, speckled
D. 1:40, homogenous

Question 3

Which of the following can be a distinguishing laboratory finding in lupus erythematosus?

A. Normal WBC count
B. Reduced erythrocyte sedimentation rate
C. Positive antinuclear antibody titer
D. Negative ENA panel


Scl70
CRP
IgG
RF
ANA
JIA
RA
RNP
SLE
ANCA
Ro-SSA
HLA-B27
anti-CCP
CH50
MCTD
ACA
CBC
ds-DNA
ESR
Jo-1
OA
La/SSB

Terminology Review

Specificity and Sensitivity

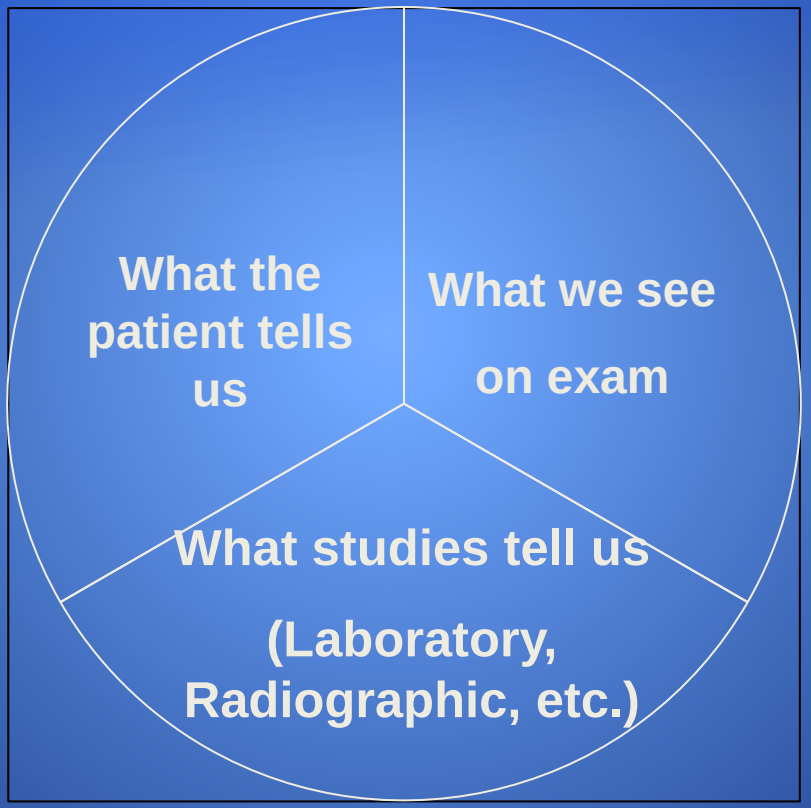
- Sensitivity
  - Percent of positive results in people with the disease
  - ↓ sensitivity, ↑ chance for false negative results
- Specificity
  - Percent of negative results in people without the disease
  - ↓ specificity, ↑ chance for false-positive results

The Benefit of Laboratory Testing

- Establish a diagnosis
- Determining prognosis
- Monitoring disease activity, prognosis, or damage
- Monitoring drug or therapeutic toxicities
- Establishing complications of the underlying disease process
- Excluding alternative diagnoses or complications

Bartlett, Susan J., PhD, editor. Clinical Care in the Rheumatic Diseases, 3rd edition. Atlanta: Association of Rheumatology Health Professionals; 2006.

Putting the Pieces Together



Case 1

- Clinical presentation
  - 45 yo male with left great toe pain and erythema X 1 day, unable to ambulate, hard to sleep with sheet touching toe
- 3 previous episodes, HTN on HCTZ
- PMH: HTN, seasonal allergies
- SH: 6 pack of beer/day, no TOB
- PE: Unremarkable except for Left podagra

Case 1

- What lab would be helpful?
- When should this lab be ordered?

Uric Acid

- Elevated levels of uric acid are often associated with gout, but other pathologies can cause the uric acid to be high:
  - Chronic renal disease (most common cause)
  - Leukemia
  - Polycythemia vera
  - Therapy with thiazide diuretics
  - Eclampsia
- Uric acid crystals in joint fluid diagnostic for gout
- Best ordered when?

