Autoimmune (AI) Disorders

- Affect up to 50 million people in the U.S.
- 80–100 types, dozens more suspected
- #2 cause of chronic illness

- Women are more likely to be affected than men
- Symptoms overlap and are nonspecific
- Patients with one AI or AI disorder in their family are at higher risk

- Rheumatoid arthritis
- Scleroderma
- Systemic lupus erythematosus
- Sjögren's syndrome
- Systemic sclerosis
- Mixed connective tissue disease
- Polymyositis
- Dermatomyositis

What You Do Matters!

• Diagnosis is often delayed (average 5 years)
  – Delay in presentation to PCPs
  – Non-specific presentations
  – Non-availability of one single diagnostic test
  – Multiple tests combined with clinical findings are required to make a diagnosis

• However, prompt diagnosis and treatment of rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and other autoimmune diseases will lead to improved long-term prognosis

## Conditions Associated with a Positive Antinuclear Antibody (ANA) Test

<table>
<thead>
<tr>
<th>Disease</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic lupus erythematosus</td>
<td>93 – 95</td>
<td>57</td>
</tr>
<tr>
<td>Sjögren's syndrome</td>
<td>48</td>
<td>52</td>
</tr>
<tr>
<td>Systemic sclerosis</td>
<td>85</td>
<td>54</td>
</tr>
<tr>
<td>Juvenile idiopathic arthritis</td>
<td>57</td>
<td>39</td>
</tr>
<tr>
<td>Juvenile idiopathic arthritis with uveitis</td>
<td>80</td>
<td>53</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>41 – 86</td>
<td>56</td>
</tr>
<tr>
<td>Polymyositis/dermatomyositis</td>
<td>61</td>
<td>63</td>
</tr>
<tr>
<td>Drug-induced lupus*</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Mixed connective tissue disease*</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*For both drug-induced lupus and mixed connective tissue disease, the diagnostic criteria require a positive ANA, and therefore specificity and sensitivity cannot be determined.

ANA IFA vs ANA ELISA: Which Lab Test?

**ANA IFA**
- Still considered the gold standard by ACR
- Higher titers are generally associated with greater likelihood of AI disease, but do not reflect disease activity
- When positive, results reported as a titer with a particular type of immunofluorescence pattern
- Different patterns are associated with a variety of autoimmune disorders
- Automated tiered testing possible when positive results obtained

**ANA ELISA**
- More economical
- Allows for large volume of testing
- Less labor-intensive
- Tests for only several biomarkers at a time
- Reports a number for positivity
- Reliability and accuracy system-dependent
- Results in comparison with IFA variable
- Tiered testing possible when positive results obtained

ELISA=enzyme-linked immunosorbent assay; IFA=immunofluorescence. 
ACR Position Statement: Methodologies of Testing for Antinuclear Antibodies

- ACR supports the ANA IFA test using Human Epithelial type 2 (HEp-2) substrate as the gold standard.
- Laboratories should specify the methods utilized for detecting ANAs.
- Laboratories using alternative multiplex platforms or other assays for detecting ANAs must provide requested data that the assay has the same or improved sensitivity compared to ANA IFA.
- In-house assays for detecting ANA as well as anti-DNA, anti-Sm, anti-RNP, anti-Ro/SS-A, anti-La/SS-B, etc, should be standardized according to national (eg, CDC) and/or international (eg, WHO, IUIS) standards.
ANA by Immunofluorescence Antibody: Reporting Titer and Pattern

• ANA titers (generally):
  ▪ <1:40 negative
  ▪ 1:40–1:80 low antibody level
  ▪ >1:80 elevated antibody level

• Any titer above 1:40 along with pattern interpretations is reported

• Patterns aid in differential diagnosis

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Picture</th>
<th>Antibody</th>
<th>Disease State(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rimmed/peripheral</td>
<td><img src="image1.png" alt="Image" /></td>
<td>Anti-DNA</td>
<td>SLE</td>
</tr>
<tr>
<td>Homogenous</td>
<td><img src="image2.png" alt="Image" /></td>
<td>Anti-DNA Anti-histone</td>
<td>RA &amp; SLE Misc. Disorders</td>
</tr>
<tr>
<td>Speckled</td>
<td><img src="image3.png" alt="Image" /></td>
<td>Anti-Sm &amp; RNP Anti-Ro &amp; La Anti-Jo-1 &amp; Mi-2 Anti-Scl-70</td>
<td>SLE &amp; SSc PM/DM</td>
</tr>
<tr>
<td>Centromere</td>
<td><img src="image4.png" alt="Image" /></td>
<td>Anti-centromere</td>
<td>SSc &amp; Sjögren’s</td>
</tr>
<tr>
<td>Nucleolar</td>
<td><img src="image5.png" alt="Image" /></td>
<td>Anti-nucleolar</td>
<td>SLE &amp; SSc</td>
</tr>
</tbody>
</table>

DM=dermatomyositis; lcSSc=limited cutaneous of systemic sclerosis; PM=polymyositis; RA=rheumatoid arthritis; RNP=ribonucleoprotein; SSc=systemic sclerosis; SLE=systemic lupus erythematosus.
A tiered approach to testing may be used to identify autoimmune (AI) disorders when ANA is positive.

