Testing for Rheumatologic Diseases: Finding The Signal Through The Noise

Ari Weinreb M.D./Ph.D.
Associate Chief of Rheumatology, VAGLAHS
Associate Professor of Medicine
David Geffen School of Medicine at UCLA
When a patient with arthritis comes in my front door, I try to go out the back door.

-Sir William Osler
Topics to Cover

1. Autoimmunity
   - How does the development of autoimmunity relate to immunologic test interpretation?

2. Bedside Test Statistics
   - Why is it important to know what you are really testing for?

3. Rheumatoid Factor (RF) and Anti-Cyclic Citrullinated Peptide Antibody (ACPA)
   - Is that joint pain really due to rheumatoid arthritis?

4. Antinuclear Antibody (ANA)
   - It must be lupus, SSRD (Some Sort of Rheumatologic Disease), or maybe something else?
The Immune System

- **Innate immunity**
  - Microbe
  - Epithelial barriers
  - Phagocytes
  - Complement
  - NK cells
  - **Hours:**
    - 0, 6, 12

- **Adaptive immunity**
  - B lymphocytes
  - Antibodies
  - T lymphocytes
  - Effector T cells
  - **Days:**
    - 1, 3, 5
  - **Time after infection**:

A non-specific and rapid 1° line of defense; effector of autoimmunity (and autoinflammatory diseases)

Specific recognition of antigens by antibodies and T-cell receptors confer a more specific and effective 2° line of defense; specific self-reactive antibodies and T-cells define the presence of an autoimmune process.
Adaptive Immunity: Immunoglobulins and T-Cell Receptors

- **diversity** of the adaptive immune response is determined by the **variable regions** of the immunoglobulin and T-cell receptor molecules.

- **constant regions** determine other antibody and T-cell receptor related functions.
Adaptive Immunity: Diversity

V, D, J Chromosomal Gene Rearrangement
-occurs during development of the immune system

-creates the diversity of the variable region antigen binding sites of antibodies (IGs) and T cell receptors

-random process
-generates diverse B cell and T cell clones with antibody molecules and T-cell receptors reactive to both foreign antigens and self-antigens
Tolerance vs. Autoimmunity

-tolerance
- elimination or inactivation of autoreactive lymphocytes
- not perfect: a low level of non-pathogenic autoreactive lymphocytes persist
- possible physiologic role for autoreactivity (e.g. clearance of cellular debris and immune complexes)

-autoimmunity
- results from failure of tolerance mechanisms and activation, expansion, and evolution of pathologic autoreactive lymphocytes
Autoimmunity: The Current Pathophysiologic Model

Genetics: predisposing alleles that alter cytokines, signaling apoptosis, and other immune related functions

Environment: cross-reactive infectious antigens or exposure of hidden self-antigens

Altered Immune Responses: stochastic or random immune system responses to the various antigens, cytokines, and other signals create an inflammatory state

Autoimmunity: self-reactive lymphocytes in the setting of autoimmune driving genes and the appropriate inflammatory environment result in autoimmunity
Autoimmune disease development results from the interaction of an individual’s genetics, environmental exposures, and the unpredictable response of the immune system. We are still at the early stage identifying these factors and how they interact.
How Does Autoimmunity Present Clinically in a Patient?

- **serum autoimmunity**
  - presence of autoantibodies with or without clinical findings (clinical testing for autoreactive T-cells not available)

- **clinical autoimmunity**
  - non-specific constitutional symptoms, single sites of inflammation, and/or systemic inflammation (multiple organ systems)
  - caveat: nonautoimmune processes can stimulate an inflammatory response (injury, infection, neoplasm, etc.)

- **ROS:** disease specific autoimmune findings are most helpful in determining the presence of an autoimmune process
  - e.g. palpable purpura, mucocutaneous ulcers, pleuritic chest pain, hemoptysis, hematuria/proteinuria, inflammatory arthritis, hemolytic anemia, etc.)
Statistics at the Bedside
Types of Tests

- assessment of organ function
- diagnosis of a disease
- screening for a disease
- prognostication
- assessing disease activity
- disease risk determination

The reason for performing a test impacts on the interpretation of the results.
Some Issues With Rheumatologic Tests That Complicate Interpretation