Monosodium Urate crystals

Strongly negatively birefringent

Perpendicular=blue

Parallel=bright yellow

Calcium Pyrophosphate Dihydrate crystals

Weakly positive birefringence

Perpendicular=yellow

Parallel=blue

SYNOVIAL FLUID ANALYSIS

Condition	Color	Clarity	WBC	Crystals	C&S
OSTEO	Amber	Clear	200 -2,000	–	–
TRAUMA	Pink Red	Clear-opaque	<2,000	–	–
INFLAM-MATORY	Yellow	Cloudy	2000-100,000	– +	–
INFECTION	Purulent	Opaque	>50,000 (>90%PMNs)	– +	+

Case 2

- Clinical presentation
  - 35 yo female with 6 week onset of pain in bilateral MCPs, PIPs, MTPs. 2 hours of morning stiffness. Steroid dose pack from PCP helped until complete dose pack. Difficult ambulation with symptoms. Symptoms worsen after prolonged sitting.
- PMH: no prior medical problems. G3P3
- SH: Wine with dinner, 1 glass. No TOB
- FH: Maternal GM-RA
- PE: Unremarkable except for tenderness with palpation of bilateral wrists, MCPs and PIPs. Synovitis noted in left 2-5 MCPs, and bilateral MTPs. Positive squeeze test of MCPs and MTPs.

Case 2

- What lab tests would assist with diagnosis?
- What other lab tests are needed to establish a baseline for therapy?
- What lab tests can be used to monitor therapy?

Rheumatoid Factor (RF)

- RF not conclusive of rheumatoid arthritis (RA)
- RF not used to measure RA disease activity, but higher titers can be associated with disease severity, erosions, extra-articular manifestations, disability.
- RA nodules and RA vasculitis almost exclusively in RF (+) RA

Rheumatoid Factor in other diseases

CH-Chronic disease
- *hepatic (PBC)
- *pulmonary (IPF, silicosis, asbestosis)

R-Rheumatoid Arthritis

O-Other rheumatic disease
- *SLE
- *Systemic sclerosis
- *MCTD
- *Sjögren's
- *Polymyositis
- *Sarcoid

N-Neoplasm, especially after XRT or chemo

I-Infections
- *AIDS
- *Mononucleosis
- *Parasitic infections
- *Chronic Viral
- *Hepatitis B/C
- *Chronic bacterial (SBE, syphilis, mycobacteria)

C-Cryoglobulienmia (esp with Hep C)

Specific autoantibodies precede the symptoms of rheumatoid arthritis: A study of serial measurements in blood donors

Nielen, MM, van Schaardenburg, D, Reesink, HW, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum 2004; 50:380.

Serum of 79 RA pts (matched controls)

*Blood donors

*Tested for IgM RF and CCP

Results-

*Median age before sxs of samples-7.5 yrs (range 0.1-14.5 yrs)

*49% (+) RF/CCP at least once before sxs-median 4.5 yrs before sxs (range 0.1-13.8 yrs)

*Controls---(+) RF-1.1%, (+) CCP-0.6%

Conclusion-

"Approximately half of patients with RA have specific serologic abnormalities several years before the onset of symptoms. A finding of an elevated serum level of IgM-RF or anti-CCP in a healthy individual implies a high risk for the development of RA. We conclude that IgM-RF and anti-CCP testing with appropriately high specificity may assist in the early detection of RA in high-risk populations."

Anticyclic Citrullinated Peptide Antibodies (anti-CCP)

- ELISA detects antibodies directed against filaggrin
- Anti-CCP found in most patients with RA
  - Specificity for RA – 90-96%
  - Sensitivity 47-76%
- Combination of positive RF and positive anti-CCP has 99.5% specificity for RA
- (+) CCP – more likely to have aggressive disease and progressive radiographic joint damage

Anti-CCP

Can occur in

- Tuberculosis
- SLE
- Sjogren's
- Polymyositis
- Dermatomyositis
- Scleroderma

Acute-Phase Reactants

- Erythrocyte Sedimentation Rate (ESR) and C-Reactive Protein (CRP)
  - Estimate extent of inflammation
  - Monitor disease activity over time
  - Assess prognosis
  - Nonspecific