<table>
<thead>
<tr>
<th>After Positive ANA</th>
<th>Antibodies tested</th>
<th>Potential Diagnosis when Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tier 1</td>
<td>Chromatin, dsDNA, RNP, Sm, and Sm/RNP</td>
<td>SLE, MCTD</td>
</tr>
<tr>
<td>Tier 2</td>
<td>Jo-1, Scl-70, SS-A, and SS-B</td>
<td>Sjögren’s, SSc, Polymyositis/Anti-synthetase syndrome</td>
</tr>
<tr>
<td>Tier 3</td>
<td>Centromere B and ribosomal P</td>
<td>Limited-cutaneous SSc, Neurologic SLE</td>
</tr>
</tbody>
</table>

MCTD = mixed connective tissue disease.
ANA IFA: Subserologies (cont.)

ANA Screen (IFA)

- **Negative**: Rheumatic disease less likely
- **Positive**: Titer and Pattern
  - Tier 1: Chromatin, dsDNA, RNP, Sm, and Sm/RNP antibodies
## ANA IFA: Subserologies (cont.)

### Tier 1
**Chromatin, dsDNA, RNP, Sm, and Sm/RNP antibodies**

### Tier 2
**Jo-1, Scl-70, SS-A, and SS-B antibodies**

<table>
<thead>
<tr>
<th>Antibody Test</th>
<th>Systemic Lupus Erythematosus</th>
<th>Mixed Connective Tissue Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>dsDNA</td>
<td>+ (high specificity)</td>
<td>–</td>
</tr>
<tr>
<td>Chromatin</td>
<td>+ (high specificity)</td>
<td>–</td>
</tr>
<tr>
<td>Sm</td>
<td>+ (high specificity)</td>
<td>–</td>
</tr>
<tr>
<td>Sm/RNP</td>
<td>+</td>
<td>+ (high titer)</td>
</tr>
<tr>
<td>RNP</td>
<td>+</td>
<td>+ (high titer)</td>
</tr>
</tbody>
</table>
### ANA IFA: Subserologies (cont.)

#### Tier 2

**Jo-1, Scl-70, SS-A, and SS-B antibodies**

#### Tier 3

**Centromere B and ribosomal P antibodies**

<table>
<thead>
<tr>
<th>Antibody Test</th>
<th>Sjögren’s Syndrome</th>
<th>Systemic Scleroderma</th>
<th>Polymyositis</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS-A</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>SS-B</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Scl-70</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Jo-1</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>
No evidence of rheumatic disease shown by analytes tested

Tier 3
Centromere B and ribosomal P antibodies

<table>
<thead>
<tr>
<th>Antibody Test</th>
<th>Limited cutaneous SSc</th>
<th>Neurologic SLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centromere</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Ribosomal P</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
ACR Choosing Wisely Recommendation When Considering ANA Testing

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

- Tests for anti-nuclear antibody (ANA) sub-serologies are usually negative if the ANA is negative
- Broad testing of autoantibodies should be avoided; instead the choice of autoantibodies should be guided by the specific disease under consideration
  - Exceptions include anti-Jo1, which can be positive in some forms of myositis, or occasionally anti-SSA, in the setting of lupus or Sjögren’s syndrome

ACR. Available at: http://www.choosingwisely.org/societies/american-college-of-rheumatology
Potential for Combination Serological Assessment To Improve Diagnostic Utility in Early RA Detection

Early RA (n=99) vs healthy controls (n=189)

Sensitivity ratios for RF, ACPA, and RF and/or ACPA

New Panel for Suspected Rheumatoid Arthritis

RF, CCP & 14-3-3η

- Negative: RA disease less likely
  Consider other rheumatic diseases and test with ANA screen, IFA, reflex titer/pattern and reflex to multiplex 11 Ab cascade (Test code 16814)

- Positive: Consider RA
14-3-3\(\eta\) Is also a Marker for Joint Damage…

- A positive 14-3-3\(\eta\) test and higher titers at baseline indicate high joint damage progression risk 5 years out

- **Persistent negative 14-3-3\(\eta\) values show better outcomes**
  - A higher percentage of patients achieved SDAI remission based on a persistently negative test for 14-3-3\(\eta\)

Testing for Sjögren’s Syndrome

**Diagnostic**
- SS-A (Ro)
  - The main antibody used to aid in diagnosis
- SS-B (La)
  - The significance of a positive SS-B in the setting of a negative SS-A is debated
- Rheumatoid factor and ANA
  - Each may be positive in about ½ of Sjögren's patients
  - Having both +ANA ≥1:320 and a +RF may be used to aid in diagnosis

**Additional testing**
- Lip biopsy
- Ocular staining for integrity of tear film
- Schirmer test
Testing for Systemic Lupus Erythematosus (SLE)

