LABORATORY TESTS IN RHEUMATOLOGY: A PRIMER
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LIMITATIONS TO THE USE OF AUTOANTIBODY TESTING

- Autoantibodies may be associated with a particular disease process. Their sensitivity and specificity vary.
- Lab tests are not the gold standard for the diagnosis of an autoimmune illness.
- They are an adjunct used in combination with both the history and physical exam.
- Each rheumatic disease has a set of criteria used to make the diagnosis of that particular disease process. A lab test is just a small portion of that.
- Normal individuals may have positive autoantibody tests without any disease process.
- None of these tests is perfect.
**Rheumatoid Factor (RF)**

- Antibody directed against the Fc portion of IgG.
- Measured by latex agglutination, nephelometry or turbidometric assay.
- Test usually measures the IgM RF, but there are tests that measure the IgG, IgA, and IgE RF’s.
- 80% of patients with Rheumatoid arthritis are RF positive.
- RF may be seen in other autoimmune illnesses, as well as chronic liver or pulmonary diseases.
# Frequency of Rheumatoid Factors in Autoimmune Disease

<table>
<thead>
<tr>
<th>Disease Process</th>
<th>Percent RF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatoid Arthritis</td>
<td>50-90%</td>
</tr>
<tr>
<td>Systemic Lupus Erythematosus</td>
<td>15-25%</td>
</tr>
<tr>
<td>Sjogren’s Syndrome</td>
<td>20-30%</td>
</tr>
<tr>
<td>Cryoglobulinemia</td>
<td>40-100%</td>
</tr>
<tr>
<td>Polyarticular JIA</td>
<td>25%</td>
</tr>
<tr>
<td>Systemic Sclerosis</td>
<td>20-30%</td>
</tr>
<tr>
<td>Polymyositis/Dermatomyositis</td>
<td>5-10%</td>
</tr>
<tr>
<td>Healthy Individuals</td>
<td>2-10%</td>
</tr>
</tbody>
</table>
**ANTI-CYCLIC CITRULLINATED PEPTIDE ANTIBODIES (ANTI-CCP)**

- Used to aid in the diagnosis of Rheumatoid Arthritis.
- Directed against citrulline residues from the amino acid, Arginine.
- Highly specific- 98%
- Only moderately sensitive- 65%
- Higher levels may correlate with severity of disease.
- May see a positive anti-CCP with a negative RF.
- More likely to be positive in patients with RA who are smokers.
The hypothesized role of citrullination in RA

ANTI-NUCLEAR ANTIBODIES (ANA)

- Diverse group of autoantibodies directed against antigens in the nucleus and cytoplasm.
- Antigens are common to all nucleated cells, and may play a role in transcription or translation, in the cell cycle or as structural proteins.
- The antigens are named by:
  - Chemical structure (dsDNA)
  - Disease association (SSA/SSB in Sjogren’s syndrome)
  - Cytological location (nucleolar or centromere)
- Used as a screening test.
DETECTION OF ANA

- Indirect Immunofluorescence (IIF)
  - Uses HEp2 cells (human epithelial cancer cells).
  - IIF will give a titer and a pattern.
  - Best test to use when screening a patient.

- ELISA (Enzyme linked immunoassay)
  - Increasingly available.
  - Ease of use (tech is not required to evaluate pattern).
  - No titer, just a number.

- Other methods to detect ANA less commonly used:
  - Ouchterloney double immunodiffusion
  - Western blotting
  - Countercurrent Immunoelectrophoresis (CIE)
## ANA PATTERNS

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Antigen</th>
<th>Disease Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenous (Diffuse)</td>
<td>DNA Histone</td>
<td>SLE Drug induced SLE</td>
</tr>
<tr>
<td>Peripheral (Rim)</td>
<td>DSDNA Sm RNP</td>
<td>Specific for SLE</td>
</tr>
<tr>
<td>Speckled Fine Coarse</td>
<td>SSA/SSB Sm and RNP</td>
<td>Sjogren’s and SLE SLE and SLE/SS overlap</td>
</tr>
<tr>
<td>Centromere</td>
<td></td>
<td>CREST syndrome</td>
</tr>
<tr>
<td>Nucleolar</td>
<td>Nucleolar RNA</td>
<td>Systemic Sclerosis</td>
</tr>
<tr>
<td>Cytoplasmic</td>
<td>Histidyl-t-RNA synthetase (Jo-1)</td>
<td>Polymyositis</td>
</tr>
</tbody>
</table>
ANA PATTERNS