ESR

- ESR normal values
  - Increases with age
    - Male = age/2
    - Female = (age + 10)/2
- Increased in:
  - RA, MI, TB, multiple myeloma, pregnancy, malignancy, bacterial infection, connective tissue disease, inflammation

C-Reactive Protein (CRP)

- CRP Normal Values
  - Male = age/50
  - Female = (age/50) + 0.6
- CRP concentrations increase in response to tissue injury and infection
  - Rises and falls more quickly than ESR
  - Serial assays most valuable
  - Not disease specific

Recommended laboratory evaluation for starting, resuming or significant dose increase for selected medications (RA pts)

THERAPEUTIC AGENTS	CBC	Transaminases	Creatinine	Other
HCQ	X	X	X	Eye exam
LEF	X	X	X	Hepatitis serologies if pt at risk
MTX	X	X	X	Hepatitis serologies if pt at risk
Minocycline	X	X	X	
SSZ	X	X	X	
Biologics	X	X	X	(Hepatitis serologies)

Saag KG, et al. American College of Rheumatology 2008 Recommendations for the Use of Nonbiologic and Biologic Disease-Modifying Antirheumatic Drugs in Rheumatoid Arthritis. *ArthritisReum.* 2008; 59: 762-784.

Recommended optimal laboratory follow-up monitoring (CBC, kidney and liver fxn) for selected medications (RA pts)

Therapeutic Agent	<3 months	3-6months	>6 months
HCQ	None after baseline	None	None
LEF	2-4 weeks	6-12 weeks	12 weeks
MTX	2-4 weeks	6-12 weeks	12 weeks
Minocycline	None after baseline	None	None
SSZ	2-4 weeks	6-12 weeks	12 weeks

Saag KG, et al. American College of Rheumatology 2008 Recommendations for the Use of Nonbiologic and Biologic Disease-Modifying Antirheumatic Drugs in Rheumatoid Arthritis. *ArthritisReum.* 2008; 59: 762-784

Case 3

- Clinical presentation
  - 24 yo male with arthritis symptoms since age 13
  - Left great toe with pain/swelling, sudden onset
  - Feet, hands (MCPs, PIPs), wrists, elbows, knees, shoulder
  - Multiple right knee aspirations, inflammatory fluid
  - Diagnosis at age 13 – RA
  - Treatment: MTX/folic acid, Naproxen
- At age 16, symptoms persist with treatment
  - Tx-etanercept added, helped significantly (pt reduced dose from Q week to Q month)
- At age 22, onset of atraumatic low back pain
  - ↑-inactivity, in the morning
  - ↓-activity, exercise, stretching

Case 3

- PMH: eczema, otherwise negative
- Social History: (-)tobacco, EtOH
- Family History: maternal aunt-RA
- Physical Exam
  - Flesh colored patches on BUE proximally and peri-axillae area.
  - No synovitis in peripheral joints.
  - No secondary degenerative arthritis changes.
  - Mild tenderness with direct palpation over bilateral SI joints.
  - Otherwise (-)
- Labs
  - (-) RF/CCP
  - CBC/CMP wnl, except ALT = 51

Case 3

What is your next step?

A. Continue current regimen without change as diagnosis is RA.
B. Ask patient to take etanercept weekly as approved by FDA for RA.
C. Order additional diagnostic studies for low back pain.
D. Order L-spine MRI for low back pain.

Human Leukocyte Antigen B27 (HLA-B27)

- Qualitative test (positive or negative)
- Associated with seronegative spondyloarthropathies
  - Assists with ankylosing spondylitis, Reiter's syndrome, or anterior uveitis diagnosis
- Presence is genetically determined

SI Joint X-rays

Case 4

- Clinical presentation
  - 20 yo female with arthralgia, fatigue and red rash in photo-exposed distribution on neck and chest. Known (+)ANA 1:1280 homogeneous.
- PE: No synovitis on joint exam. Erythematous facial rash in malar distribution and erythema on posterior neck and anterior chest.
- What other lab should be ordered to support diagnosis?

Anti-Nuclear Antibodies (ANA)

1948---LE cell test (phagocyte with ingested nucleus)

Hargraves MM. Discovery of the LE cell and its morphology. Mayo Clin Proc. 1969;44:579-99.