**Diagnostic**
- An ANA is reported to be “positive”
  - Titer = 1:320
  - Pattern = Homogeneous or speckled
- Specific antibodies-SS-A/SS-B; Smith (Sm); anti-DNA (dsDNA); anti-chromatin
- Antiphospholipid antibody positivity
- Complement levels (C3/C4/CH50)
- Complete blood count with differential & platelets
- Creatinine & urinalysis
- Erythrocyte sedimentation rate/C-reactive protein (ESR/CRP)

**Disease Activity**
- Anti-native DNA antibody (dsDNA)
- C3/C4
- CBC w/ diff & platelets
- Creatinine
- ESR/CRP
- Urinalysis, urine protein/creatinine ratio

**ANA:** Once this is positive, there is no need to repeat. Continue to treat or refer to a Rheumatologist.
# Drugs Implicated in the Development of Drug-induced SLE

<table>
<thead>
<tr>
<th>Definite</th>
<th>Probable</th>
<th>Possible</th>
<th>Case Reports</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydralazine</td>
<td>Sulfasalazine</td>
<td>Antibiotics</td>
<td>Infliximab</td>
</tr>
<tr>
<td>Procainamide</td>
<td>Antithyroid</td>
<td>Ciprofloxacin</td>
<td>Etanercept</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>Anticonvulsants</td>
<td>Penicillin</td>
<td>Interleukin-2</td>
</tr>
<tr>
<td>Metyldopa</td>
<td>Ethosuximide, Phenytoin</td>
<td>Tetracycline</td>
<td>Zafirlukast</td>
</tr>
<tr>
<td>Quinidine</td>
<td>Primidone, Valproate</td>
<td>Nitrofurantoin</td>
<td>Clobazam</td>
</tr>
<tr>
<td>Minocycline</td>
<td>Zonisamide, Carbamazepine</td>
<td>Cefepime</td>
<td>Tocainide</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td></td>
<td>Cefuroxime</td>
<td>Lisinopril</td>
</tr>
<tr>
<td></td>
<td>Statins</td>
<td>NSAIDS</td>
<td>Bupropion</td>
</tr>
<tr>
<td></td>
<td>Lovastatin, Simvastatin</td>
<td>Ibuprofen</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fluvastatin, Pravastatin</td>
<td>Diclofenac</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Atorvastatin</td>
<td>Miscellaneous</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Terbinafine</td>
<td>Lithium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Penicillamine</td>
<td>Interferons</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fluorouracil agents</td>
<td>Gold salts</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hydrochlorothiazide</td>
<td></td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

Cessation of offending therapy offers the best outcome


Systemic Sclerosis – Diffuse Cutaneous

- Skin tightness proximal to elbows or knees often with truncal involvement
- “Salt and pepper” pigment changes
- Scl-70 positive
- Pulmonary fibrosis
  - Secondary pulmonary arterial hypertension
- Renal/hypertensive crisis
- Raynaud’s phenomenon
- GERD
Testing for Systemic Sclerosis

• Serologic
  • ANA positive
    ▪ Titer=1:320
    ▪ Pattern=Speckled, nucleolar, or centromere
  • Anti-centromere antibody
    ▪ Limited cutaneous systemic sclerosis (formerly CREST)
    ▪ Associated with higher risk of pulmonary hypertension

• Anti-nucleolar antibody
  ▪ Diffuse cutaneous systemic sclerosis (formerly PSS)

• Anti-Scl-70 (Topoisomerase I)
  ▪ Suggests high risk of pulmonary involvement with fibrosis

• RNA polymerase 3
  ▪ Associated with higher risk of developing diffuse cutaneous disease, renal crisis, and a temporal relationship to malignancy

Continue to treat or refer to Rheumatologist or refer to Scleroderma Center
### Rheumatic Diseases to Consider when ANA Test is Negative

<table>
<thead>
<tr>
<th>Autoimmune thyroid disease</th>
<th>Autoimmune neuropathies and vasculitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sjögren's syndrome</td>
<td>Celiac disease and bullous disease</td>
</tr>
<tr>
<td>Rheumatoid Arthritis</td>
<td>Gout / pseudogout</td>
</tr>
<tr>
<td>Ankylosing spondylitis</td>
<td>Idiopathic myositis / polymyositis (not associated with MCTD)</td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
<td></td>
</tr>
<tr>
<td>Psoriatic arthritis</td>
<td></td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td></td>
</tr>
<tr>
<td>Myasthenia gravis</td>
<td></td>
</tr>
</tbody>
</table>
When to Refer to a Rheumatologist: Treatment Help...

- Rheumatic manifestations of other diseases
  - Genetic, paraneoplastic, infections, neuropathic, and hematologic disorders
- Other inflammatory/musculoskeletal conditions
  - Complex regional pain syndrome
  - Serum sickness
  - Inflammatory eye disease
  - Vasculitis
  - Osteoarthritis
  - Metabolic bone disease
  - Fibromyalgia
  - Sarcoidosis