- **Peripheral (rim)**: anti-DNA (not seen on HEp-2), SLE
- **Homogeneous (diffuse)**: anti-DNA, anti-histone, anti-DNP (nucleosomes), RA & SLE, Misc. Disorders (anti-ssDNA)
- **Speckled**: anti-Sm & RNP, anti-Ro & La, anti-Jo-1 & Mi-2, anti-Scl-70, SLE & SS, PM/DM, PSS (Systemic)
- **Centromere**: anti-centromere, PSS (CREST)
- **Nucleolar**: anti-nucleolar, SLE & PSS
ANA PATTERNS

Peripheral

Diffuse

Speckled

Nucleolar
CONDITIONS ASSOCIATED WITH A POSITIVE ANA

- SLE
- MCTD
- Scleroderma
- Sjogren’s syndrome
- Rheumatoid Arthritis (30%)
- Polymyositis
- Dermatomyositis
- Discoid Lupus (5%)
- Autoimmune thyroid disease
- Autoimmune hepatitis
- Primary biliary cirrhosis
- Autoimmune cholangitis
- Drug-induced SLE
- Asymptomatic drug-induced ANA
- Chronic infection
- Idiopathic pulmonary fibrosis
- Primary pulmonary hypertension
- Lymphoproliferative disorders
- Normal patients (5%)
Anti-double stranded DNA (DS-DNA)

- Strongly associated with SLE
- Reported in 60% of patients with SLE
- Performed by three methods
  - Farr assay (radioimmunoassay)-detects high-avidity antibodies, and has highest specificity for SLE and has greatest correlation with disease activity (rarely used due to concerns over radiolabelling.
  - IIF using Crithidia (parasite with dsdna in its kinetoplast)-very specific, but has low sensitivity.
  - ELISA- very sensitive, but many false positives as well. Mostly commonly used by lab. Ask for confirmation of a positive with IIF using Crithidia.
CRITHIDIA LUCILAE
EXTRACTABLE NUCLEAR ANTIGENS (ENA’S)

- Group of antigens in the nucleus of a cell.
- Tested mostly by ELISA at this time
- Antigens include
  - SSA (Ro)
  - SSB (La)
  - Sm (Smith)
  - RNP (ribonucleoprotein)
  - Scl-70
  - Centromere
  - Jo-1
ANTI-SSA (Ro)

- Found in 35% of patients with SLE
- Found in 60% of patients with primary Sjogren’s syndrome
- Associated with congenital heart block and neonatal lupus in mother’s who have the antibody.
  - Incidence of heart block is 3% in children whose mother has not had a child with heart block, and as high as 33% in a child whose mother has had a previous pregnancy with fetal heart block.
  - Incidence of neonatal lupus is 1 in 15
ANTI-SSB (La)

- Found in 40% of patients with primary Sjogren’s syndrome.
- May be seen in SLE, RA, Polymyositis and Autoimmune Hepatitis.
**ANTI-SM (SMITH)**

- Sm antigen is a complex group of four proteins
- Many patients with anti-Sm antibodies will have an anti-RNP antibody.
- Very specific for SLE (95%)
- Not very sensitive (20-30%)
Anti-RNP (Ribonucleoprotein)

- Known by many names
  - nRNP
  - snRNP
  - U-snRNP
  - U1-snRNP

- High specificity for Mixed Connective Tissue Disease (85-100%)
  - A mixture of SLE, Scleroderma, and Polymyositis
  - Not every patient with an RNP antibody has MCTD.
  - Many false positives by ELISA
ANTI-SCL-70 (SCLERODERMA ANTIBODY)

- Found in 20-25% of patients with Systemic Sclerosis.
- May produce a nucleolar pattern on ANA testing.
- High specificity for Systemic Sclerosis (100%)
- May be associated with pulmonary fibrosis.
- Associated with diffuse cutaneous involvement in patients with Scleroderma.
- Patients with Scl-70 antibodies and Scleroderma have more organ involvement, and therefore have a poorer prognosis.
ANTI-JO-1 ANTIBODY

- Group of antibodies directed against aminoacyl-tRNA synthetases.
- Found in both Polymyositis and Dermatomyositis (20%)
- Associated with the anti-synthetase syndrome in patients with PM/DM.
  - Fever
  - Interstitial lung disease
  - Raynaud’s
  - Inflammatory polyarthritis
  - Mechanic’s hands
ANTI-CENTROMERE ANTIBODY