Today's techniques

- *Immunofluorescent microscopy (rodent liver or kidney, Hep-2 cell lines)
- *Immunodiffusion
- *Hemagglutination
- *Complement fixation
- *Solid-phase immunoassay (ELISA or immunoblotting)
- *Radioimmunoassays

ACR Position Statement

Methodology of Testing for Antinuclear Antibodies

~~Approved 8/2015~~

The ACR supports the immunofluorescence antinuclear antibody (ANA) test, using Human Epithelial type 2 (Hep-2) substrate, as the gold standard for ANA testing.

Hospital and commercial laboratories using alternative bead-based multiplex platforms or other solid phase assays for detecting ANAs must provide data to ordering healthcare providers on request that their alternative assay has the same or improved sensitivity compared to the IF ANA.

In-house assays for detecting ANA as well as anti-DNA, anti-Sm, anti-RNP, anti-Ro/SS-A, etc. should be standardized according to national (e.g., CDC) and/or international (e.g., WHO, IUIS) standards.

Laboratories should specify the methods utilized for detecting ANAs when reporting their results.

http://www.rheumatology.org/Portals/0/Files/Methodology%20of%20Testing%20Antinuclear%20Antibodies%20Position%20Statement.pdf

Accessed 08 May 2020

ANA Patterns

Peripheral or “rim”

Homogeneous (Diffuse)

Speckled

Nucleolar

Centromere

ANA

- 95 - 99% sensitivity for SLE, discoid (15%), drug induced (100%)
- High titers > 1:640 raise suspicion for an autoimmune disorder
- Positive titers remain constant over time
- <1:160 titers less clinically significant titer
- Titers are not a measure of disease activity

Conditions associated with a (+) ANA

Very useful for Dx
- SLE
- PSS

Somewhat useful for Dx
- Sjogren's
- Polymyositis/Dermatomyositis

Useful for monitoring or prognosis
- Juvenile ideopathic arthritis
- Raynaud's phenomenon

Critical part of Diagnostic criteria
- Drug-associated lupus
- MCTD
- Autoimmune hepatitis

Not useful or has no proven value for diagnosis, monitoring or prognosis
- RA
- MS
- Thyroid disease
- Infectious disease
- ITP
- FMS

Solomon DH, et al. Evidence Based Guidelines for the use of immunologic tests: Antinuclear antibody testing. *Arthritis Rheum*. 2002; 47: 434-444.

If ANA positive, consider

-ds DNA

-SS-A/SS-B

-ENA (Sm, RNP)

-Scl-70

-Other lab….

Depending on history, exam and titer

Choosing Wisely®

An initiative of the ABIM Foundation

AMERICAN COLLEGE OF RHEUMATOLOGY

-Don't test ANA sub-serologies without a positive ANA and clinical suspicion of immune-mediated disease.

-Don't test for Lyme disease as a cause of musculoskeletal symptoms without an exposure history and appropriate exam findings.

-Don't perform MRI of the peripheral joints to routinely monitor inflammatory arthritis.

- Don't prescribe biologics for rheumatoid arthritis before a trial of methotrexate (or other conventional non-biologic DMARDs).

-Don't routinely repeat DXA scans more often than once every two years.

Drug Induced Lupus

Definite — Procainamide, hydralazine, minocycline, diltiazem, penicillamine, isoniazid, quinidine, anti-tumor necrosis factor alpha therapy, interferon-alfa, methyldopa, chlorpromazine, and practolol.

Probable — Anticonvulsants (phenytoin, mephenytoin, trimethadione, ethosuximide), antithyroid drugs, antimicrobial agents (sulfonamides, rifampin, nitrofurantoin), beta blockers, lithium, paraaminosalicylate, captopril, interferon gamma, hydrochlorothiazide, glyburide, sulfasalazine, terbinafine, amiodarone, ticlopidine, and docetaxel.

Possible — Gold salts, penicillin, tetracycline, reserpine, valproate, statins (eg, lovastatin, simvastatin, and atorvastatin) griseofulvin, gemfibrozil, valproate, lamotrignine, ophthalmic timolol, and 5-aminosalicylate.

