The black hole of medical knowledge: An internist’s view of rheumatologic lab tests

The ABIM’s view of rheumatologic lab testing

Demystifying Rheumatology Lab Tests

• Understand basic principles of how given test is performed
  – What type of test is it?
  – What does the test measure?
  – What are the test’s limitations?

• Know the patients being tested
  – Pretest likelihood that they have disease for which they are being tested?


**Sedimentation Rate**

Sample question: What is the highest Erythrocyte sedimentation rate ever recorded?

a) 100  
b) 200  
c) 400  
d) I have no Idea!!!!!

Answer:  
Technically speaking: 200 MM/hr  
Practically speaking: About 150

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**ESR: Technique**

- Aspirating the diluted EDTA-blood (in citrate) to the 200 mm mark of a Westergren tube
- Placing the tube in a vertical position in a Westergren rack in a location that is free of vibration and that is not exposed to direct sunlight.
- After exactly one hour, reading the distance the erythrocytes have fallen.

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**What does an ESR Measure?**

- Measures Acute Phase Proteins  
  - Fibrinogen most common  
  - Produced in liver as part of an inflammatory response under control of cytokines like IL-6, IL-1, TNF
- RBC’s serve as proxy for fibrinogen levels  
  - Fibrinogen interacts with RBC to make them sediment faster
- Many other factors that affect serum fibrinogen levels or RBC morphology can affect the ESR
Causes of Elevated ESR’s

- Pregnancy (increased Fib levels)
- Anemia (Plasma counter flow altered)
- Macrocytosis (cells fall faster)
- Diabetes
- End Stage Renal Failure
- Malignancy
- Infections
- Autoimmune inflammatory diseases
  - Especially Vasculitis, PMR, RA

ESR - Tidbits

- Women generally have slightly higher ESRs then Men
- ESRs rise with age: \( \text{ESR} \leq \frac{\text{Age}}{2} + 5 \) in women
- ESRs can be affected by room temperature and laboratory technique
- Although ESRs are non-specific…..
  - ESRs part of diagnostic criteria for Polymyalgia Rheumatica & Giant Cell Arteritis
  - ESRs can be useful in following disease activity or response to therapy for rheumatoid arthritis and osteomyelitis

C Reactive Protein

- What is it?
  - Acute phase protein produced by the liver
- How is it measured?
  - Directly via an ELISA or nephelometrey (unlike ESR)
- Advantages
  - Rises and falls more rapidly in association with acute phase response
  - Not affected by anemia, renal failure, or other conditions that affect ESR
  - Unclear if always more sensitive than ESR for various CVD's

Measuring the Acute Phase Response Directly
Timing of CRP vs. ESR Response

Comparison Between ESR & CRP

<table>
<thead>
<tr>
<th>Results affected by</th>
<th>ESR</th>
<th>CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Age</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Temperature</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Drugs (e.g., steroids, salicylates)</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Smoking</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

- CRP and ESR measure somewhat different aspects of inflammatory response.
- They usually but not always correlate with each other.

Autoantibodies: Target self-antigens
Self Antigens: Components of cells

Examples of Autoantibodies

- Plasma Membrane
- Antiphospholipid
- Cytoplasm
- Antimitochondrial
- Nucleolus
- Anti Topoisomerase I
- Neutrophilic Cytoplasm
- Anti Pr3 (ANCA)
- Nucleus
- Anti dsDNA

Complex Organelles
1,000's of proteins
Complex Ribonuclear Proteins
Nucleic Acids
Phospholipids
What is an Anti-Nuclear Antibody?

- Autoabs directed specifically against intra-nuclear antigens
- Most commonly (not always) detected by immunofluorescence on intact cells
- If an ANA is detected, the specific antigen may or may not be known (most ANA’s aren’t known – only detected by fluorescence inside of an intact nucleus)
- When an ANA screen is positive, one then uses more specific tests against known antigens to determine if that ANA is relevant to medical disease (Subserology)

How is an ANA Performed??