- Associated with CREST
  - C- Calcinosis
  - R- Raynaud’s
  - E- Esophageal dysmotility
  - S- Sclerodactyly
  - T- Telangiectasias
- Found in 80% of patients with CREST
- Associated with an increase in the development of pulmonary hypertension in patients with CREST
ANTI-HISTONE ANTIBODIES

- Produce a homogenous pattern on ANA
- Found in patients with SLE and drug-induced SLE.
- Drugs associated with drug-induced SLE include
  - Procainamide
  - Hydralazine
  - Minocycline
  - TNF inhibitors (Enbrel/Humira/Remicade)
  - INH
  - Quinidine
  - Alpha methyldopa (Aldomet)
  - Chlorpromazine
  - Penicillamine
  - Chlorpromazine
  - Amiodarone
ANTI-NEUTROPHIL CYTOPLASMIC ANTIBODIES (ANCA’S)

- Function of the neutrophil is to infest and destroy antigens.
- Neutrophils contain damaging enzymes such as Proteinase-3 (PR-3), Myeloperoxidase (MPO), and Elastase
- Antibodies to these enzymes have been found to be associated with primary systemic vasculitis
- Done by ELISA and Indirect Immunofluorescence (IIF). IIF detects the ANCA and ELISA, detects the specific antigen (PR-3 or MPO).
- Two staining patterns
  - C-ANCA (cytoplasmic)
  - P-ANCA (perinuclear)
C-ANCA (CYTOPLASMIC)

- Usually associated with proteinase-3 (PR-3) antibodies.
- Usual disease association is Granulomatosis with Polyangiitis (GPA).
- Sensitivity in GPA is 80-90%
- C-ANCA and PR-3 antibodies may be seen in other disease processes.
- Clinical correlation is a must, as C-ANCA’s may be found in patients with no clinical disease.
**P-ANCA (PERINUCLEAR)**

- Usually associated with the Myeloperoxidase antibody (MPO)
- May be seen in patients with Microscopic polyangiitis (50%)
- May also be found in patients with Ulcerative colitis, Sclerosing cholangitis, and atypical pneumonias. Not associated with MPO antibodies.
ANCA PATTERNS
### Disease Associations with PR-3 and MPO Antibodies

<table>
<thead>
<tr>
<th>Disease Entity</th>
<th>Anti-PR-3</th>
<th>Anti-MPO</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPA</td>
<td>85%</td>
<td>10%</td>
</tr>
<tr>
<td>MPA</td>
<td>45%</td>
<td>45%</td>
</tr>
<tr>
<td>Idiopathic crescentic glomerulonephritis</td>
<td>25%</td>
<td>65%</td>
</tr>
<tr>
<td>Churg-Strauss (EPA)</td>
<td>10%</td>
<td>60%</td>
</tr>
<tr>
<td>Polyarteritis nodosa</td>
<td>5%</td>
<td>15%</td>
</tr>
</tbody>
</table>
Drug-Induced ANCA Positive Vasculitis

- Certain medications may induce forms of vasculitis associated with an ANCA.
- Usually associated with a P-ANCA and MPO antibodies, but some associated with C-ANCA and PR-3 antibodies.
- Drugs associated with an ANCA positive vasculitis include
  - Propylthiouracil (PTU)
  - Minocycline
  - Hydralazine
  - Cocaine contaminated with Levamisole
Antiphospholipid Antibodies

- Also known as anticardiolipin antibodies.
  - May cause false positive RPR or VDRL
- Antibodies against a variety of phospholipid antigens to include:
  - Phosphatidylcholine
  - Phosphatidyl-serine
  - Phosphatidylethanolamine
- Beta-2, glycoprotein-1 (B2GP1) is the major phospholipid binding protein and antibodies to it, may be responsible for hypercoaguability.
- Antibodies may be associated with:
  - Recurrent fetal loss
  - Thrombocytopenia
  - Hypercoaguability (both venous and arterial)
- Measured by ELISA- IgG and IgM.
- False positives with infection and inflammation.
LUPUS ANTICOAGULANT

- An antiphospholipid antibody that may cause a hypercoaguable state.
- Baseline aPTT prolonged and does not correct with normal plasma (1:1 mix).
- Confirmed with the dilute Russell Viper Venom test (DRVVT)
  - Prolonged aPTT despite the addition of Russell Viper Venom (ratio calculated)
  - Must have 2 positives several weeks apart.
- Anticoagulation may interfere with test results.
  - Aspirin and Clopidogrel do not interfere with test results.
COMPLEMENT