1. Crossreactivity
   - rheumatologic and non-rheumatologic conditions can share positive autoantibody reactivities:
     autoantibody tests should not the sole means of making a diagnosis

2. Interlaboratory Variability
   - variability in reagents, assay methods, measurement, and interpretation:
     may make low titer positive results less certain

3. Bias and Generalizability
   - the population characteristics used to design a test may differ from the actual test population
     know who you are testing
Test Cutoffs and Overlap: FPs and FNs

Cutoff Value for Test: determined by the desired sensitivity and specificity for the test.
(< value=normal, > value=abnormal)

- overlap of test results in healthy and disease populations leads to FP and FN results
- the more tests ordered, the more likely a positive result will occur by chance (=FP).

false positives (FP, healthy, but test positive)
false negatives (FN, with disease, but test negative)

Prevalence =

individuals who are healthy
individuals with disease
## Sensitivity, Specificity, and Prevalence

<table>
<thead>
<tr>
<th></th>
<th>Disease Present</th>
<th>Disease Absent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Positive</td>
<td>a</td>
<td>b (FP)</td>
<td>a+b</td>
</tr>
<tr>
<td>Test Negative</td>
<td>c (FN)</td>
<td>d</td>
<td>c+d</td>
</tr>
<tr>
<td>Total</td>
<td>a+c</td>
<td>b+d</td>
<td>a+b+c+d</td>
</tr>
</tbody>
</table>

**Sensitivity:** percent of patients with disease who are correctly identified with a positive test result = \( \frac{a}{a+c} \)

**Specificity:** percent of patients without disease who are correctly identified by a negative test result = \( \frac{d}{b+d} \)

**Disease Prevalence:** percent of population with the disease = \( \frac{a+c}{a+b+c+d} \times 100 \)

### The Key Points on Sensitivity and Specificity:
1. Determined by the test: population, methodology, cutoffs
2. Not affected by disease prevalence/pretest probability
3. Do not predict disease given a test result.
# Positive and Negative Predictive Values (PPV and NPV)

<table>
<thead>
<tr>
<th></th>
<th>Disease Present</th>
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<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test Positive</strong></td>
<td>a</td>
<td>b</td>
<td>a+b</td>
</tr>
<tr>
<td><strong>Test Negative</strong></td>
<td>c</td>
<td>d</td>
<td>c+d</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>a+c</td>
<td>b+d</td>
<td>a+b+c+d</td>
</tr>
</tbody>
</table>

**Positive Predictive Value:** % of patients with a positive test with the disease = \( \frac{a}{a+b} \)

**Negative Predictive Value:** % of patients with a negative test result without the disease = \( \frac{d}{c+d} \)

## The Key Points to Know
1. determined by the sensitivity, specificity, and disease prevalence/pretest probability
2. addresses the clinical question for which the test was ordered
What Effect Does Disease Prevalence/Pretest Probability Have on the PPV and NPV Of The Test?

### Uncommon Disease: Low Pretest Probability (0-20%)

<table>
<thead>
<tr>
<th></th>
<th>Disease Present</th>
<th>Disease Absent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test Positive</strong></td>
<td>90</td>
<td>90</td>
<td>180</td>
</tr>
<tr>
<td><strong>Test Negative</strong></td>
<td>10</td>
<td>810</td>
<td>820</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
<td>900</td>
<td>1000</td>
</tr>
</tbody>
</table>

Population Size=1000
Sensitivity=90%
Specificity=90%
Prevalence/Pretest Probability=10%

Positive Predictive Value=90/180x100=50%
Negative Predictive Value=810/820x100=98.8%
FPR=90/180x100=50%

### More Common Disease: Midrange Pretest Probability (21-70%)

<table>
<thead>
<tr>
<th></th>
<th>Disease Present</th>
<th>Disease Absent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test Positive</strong></td>
<td>450</td>
<td>50</td>
<td>500</td>
</tr>
<tr>
<td><strong>Test Negative</strong></td>
<td>50</td>
<td>450</td>
<td>500</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>500</td>
<td>500</td>
<td>1000</td>
</tr>
</tbody>
</table>

Population Size=1000
Sensitivity=90%
Specificity=90%
Prevalence/Pretest Probability=50%

Positive Predictive Value=450/500x100=90%
Negative Predictive Value=450/500x100=90%
FPR-50/500x100=10%