- Hep-2 cells fixed to slide & permeabized
- Incubated in patient serum
- Washed vigorously to remove serum
- Fluorescently labeled Anti-hum Ig secondary Ab
- Wash again
- Detect florescence of bound secondary Ab

ANA Patterns

- Depends upon what molecule(s) are recognized by patient antibodies
  - DNA is homogeneously distributed
  - Centromeres seen in dividing cells
  - Extractable nuclear antigens are speckled throughout cell

ANA Patterns: Homogenous
Testing for Anti-Nuclear Abs

- General screening test for antibodies against most nuclear antigens
- Most of the other specific antibody tests for SLE are test for ANA’s
- If ANA negative, with few exceptions (SSA), No need to test for other antibodies
- Newest generation of IIF ANA’s, use human cell lines, are 95-99% sensitive for SLE
- ANA negative SLE is rare

More ANA Facts

- ANA is not nearly as specific for SLE as it is sensitive
  - Autoimmune thyroid disease
  - Other Collagen-Vascular diseases (>90% of SSc)
  - Medications
  - Malignancies
  - Infections (viral)
  - Normal people (especially low titers)
Antinuclear Antibodies and SLE

- Only one of eleven ACR classification criteria for SLE
  - 2/11 criteria .....................50% Specificity
  - 3/11 criteria .....................75% Specificity
  - 4/11 criteria .....................95% Specificity

- When working up SLE, the ANA should only be ordered with good pretest, clinical suspicion for SLE
  - In a patient with arthritis, ANA is no better than coin flip

- If ANA negative, no need to check ANA “panel.”

When the ANA is Positive

- Further differentiating the specific target may be of use, in the right clinical context

- Most tests/sub-serologies are done by specific ELISA or immunoblot
  - Patient serum is incubated with target antigen
  - Antibodies remaining bound to the target antigen are detected with labeled antisera

- If detected, the specific target of the ANA, with the right clinical picture, can help clarify a diagnosis and/or serve a predictive role

Homogeneous Patterns: Anti-dsDNA Abs

- 50-60% sensitive for SLE
- 90-95% specific for SLE
- 1/11 SLE “criteria”
- Presence and titer can correlate with renal/systemic disease flares
- Possible direct implication in GN

ABIM Choosing Wisely Campaign 2013
http://www.choosingwisely.org/

- “An initiative of the ABIM Foundation…specialty societies have created lists of “Things Physicians and Patients Should Question” — evidence-based recommendations that should be discussed to help make wise decisions about the most appropriate care based on a patients’ individual situation.”
Anti-Histone Antibodies (Histones are bound to DNA)

- Directed against one or more proteins or protein-DNA complexes in nucleosome (histone + dsDNA)
- Can be seen in SLE and Drug-induced LE
  - Not specific for Drug-LE
  - Very Sensitive (practically required to even consider the diagnosis of drug-induced LE)
    - Strong negative predictive value (not positive)
    - Can be seen with or without disease, with other diseases (SLE)
    - 95% cases of procainamide LE
    - Hydralazine, INH, Aldomet, Dilantin, Tegretol

Speckled: Extractable Nuclear Antigens

- Acid extractable nuclear antigens
  - U1SNRnP
    - Anti-Smith
    - Anti-RNP
  - SSA (RO)
  - SSB (La)

U1snRNP Particle

- Complex macromolecule of RNA and proteins
- Includes target sites for both anti-Smith and anti-RNP Abs
- Helps explain why many SLE patients have antibodies to both Smith and RNP

Anti-Smith Antibodies

- Poor sensitivity for SLE (20-30%)
- Very high Specificity for SLE (95-99%)
- May identify a subset of patients with more severe disease and/or renal involvement
**Anti-RNP Antibodies**

- 100% sensitivity for patients with MCTD (diagnostic criterion)
- 40-60% patients with SLE
  - More raynaud’s phenomenon, less renal involvement, “less severe disease”
  - More interstitial lung disease
  - Features of myositis, scleroderma, and arthritis

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**Anti-SSA (Ro) and SSB (La)**

**Key Associations You Have to Know**

- Sjogren’s syndome
  - 88-96% of patients with primary SS have SSA
  - 70-80% with primary SS have SSB
  - Much lower percentage for secondary SS pts.
  - Primary SS usually dual Ab positive
    - Increased incidence of vasculitis, purpura, lymphoma, etc...
- Associated with neonatal lupus
  - Implicated in pathogenesis, although not only factor
  - Mothers with SLE, Sjogren’s, or asymptomatic
  - Rash and congenital heart block

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**Anti-Ro (SSA) Skin Disease**

Subacute cutaneous lupus erythematosus

- Papulosquamous
- Annular

*Courtesy ACR Image Bank*

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**Anti-Centromere Antibodies**

- Newer ANA assays use a cell line that rapidly divides
- ANA’s may recognize components of mitotic spindle
- Most IIF can detect Anti-Centromere Abs now
- Any doubt, order specific ELISA
Anti-Centromere Antibodies

- Classically associated with Limited Scleroderma “CREST” syndrome (60-80%)
- Diagnostic/prognostic significance:
  - Isolated Raynaud’s 25% (? Predict future?)
  - Pulmonary HTN
  - Incomplete “CREST” features
  - Progressive Systemic Sclerosis continuum