- Cascade of proteins that can be activated by a variety of agents including immune or antigen-antibody complexes (infection and autoimmune illness).
- Effector functions include opsonization, chemotaxis, activation of leukocytes, lysis of bacteria, and clearance of immune complexes.
- C3 and C4 are most commonly measured, and may be low in SLE with renal involvement.
- Congenital deficiencies of C2 or C4 may be associated with SLE.
- Terminal complement deficiencies (C5b-9) associated with recurrent Neisserial infection
- May be low in some systemic vasculitides.
IMMUNE COMPLEX DISEASES ASSOCIATED WITH HYPOCOMPLEMENTEMIA

- Systemic Lupus Erythematosus
- Vasculitis
  - Hypocomplementememic urticarial vasculitis
  - Polyarteritis nodosa (Hepatitis B associated)
- Gomerulonephritis
  - Poststreptococcal
  - Focal and Diffuse proliferative associated with SLE
- Cryoglobulinemia (types I and II)
- Infective endocarditis
- Serum sickness
**Erythrocyte Sedimentation Rate (ESR)**

- A test based on elevation of acute phase proteins.
  - C-reactive protein
  - Haptoglobin
  - Transferrin
  - Fibrinogen-(most important)
  - Ceruloplasmin, Serum Amyloid A

- Measures rate of gravitational settling of RBC’s and rouleaux formation, which may be accelerated by a variety of factors.

- Usually done by the Westergren method (0-200mm)- 1 hour waiting period.

- Increases with advancing age.
NORMALS

- **Men- 0-20mm**
  - Correction for older men is to take their age and divide by 2 \((\text{Age}/2)\)

- **Women- 0-30**
  - Correction for older women is to take their age, add 10 and divide by 2 \(((\text{Age}+10)/2)\).
ESR Increases

- Acute or Chronic inflammatory diseases.
- Tissue injury (MI, Malignancy)
- Infection
- Pregnancy
- Nephrotic syndrome
  - 1 gram of proteinuria may increase ESR by 10 mm.
- Hypergammaglobulinemia or Myeloma.
- Thyroid disease (both hyper and hypothyroidism)
- 10% lack an identifiable etiology and may need to be repeated in 3 months.
Erythrocytes

Rouleaux
ESR DECREASES

- Polycythemia
- Sickle Cell disease
- Hypofibrinogenemia
- Congestive heart failure
- Other hyperviscosity syndromes
C-REACTIVE PROTEIN (CRP)

- Has physiologic role in the innate immune response to infection and may participate in the clearance of necrotic and apoptotic cells.
- Synthesized in the liver.
- Rises and falls early- half-life of 18 hours.
- Increase with age and body mass index.
- Useful in monitoring RA and systemic vasculitis.
- May be normal in patients with SLE, Polymyositis, Systemic Sclerosis, and Psoriatic arthritis.
**Myositis Specific Antibodies**

- **Anti-Jo-1** (antisynthetase syndromes)
- **Anti-Signal Recognition Protein** antibody (SRP)
  - Associated with acute onset and severe disease.
- **Anti-Mi-2** antibody
  - High specificity for Dermatomyositis.
  - Seen in 15-20% of patients with Dermatomyositis.
Cryoglobulins

- Cold-insoluble immunoglobulins
- Precipitate with cold and dissolve on rewarming.
- Should be drawn in pre-warmed tubes and kept warm until clotting occurs.
- Analysis done by immunofixation electrophoresis (IFE) which permits classification into 3 types.
**Type I Cryoglobulins**

- Monoclonal immunoglobulin
- Precipitates with cold
- Associated with underlying lymphoproliferative disorders
- May cause a cold-induced hyperviscosity syndrome
**Type II Cryoglobulins**

- Immune complexes composed of a monoclonal immunoglobulin (usually IgM kappa) with Rheumatoid factor and polyclonal IgG.
- Associated with Hepatitis C.
- Exam findings include palpable purpura.
- May be associated with low C4.
**Type III Cryoglobulins**

- Immune complexes composed of a polyclonal Rheumatoid factor and a polyclonal IgG.

- May occur with:
  - Hepatitis C
  - Other chronic infections
  - Infective endocarditis
  - SLE
  - RA
QUESTIONS