### Very Common Disease: High Pretest Probability (71-100%)

<table>
<thead>
<tr>
<th></th>
<th>Disease Present</th>
<th>Disease Absent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test Positive</strong></td>
<td>720</td>
<td>20</td>
<td>740</td>
</tr>
<tr>
<td><strong>Test Negative</strong></td>
<td>80</td>
<td>180</td>
<td>260</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>800</td>
<td>200</td>
<td>1000</td>
</tr>
</tbody>
</table>

Population Size=1000
Sensitivity=90%
Specificity=90%
Prevalence/Pretest Probability=80%

Positive Predictive Value=720/740x100=97.3%
Negative Predictive Value=180/260x100=69.2%
FPR=20/740x100=2.7%

### The Key Points to Know:
1. The pretest probability determines the strength of the test result in ruling in or ruling out the disease
2. The history and exam are the most important determinants of the pretest probability
Rheumatologic Disease Prevalences

**Rheumatoid Arthritis:**
- Caucasians: ~1%
- Rural Africans: 0.01%
- Pima, Blackfeet, and Chippewa Indians: 5%

**Lupus (U.S.):**
- Overall: 0.02-0.15%
- Women: white- 0.164% AA- 0.406%
- Increased prevalence in Asian, African-American, African-Caribbean, Hispanic American

**Sjogren’s:**
- 0.01-0.09%

**Systemic Sclerosis:**
- North American- 0.0276-0.0443%
- Global- 0.0038-0.066%

**Psoriatic Arthritis:**
- Overall- 0.3-1%
- With psoriasis- 7-42%

**AAV:**
- GPA- 0.0065-0.016%
- MPA- 0.0039-0.0094%
- EGPA- 0.0011-0.0046%

**Gout:**
- US- 2007-2008: 3.9% (8.3 million), >60 yrs 9.3% (4.7 million)
- UK- 1.4%
- Taiwan- 3.4%

Example of the problem of “screening” for rheumatologic diseases with serologic tests:

For an AA woman w/o symptoms on screening for SLE with a +ANA (sens 90%, spec 90%), the PPV would be 3.54%
**Bayes' post-test probability calculator**

By Keiji Matsui

This app is only available on the App Store for iOS devices.

**Description**

Bayes' post-test probability calculator, an iPhone / iPod touch application, calculates the post-test probability of diseases from pre-test probability and sensitivity of the test and specificity of the test.

**What's New in Version 3.0**

It can be used on an Apple Watch.

**Screenshots**

- Bayes' post-test probability
  - pre-test probability: 65%
  - sensitivity: 75%
  - specificity: 85%

**Customer Ratings**

This application hasn't received enough ratings to display a summary.
Arthritis Classification and Patterns of Joint Involvement
modified from John Brown

Is the joint pain articular or periarticular?

- bone, soft tissue, nerve

**Monoarticular (1 joint)**
- septic arthritis
- crystal arthropathy
- other monoarthritis

**Polyarticular (>1 joint)**

**Autoimmune +/- Systemic Inflammation**

**Symmetric/Small Joint**
- rheumatoid arthritis
- SLE
- systemic sclerosis/scleroderma
- Sjogren's syndrome
- other collagen-vascular diseases

**Asymmetric/Large Joint**
- seronegative spondyloartropathy (oligoarticular, 2-4 joints):
  - ankylosing spondylitis
  - reactive arthritis (Reiter's syndrome)
  - psoriatic arthritis
  - inflammatory bowel disease arthritis

**Nonautoimmune +/- Local Inflammation**
- degenerative/osteoarthritis

Exceptions to keep in mind:
1. early presentations
2. chronic presentations
3. undifferentiated forms
4. variable presentations

RF/anti-CCP
ANA
Diagnosing Rheumatoid Arthritis... and Other Things:

The Rheumatoid Factor (RF) and Anti-Cyclic Citrullinated Peptide Antibody (ACPA)
DEVELOPMENT OF AUTOIMMUNITY AND DISEASE IN RA PATIENTS

Autoimmune disease

Diagnosis by physician

First symptoms recognized by patient

Unspecific arthritis/arthralgia

Autoimmunity

Normal

Time (yr)

### RA Classification Criteria

**Table 3** The 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for RA

<table>
<thead>
<tr>
<th>Target population (Who should be tested?): Patients who</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) have at least 1 joint with definite clinical synovitis (swelling)*</td>
</tr>
<tr>
<td>2) with the synovitis not better explained by another disease†</td>
</tr>
</tbody>
</table>