Merola JF, et al. Drug-induced lupus. UpToDate. Accessed 08 May 2020.

Double-stranded DNA antibodies (anti-dsDNA)

- Specific for SLE
  - >97% specificity, 70% sensitivity
  - Low titers in other autoimmune diseases and patients receiving drugs for rheumatic diseases
- Titers rise with disease flares
  - May be a measure of disease activity when considered with other measures of disease activity (renal)
- Can be seen in those taking minocycline, etanercept, infliximab and penicillamine.
- Can be seen in normal individuals, particularly first degree relatives of patients with lupus and some laboratory workers.

Anti-Smith and Anti-RNP

Smith antibodies (anti-Sm)

- High specificity, low sensitivity for SLE
- Titers remain positive after disease activity subsides and anti-dsDNA titers decline, so not useful for following disease course or predicting disease activity

Ribonuclearprotein antibodies (anti-U1 RNP)

- Found in many patients with SLE
- Low titers in other rheumatic diseases
- Hallmark feature of MCTD - sensitivity 100%

Anti-SS-A (Ro)

- Helpful in diagnosing Sjögren's and SLE, but not specific
- Occurs in 60% of ANA-negative SLE patients
- Sensitivity
  - 1° Sjogren's: 70-97%
  - 2° Sjogren's w/RA: 10-15%
- Concern for congenital heart block/neonatal lupus, photosensitivity, cutaneous vasculitis (palpable purpura), interstitial lung disease

Anti-SS-B (La)

- Helpful in diagnosing Sjögren's and SLE, but not specific
- Sensitivity
  - 1° Sjogren's: 70-95%
  - 10-35% in SLE
- Unusual to detect SS-B without SS-A. When found, consider PBC or autoimmune hepatitis.

Development of autoantibodies before the clinical onset of systemic lupus erythematosus.

N Engl J Med. 2003 Oct 16;349(16):1526-33

Department of Defense Serum Repository
Serum of 130 persons before SLE dx (matched controls)

Results-115/130---at least one autoantibody before SLE dx (up to 9.4 yrs, mean 3.3 yrs)

-ANA-78% (dilution of ≥1:120)
-dsDNA-55%
-SS-A-47%
-SS-B-34%
-Antiphospholipid ab-18%

3.4 yrs before dx

-Sm-32%
-RNP-26%

1.2 yrs before dx

Control group---3.8% (+) for one or more autoantibody

CONCLUSION- "Autoantibodies, are typically present many years before the diagnosis of SLE…"

See also---Heinlen, LD, McClain, MT, Merrill, J, et al. Clinical criteria for systemic lupus erythematosus precede diagnosis, and associated autoantibodies are present before clinical symptoms. Arthritis Rheum 2007; 56:2344.

Complement and Acute Phase Reactants

- Complement
  - C3 and C4 levels are decreased in active disease
- ESR and CRP
  - Elevated in many SLE patients, but may have normal CRP levels
  - Elevated CRP may be due to infection or active SLE

Other Tests to consider with SLE

- Antiphospholipid antibodies can be positive
  - Idiopathic or in patients with SLE
- CBC
- Serum chemistries
- Urinalysis

Rheumatology Testing Checklist

Questions to ask when ordering lab tests for a patient with suspected rheumatologic disorder

- What is the patient's clinical picture?
- What previous testing has been done?
- How much time has elapsed since last test?
- How will results change outcome?
- What is the benefit to the patient?

How do you communicate lab results to patients?

-Phone call

-Letter

-Discuss at next follow-up visit

-No communication

Frequency of Failure to Inform Patients of Clinically Significant Outpatient Test Results

Arch Intern Med. 2009;169(12):1123-1129.

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B. Reduced erythrocyte sedimentation rate
C. Positive antinuclear antibody titer
D. Negative ENA panel

Lessons for Practice

-The appropriate use of laboratory diagnostics is vital when caring for those with rheumatic disease.

-ANAs, RFs, and CCPs are helpful diagnostic laboratory, but are not used to measure disease activity.

-The immunofluorescence ANA test is the gold standard for ANA testing.

-When ordering a rheumatoid factor, consider also ordering a CCP.


Scl70
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The ACR's *Simple Tasks* Campaign

www.SimpleTasks.org

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