Nucleolar ANA’s: Systemic Sclerosis and Idiopathic Inflammatory Myopathies

- ANA is also 90-95 % sensitive for progressive systemic sclerosis
  - Not just a test for SLE
  - ANA negative PSS is relatively rare
- Anti-Topoisomerase I (SCL-70)
  - 25% sensitivity
  - 90+% specificity
  - Risk for more sub-acute, progressive, and systemic organ involvement (renal crisis, ILD, GI)

Not all antigens are in the Nucleus

- Sometimes IIF fails to stain the nucleus, but stains the cytoplasm instead
- TRNA synthetases: Most clinically relevant myositis
  - Jo-1 (histadyl TRNA synthetase)
  - Anti Jo-1 syndrome
  - Myositis, Raynaud’s, Arthritis, ILD (prognosis)
- Mitochondria (primary biliary cirrhosis)

Features of Anti-Jo1 (synthetase) Syndrome

- 20 TRNA synthetases: each charges a separate TRNA molecule with a specific amino acid for its RNA codon
  - Jo-1 (histadyl TRNA synthetase)
  - Anti Jo-1 syndrome
  - Myositis, Raynaud’s, Arthritis, ILD (prognosis)
  - Mitochondria (primary biliary cirrhosis)
Growing list of Anti-synthetase antibody associated diseases

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Serum anti-</th>
<th>Patients</th>
<th>Age</th>
<th>Female</th>
<th>Lung Involvement</th>
<th>Myositis</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-PL-12</td>
<td>Actin</td>
<td>52</td>
<td>56</td>
<td>96</td>
<td>60</td>
<td>52</td>
<td>56</td>
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<tr>
<td>Anti-PL-12</td>
<td>Alkaline</td>
<td>47</td>
<td>63</td>
<td>84</td>
<td>84</td>
<td>47</td>
<td>63</td>
</tr>
<tr>
<td>Anti-O2</td>
<td>Uroplakin</td>
<td>53</td>
<td>67</td>
<td>56</td>
<td>100</td>
<td>53</td>
<td>67</td>
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<tr>
<td>Anti-O2</td>
<td>Aspartate</td>
<td>51</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>51</td>
<td>100</td>
</tr>
<tr>
<td>Anti-O2</td>
<td>Glycine</td>
<td>57</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>57</td>
<td>100</td>
</tr>
<tr>
<td>Anti-20</td>
<td>Thymoparin</td>
<td>49</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>49</td>
<td>100</td>
</tr>
</tbody>
</table>

Rheumatoid Factor

- Usually IgM directed against Fc domain of one’s own IgG
- RF may be natural response of body to downregulate normal antibody responses
- Usually low titer, low affinity, more transient antibodies

Rheumatoid Factor – but not RA!

- In collagen vascular disease and RA, higher titer RF with stronger affinity for IgG
- Present in 1-5% of normal population
- Incidence increases with age – 10% prevalence over age 65
- Association with rheumatoid arthritis, but hardly exclusive to RA

Rheumatoid Factor – but not RA!

- Collagen Vascular Diseases Preval.
  - Sjogren’s Syndrome 20-30%
  - SLE 15-35%
  - Cryoglobulinemia 40-100%
  - Scleroderma 20-30%
  - Poly/Dermatomyositis 5-10%

All rheumatoid factor positive arthritis is NOT rheumatoid arthritis!!!
Rheumatoid Factor and Non-Rheumatic Diseases

- Overriding principle: Chronic antigenemia can lead to rheumatoid factor production

- Chronic Infections
  - Chronic infections elicit chronic immune responses, including rheumatoid factor
  - Includes: hepatitis C, endocarditis, osteomyelitis, syphilis
  - Antigen-antibody complexes usually contain RF

- IPF, cirrhosis, sarcoid, etc…

Rheumatoid Factor and RA

- Prevalence increases with disease duration
  - 33-50% positive at disease onset
  - 75% positive after one year
  - 80+% positive at 18 months

- Prognostic significance
  - More severe, erosive, difficult to treat, and extraarticular disease
  - Titers not usually followed (only occasionally correlate with dz activity)

Anti-CCP antibodies

- Originally identified over 40 years ago

- Recognized by indirect immunoflorescence on epidermal (skin) cells

- Target later identified as filaggrin:
  - Filaggrin: form of keratin where the amino acid arginine has been modified into citrulline
  - 1990's-2000: Recognized that RA patients make antibodies not only to filaggrin, but to many proteins that contain citrulline
  - Specific target of anti-citrullinated protein antibodies is not known

Citrulline

- Not one of 20 AA’s coded for in DNA/RNA

- Mamalians possess Peptidyl Arginine Deiminase that can convert arginine residues within proteins to citrulline.