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)‡

<table>
<thead>
<tr>
<th>A. Joint involvement§</th>
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<tbody>
<tr>
<td>1 large joint¶</td>
</tr>
<tr>
<td>2–10 large joints</td>
</tr>
<tr>
<td>1–3 small joints (with or without involvement of large joints)***</td>
</tr>
<tr>
<td>4–10 small joints (with or without involvement of large joints)</td>
</tr>
<tr>
<td>&gt;10 joints (at least 1 small joint)††</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Serology (at least 1 test result is needed for classification)‡‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative RF and negative ACPA</td>
</tr>
<tr>
<td>Low-positive RF or low-positive ACPA</td>
</tr>
<tr>
<td>High-positive RF or high-positive ACPA</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C. Acute-phase reactants (at least 1 test result is needed for classification)§§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal CRP and normal ESR 0</td>
</tr>
<tr>
<td>Abnormal CRP or normal ESR 1</td>
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</table>

<table>
<thead>
<tr>
<th>D. Duration of symptoms¶¶</th>
</tr>
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<tbody>
<tr>
<td>&lt;6 weeks</td>
</tr>
<tr>
<td>≥6 weeks</td>
</tr>
</tbody>
</table>

* | † | ‡ | § | ¶ | †† | §‡ | §§ | ¶¶ |
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<thead>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>≥6/10</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Ann Rheum Dis. 2010. 69:1580.**
-1922
Kurt Meyer:
hemagglutinating factor in human sera that strongly agglutinated sheep RBCs (found in patients with cirrhosis and chronic bronchitis)

-1940
Erik Waaler:
antibody against gamma-globulins that agglutinated antibody sensitized sheep RBCs (Waaler Rose test)

-1948
H. M. Rose:
modified Waaler’s test and identified these agglutinating antibodies in patients with rheumatoid arthritis

-1952
these agglutinating antibodies were named rheumatoid factors due to their association with rheumatoid arthritis
What Is The Rheumatoid Factor (RF)?

-an IgM, IgG, or IgA antibody which binds to the Fc portion of an IgG immunoglobulin; the IgM RFs are clinically measured

-not specific for any condition: reflects a state of chronic immune activation

-an increased RF titer can occur in older individuals, with chronic infection, and in other autoimmune diseases (despite its name, an isolated positive RF is not diagnostic of RA)

-RFs can be detected by agglutination, nephelometry, or ELISA; cost ~$22-$399
# RF Associated Diseases and Conditions

- Chronic immune stimulation is a driver for the production of rheumatoid factor

**-CHRONIC**
- CH: CHronic disease
- R: Rheumatoid arthritis
- O: Other rheumatic diseases
- N: Neoplastic
- I: Infection (esp. chronic infections, HIV)
- C: Cryoglobulinemia (esp. hep C)

<table>
<thead>
<tr>
<th>Infections</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subacute bacterial endocarditis</td>
<td>40%</td>
</tr>
<tr>
<td>Chlamydia pneumoniae infection</td>
<td>8-37%</td>
</tr>
<tr>
<td>Klebsiella pneumoniae infection</td>
<td>15%</td>
</tr>
<tr>
<td>Syphilis primary-tertiary</td>
<td>15%</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>15%</td>
</tr>
<tr>
<td>Coxsackie B virus infection</td>
<td>15%</td>
</tr>
<tr>
<td>Dengue virus infection</td>
<td>10%</td>
</tr>
<tr>
<td>EBV and CMV infections</td>
<td>20%</td>
</tr>
<tr>
<td>Hepatitis A, B and C virus infection</td>
<td>25%</td>
</tr>
<tr>
<td>HCV infection</td>
<td>40-76%</td>
</tr>
<tr>
<td>Herpes virus infection</td>
<td>10-15%</td>
</tr>
<tr>
<td>HIV infection</td>
<td>10-20%</td>
</tr>
<tr>
<td>Measles</td>
<td>8-15%</td>
</tr>
<tr>
<td>Parvovirus infection</td>
<td>10%</td>
</tr>
<tr>
<td>Rubella</td>
<td>15%</td>
</tr>
<tr>
<td>Chagas</td>
<td>15-25%</td>
</tr>
<tr>
<td>Malaria</td>
<td>15-18%</td>
</tr>
<tr>
<td>Onchocerciasis</td>
<td>10%</td>
</tr>
<tr>
<td>Toxoplasmosis</td>
<td>10-12%</td>
</tr>
</tbody>
</table>

## Arthritis
- **Rheumatoid arthritis**: 70-90%
- **Juvenile idiopathic arthritis**: 5%
- **Psoriatic arthritis**: <15%
- **Reactive arthritis**: <5%

## Other connective tissue diseases
- **Primary Sjögren’s syndrome**: 75-95%
- **Mixed connective tissue disease**: 50-60%
- **Systemic lupus erythematosus**: 15-35%
- **Systemic sclerosis**: 20-30%
- **Dermato-/polymyositis**: 20%
- **Systemic vasculitides (panarteritis nodosa, Wegener’s granulomatosis)**: 5-20%

## Other diseases
- **Mixed cryoglobulinemia type II**: 100%
- **Liver cirrhosis**: 25%
- **Primary biliary cirrhosis**: 45-70%
- **Malignancy**: 5-25%
- **After multiple immunisations**: 10-15%
- **Chronic sarcoidosis**: 5-30%

## Healthy 50-year olds
- 5%

## Healthy 70-year olds
- 10-25%

- Interstitial pulmonary fibrosis (10% to 50%)
- Silicosis (30% to 50%)
- Asbestosis (30%)

Adapted from Disease Markers. Ingegnoli F et al. 2013. 35:727.
Some Anti-Cyclic Citrullinated Peptide Antibody (ACPA) History

Anti-CCP/ACPA Assay

-the anti-CCP/ACPA assay detects binding to peptides containing the citrullinated epitope from several different proteins

ACPAs and RA

- Antibodies that recognize a citrullinated epitope on several different proteins
- Epitope generated by posttranslational deamination of arginine residues by the enzyme peptidylarginine deiminase (PAD: 5 human isotypes)

- PAD4 associated with RA susceptibility in certain ethnic groups
- Subset of RA patients with severe disease have activating anti-PAD4 antibodies
- Periodontitis (RA risk factor): The periodontitis associated oral gram negative bacterium *Porphyromonas gingivalis* expresses a unique prokaryotic PAD enzyme
- Smoking (RA risk factor): Associated with increased pulmonary PAD2 and PAD4 expression and protein citrullination in the lung

- Increased PAD4 and citrullinated proteins identified in inflamed synovium
- Cost ~$79-$189
risk of developing RA: Genes, Environment and ACPAs

- The class II HLA-DR shared epitope alleles contribute strongly to the RA ACPA pathogenicity.

- Other risk alleles and environmental factors less strongly contribute to ACPA pathogenicity.

- Less clear what genetic and environmental factors contribute to ACPA formation.

Nature Reviews Immunology. doi:10.1038/nri.2016.124. Published online 5 Dec 2016.
The Role Of ACPAs in RA: The Two Hit Model

1st Hit: protein citrullination at mucosal surfaces in setting of inflammation results in ACPA production

2nd Hit: ACPAs activate macrophages promoting inflammation, activate osteoclasts promoting bony erosions, and target other citrullinated proteins in the joint contributing to tissue damage
The Evolution of ACPA Pathogenicity and the Development of RA

-early ACPAs are not pathogenic

-genetic, environmental, and other immune response factors contribute to changes in the ACPA structure conferring increased pathogenicity

-clinical anti-CCP/ACPA assays only detect their presence and titer

-clinical anti-CCP/ACPA assays do not inform about pathogenicity: Interpretation requires clinical context!
ACPAs and RA Preclinical Autoimmunity

Prior to the development of RA (day 0):
- ACPAs to many different citrullinated proteins are present
- The number and concentration of ACPAs to different citrullinated proteins increases as the time to diagnosis approaches

ACPAs consist of multiple antibodies that specifically recognize several different citrullinated proteins.
ACPA Titer

# of ACPAs

# of Cytokines

ACPAs and Cytokines and RA Progression
# ACPA Prevalence In Other Rheumatologic Conditions