- May create new targets for autoimmunity
**RA-associated autoantibodies that recognize peptides containing citrulline**

<table>
<thead>
<tr>
<th>Peptide sequence</th>
<th>Antibody recognition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESSRDGSRHPRSHD</td>
<td>No</td>
</tr>
<tr>
<td>ESSRDGScitHPRSHD</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Actual inciting citrullinated antigen is not known

**Anti-CCP antibodies**

- CCP test is an artificial proxy for a family of antibodies recognizing citrullinated proteins
- Diagnostic ELISA tests created using synthetic citrulline containing peptides
  - Different diagnostic companies market their own proprietary “cocktail” of artificial CCPs
- Have greatest sensitivity and specificity if synthetic peptides are artificially circularized

**Anti-CCP Antibodies and RA**

- 70-80% Sensitivity for RA
- 90-95% Specificity for RA
- Help distinguish RA from other diseases
- Presence can antedate and predict development of disease in undifferentiated individuals
- Highly correlated with more severe and erosive disease

**Diagnosis of early RA by ACR criteria**
van Gaalen et al Arh Rheum 50: 709, 2004

936 patients with early inflammatory arthritis

<table>
<thead>
<tr>
<th>Initial evaluation</th>
<th>After 3 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>936</td>
<td></td>
</tr>
<tr>
<td>205 RA by ACR criteria</td>
<td></td>
</tr>
<tr>
<td>318 “undifferentiated arthritis”</td>
<td>127 RA</td>
</tr>
<tr>
<td>413 other diagnoses</td>
<td></td>
</tr>
</tbody>
</table>
Factors predictive of progression from undifferentiated arthritis to RA
van Gaalen et al Arth Rheum 50: 709, 2004

<table>
<thead>
<tr>
<th>At initial evaluation</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive rheumatoid factor</td>
<td>1.7 (0.5-5.6)</td>
</tr>
<tr>
<td>Positive anti-CCP antibody</td>
<td>38.6 (9.9-151.0)</td>
</tr>
</tbody>
</table>

Anti-Neutrophil Cytoplasmic Antibodies (ANCA)

- Antibodies directed against neutrophilic antigens
  - Neutrophils chock full of proteases
- Detected by immunoflorescence performed on neutrophils fixed to slide
  - Analogous to ANA’s, except uses a neutrophil cell line
  - Detects neutrophil-specific antigens in cytoplasm
- Unknown if ANCA are directly pathogenic or epiphenomenon of disease

ANCA Immunoflorescence Patterns

- C-ANCA
  - Associated most commonly with Granulomatosis with Polyangiitis (Wegeners)
  - >90% sensitive and specific
  - Titors may correlate with disease, but unreliable
- P-ANCA
  - Microscopic Polyangiitis
  - Churg-Strauss
  - IBD
  - Drugs
  - Not commonly PAN

Specific Targets of ANCAs

- We now know specific antigens that stain in these patterns and how they correlate with specific diseases
  - C-ANCA is Proteinase 3 and correlates with WG
  - P-ANCA is usually myeloperoxidase and correlates with MPA
  - Some P-ANCA not MPO = “typical”
  - Although one can order ELISAs directly, some advocate ordering both IIF and ELISAs to enhance sensitivity, specificity and interpretation of all ANCAs
Predictive Value and Testing for Rheumatic Diseases

- Sensitivity and Specificity are intrinsic properties of a given diagnostic test
  - Never changes depending upon who/what is being screened
  - ANCA’s revolutionized diagnosis of vasculitis because of their high sensitivity and specificity
  - Also created huge problems in interpretation
    - VERY POOR PREDICTIVE VALUE!!!!!!

Predictive Value

- Describes the usefulness of a positive or negative test in ruling in or out a given disease
- Takes into account not only test’s sensitivity and specificity, but also disease prevalence
- PPV = True positives/Total positives
- Total positives = True Pos + FALSE POS!!

False positives are a problem when testing for rare diseases

- For a given rare disease that affects less than 0.01% general population (100/1,000,000)
- Even with an ANCA-like specificity of 95%, if 1,000,000 people were randomly screened, 5% false positives = 50,000/1,000,000
- True positives = 100, False positives = 50,000
- With a test that is 100% sensitive, 95% specific, PPV only roughly 100/50,100 = 0.2%!!!!

Take Home Message

- Rheumatic diseases are uncommon
- Even with very good testing, must know your patients in whom you order these tests (what is pre-test likelihood of disease?)
- Must know the qualities of test being performed
- ANA general screening test
  - If negative, with few exceptions, no need to hunt for other specific sub-serologies