<table>
<thead>
<tr>
<th>Disease</th>
<th>References</th>
<th>Patients (No)</th>
<th>Positive anti-CCP test No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic lupus erythematosus</td>
<td>17, 18, 21, 23, 24, 24, 30, 33, 34, 43, 45, 48, 49, 50, 52</td>
<td>567</td>
<td>49 (9)</td>
</tr>
<tr>
<td>Sjögren’s syndrome</td>
<td>17, 19, 23, 30, 33, 45, 46, 48, 49, 52, 68, 69</td>
<td>521</td>
<td>27 (5)</td>
</tr>
<tr>
<td>Hepatitis C virus</td>
<td>17, 41, 42, 70</td>
<td>219</td>
<td>3 (1)</td>
</tr>
<tr>
<td>Wegener’s granulomatosis</td>
<td>19, 24, 34, 48, 52</td>
<td>67</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Ankylosing spondylitis</td>
<td>18, 21, 23, 24, 43, 45, 48, 50, 52</td>
<td>181</td>
<td>5 (3)</td>
</tr>
<tr>
<td>Psoriatic arthritis</td>
<td>14, 21, 34, 46, 48, 50, 52, 66, 67</td>
<td>424</td>
<td>36 (8)</td>
</tr>
<tr>
<td>Polymyalgia rheumatica</td>
<td>47</td>
<td>49</td>
<td>0</td>
</tr>
<tr>
<td>Palindromic rheumatism</td>
<td>71</td>
<td>63</td>
<td>28 (44)</td>
</tr>
</tbody>
</table>

Diagnosis of RA: PPV and NPV of the RF and ACPA


<table>
<thead>
<tr>
<th>Pre-Test Probability</th>
<th>1%</th>
<th>50%</th>
<th>75%</th>
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</thead>
<tbody>
<tr>
<td><strong>RF</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPV</td>
<td>3.3%</td>
<td>77.4%</td>
<td>91.1%</td>
</tr>
<tr>
<td>NPV</td>
<td>99.6%</td>
<td>69.8%</td>
<td>43.5%</td>
</tr>
<tr>
<td><strong>Anti-CCP2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPV</td>
<td>3.0%</td>
<td>75.5%</td>
<td>90.2%</td>
</tr>
<tr>
<td>NPV</td>
<td>99.9%</td>
<td>95.8%</td>
<td>88.4%</td>
</tr>
</tbody>
</table>
RF and ACPA Positivity Associated with Progression to Clinical RA in Patients with Arthralgia
Increased ACPA and RF Levels Associated with Increased Risk for Progression to RA in Patients with Arthralgia

Fig. 3 Kaplan-Meier plots with (A) ACPA levels in ACPA-positive patients and (B) RF levels in RF-positive patients and risks for progression to clinical arthritis

doi:10.1093/rheumatology/kex340
High Titer RF and ACPA Positivity Associated with an Increased Risk of Erosive RA
Diagnosing SLE...and Other Things:
The Antinuclear Antibody
2019 EULAR/ACR Classification Criteria for SLE

- the diagnosis of SLE is clinical and based upon the history, exam findings, and laboratory results

- SLE is not diagnosed from a single clinical finding or single laboratory test
What Are Antinuclear Antibodies (ANA)?

-Hargraves and Morton 1948: identified the LE cell, a PMN leukocyte with a phagocytosed nucleus found to be increased in lupus patients; subsequently found to be caused by antinuclear antibody binding to nuclei promoting their phagocytosis by PMNs (opsonization)

-the ANA detects antibodies directed against several different antigens in the nucleus

-almost always positive in patients with lupus, but they are not specific for lupus; positive ANAs can be found in many other rheumatologic and non-rheumatologic conditions

LE Cell

phagocytosed nucleus
The Gold Standard of ANA Detection: Direct Immunofluorescence Assay (ANA DIFA)

- ANAs bind to the nucleus and are detected by a labelled anti-Ig antibody
- the titer is the highest serial dilution of serum giving a positive signal
- an abnormal result is usually a titer of $\geq 1:40$ ($\geq 1:80$ by new criteria)
- there are many different antigens in the nucleus that can detect many different ANAs
- cost ~$29-$159
DIFA ANA vs. ELA ANA

-some clinical labs may perform a screening ANA test by an ELISA assay
-although easier and quicker, it is less specific
-it must be followed up by a confirmatory DIFA ANA

ELISA Assay for detecting and measuring specific ANAs:
- screening ANA: each well contains a mixture of nuclear antigens bound to the bottom of the well
- specific ANA testing: an individual specific nuclear antigen is bound to the well
- although sensitive, non-specific binding increases the rate of false positive results (esp. for anti-dsDNA ABs)
ANA Patterns and Specific Antinuclear Antibodies

- different patterns of staining can be associated with different types of ANAs (e.g. homogenous pattern and anti-DNA antibodies, speckled pattern and anti-ribonucleoprotein antibodies)

- specific antinuclear antibodies are associated with certain SLE clinical features and other rheumatologic diseases
Mulitplex Bead Assay for Detection of ANAs

Problems:
- multiplex immunoassays for autoantibodies differ significantly depending on the manufacturer or kits
- do not always show the intended specificity and sensitivity
- lack of standardization of test methodology
- lack of validation against a standard assay for many of these
<table>
<thead>
<tr>
<th>Titer Range</th>
<th>Lab 1</th>
<th>Lab 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>9</td>
<td>78</td>
</tr>
<tr>
<td>$\geq 1:40$ and $&lt; 1:80$</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>$\geq 1:80$ and $&lt; 1:160$</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>$\geq 1:160$ and $&lt; 1:320$</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>$\geq 1:320$ and $&lt; 1:640$</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>$\geq 1:640$ and $&lt; 1:1280$</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>$\geq 1:1280$ and $&lt; 1:2560$</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>$\geq 1:2560$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>101</td>
<td>101</td>
</tr>
</tbody>
</table>

**Individual Patient Serum Samples Tested by Two Different Labs**

ANA results by titer at laboratories 1 and 2. This table demonstrates that in this patient cohort, ANA titers drawn at laboratory 1 were much more likely to be positive.
Some Limitations of using the ANA for the early diagnosis of SLE:
- not everyone with a positive ANA goes on to develop SLE
- early symptoms may not be associated with a positive ANA
- currently no other available tests to better stratify risk of progressing to SLE
Rheumatic Disease ANA Associations

Rheumatic Diseases (sensitivity: % with positive ANA)
- **systemic lupus erthematosus** (99%)
- **drug-induced lupus** (95-100%)
- **scleroderma/systemic sclerosis** (60-80%)
- **mixed connective tissue disease** (100%)
- **polymyositis/dermatomyositis** (61%)
- **Sjogren’s syndrome** (40-70%)
- **rheumatoid arthritis** (30-50%)

highly sensitive for SLE

positive in many other rheumatologic diseases: not very specific for any given rheumatic condition

specificities from 49-92%

Non-Rheumatic ANA Associations

Non-Rheumatic Conditions (further contributing to its non-specificity)

- normal individuals:
  - females>males, increasing age (20% in those >70yrs),
  - healthy relatives of patients with SLE (15-25%),
  - pregnancy

- liver disease

- pulmonary disease

- chronic infections

- hematologic conditions

- malignancies

- other


Healthy ("Normal") Persons

<table>
<thead>
<tr>
<th>Titers</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥1:40</td>
<td>20-30</td>
</tr>
<tr>
<td>≥1:80</td>
<td>10-12</td>
</tr>
<tr>
<td>≥1:160</td>
<td>5</td>
</tr>
<tr>
<td>≥1:320</td>
<td>3</td>
</tr>
</tbody>
</table>

titers tend to be more mildly elevated with non-rheumatic conditions
PPV and NPV of the ANA for SLE

<table>
<thead>
<tr>
<th>Pre-Test Probability*</th>
<th>0.4%</th>
<th>50%</th>
<th>75%</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPV</td>
<td>4.69%</td>
<td>92.50%</td>
<td>97.40%</td>
</tr>
<tr>
<td>NPV</td>
<td>&gt;99.99%</td>
<td>97.87%</td>
<td>93.88%</td>
</tr>
</tbody>
</table>

*for an ANA titer of 1:80, sensitivity of 99% and specificity of 92%

-the high ANA sensitivity for SLE makes a negative result very helpful for ruling out SLE

-the lower ANA specificity for SLE requires a higher pretest probability for it to be helpful in the diagnosis of SLE
### Specific Antinuclear Antibodies

<table>
<thead>
<tr>
<th>Disease</th>
<th>*Anti-dsDNA</th>
<th>Anti-RNP</th>
<th>*Anti-Sm</th>
<th>Anti-SSA</th>
<th>Anti-SSB</th>
<th>Anti-centromere</th>
<th>Anti-SCL70</th>
<th>Anti-Jo1</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE</td>
<td>60%</td>
<td>30%</td>
<td>30%</td>
<td>30%</td>
<td>15%</td>
<td>rare</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RA</td>
<td>-</td>
<td>-</td>
<td>Rare</td>
<td>rare</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MCTD</td>
<td>-</td>
<td>high titer &gt;95%</td>
<td>Rare</td>
<td>rare</td>
<td>rare</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Systemic Sclerosis</td>
<td>-</td>
<td>low titer</td>
<td>Rare</td>
<td>rare</td>
<td>10-15%</td>
<td>20-30%</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Limited Sclerosis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>60-90%</td>
<td>10-15</td>
<td>-</td>
</tr>
<tr>
<td>Sjogren’s Syndrome</td>
<td>-</td>
<td>rare</td>
<td>-</td>
<td>70%</td>
<td>60%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Polymyositis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20-50%</td>
</tr>
</tbody>
</table>

*The anti-dsDNA and anti-Sm antibodies are very specific for SLE (not typically associated with other rheumatologic diseases). That is why they are included in the immunologic criteria in the SLICC and 2019 EULAR/ACR Classification Criteria.*
# Antinuclear Antibody SLE Associations

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Frequency (%)</th>
<th>Clinical Associations</th>
</tr>
</thead>
<tbody>
<tr>
<td>dsDNA</td>
<td>60-90</td>
<td>high sensitivity and specificity; helpful diagnostically for SLE; correlates with disease activity (esp. nephritis)</td>
</tr>
<tr>
<td>ssDNA</td>
<td>90</td>
<td>low specificity, not helpful diagnostically</td>
</tr>
<tr>
<td>Histones</td>
<td>50-70</td>
<td>drug-induced lupus</td>
</tr>
<tr>
<td>Ro(SS-A)</td>
<td>20-60</td>
<td>neonatal lupus (with anti-LA), photosensitivity, subacute cutaneous lupus,</td>
</tr>
<tr>
<td>La(SS-B)</td>
<td>15-40</td>
<td>neonatal lupus (with anti-Ro)</td>
</tr>
<tr>
<td>Sm</td>
<td>10-30</td>
<td>high specificity for SLE (low sensitivity); helpful diagnostically; no SLE associations</td>
</tr>
<tr>
<td>RNP</td>
<td>10-30</td>
<td>no associations with SLE subsets</td>
</tr>
<tr>
<td>ribosomal-P</td>
<td>10-15</td>
<td>specific for SLE; ?association with psychiatric disease, particularly depression</td>
</tr>
<tr>
<td>neuronal (detected in the CSF)</td>
<td>10% SLE w/o neuropsychiatric</td>
<td>? neuropsychiatric lupus</td>
</tr>
<tr>
<td></td>
<td>75% SLE w/ neuropsychiatric</td>
<td></td>
</tr>
<tr>
<td>NMDA receptor</td>
<td></td>
<td>? association with neuropsychiatric lupus</td>
</tr>
</tbody>
</table>
The Clinical Utility of a Positive ANA Test Result: Results of Referrals for Subspecialty Evaluation of a Recently Ordered Positive ANA

- low pretest probability for an autoimmune condition
- ANAs ordered indiscriminately
Autoantibody Testing and Evaluation/Management of Rheumatologic Diseases

- Antibodies may not be pathogenic
- No other lab test available for risk stratification
- Significance of early MRI/US findings - continued interval monitoring recommended

Nature Review Rheumatology.
Take Home Points

1. most rheumatologic autoimmune/inflammatory diseases are uncommon with disease prevalences of ≤1%

2. the RF and ACPA for diagnosing RA and ANA for diagnosing SLE or other autoimmune syndromes are most helpful in the setting of a moderately raised pretest probability
   • they should not be used as screening tests
   • the history and exam should be the prime determinant of the pretest probability
   • how will positive or negative test results change management?

3. multitest rheumatologic panels should not be ordered (↑false pos rate)

4. both the autoantibody and clinical responses can evolve over time: importance of interval follow-up
Medicine is a science of uncertainty and an art of probability.

-Sir William Osler