Anti-Nuclear Antibodies

- To help establish a diagnosis in pt with clinical features suggestive of an autoimmune disorder
- To exclude such disorders in pt with uncertain findings
- To subclassify a patient with an established diagnosis
- To monitor disease activity (eg. Anti ds DNA)

Anti-Nuclear Antibodies

Diseases Associated with +ve ANA

- SLE — 93 percent
- Scleroderma — 85 percent
- Mixed connective tissue disease — 93 percent
- Polymyositis/dermatomyositis — 61 percent
- Rheumatoid arthritis — 41 percent
- Rheumatoid vasculitis — 33 percent
- Sjögren's syndrome — 48 percent
- Drug-induced lupus — 100 percent
- Discoid lupus — 15 percent
- Pauciarticular juvenile chronic arthritis — 71 percent

Diseases Associated with +ve ANA

- Hashimoto's thyroiditis — 46 percent
- Graves' disease — 50 percent
- Autoimmune hepatitis — 63 to 91 percent
- Primary biliary cirrhosis 10 to 40 percent
- Primary autoimmune cholangitis — 100 percent
- Idiopathic pulmonary arterial hypertension — 40 percent

- Chronic Active Hepatitis 100 %
- Myasthenia Gravis 50 %
- Diabetes 25 %
- Normal < 5%

1948 SLE diagnosed with LE cell
- Antibodies attack DNA complexes in nuclei
- Nuclei become damaged
- Ingested by phagocytic cells
- LE Cell - PMN with a denatured nuclei inside
Anti-Nuclear Antibodies

- Currently we use the Fluorescent Antinuclear Antibody Test (FANA)
- Antibodies attach to prepared cells from the lab
- Serum is washed off with antibodies left behind
- Antibodies are stained with fluorescent Ab
- Results are observed manually by microscope

Anti-Nuclear Antibodies

Anti-Nuclear Antibodies

Anti-Nuclear Antibodies by Immunofluorescence

Human cell
Anti-Nuclear Antibodies
The SIX (6) Fluorescence Patterns

Homogeneous Pattern
Speckled Pattern

Rim Pattern
Nucleolar Pattern

Anti-Nuclear Antibodies
The SIX (6) Fluorescence Patterns
### Anti-Nuclear Antibodies

**The SIX (6) Fluorescence Patterns**

<table>
<thead>
<tr>
<th>Patterns of ANA</th>
<th>Specific for SLE</th>
<th>Specific for SLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenous</td>
<td>SLE, DILE, Overlap (PM-Scl-SLE)</td>
<td>Chromatin, histone, dsDNA, Ku</td>
</tr>
<tr>
<td>Rim</td>
<td>SLE</td>
<td>Lamins, Nuclear pore complex</td>
</tr>
<tr>
<td>Speckled</td>
<td>Coarse - SLE (Sm, U1-RNP)</td>
<td>Fine - SS, SCLE (Ro, La)</td>
</tr>
<tr>
<td>Diffuse</td>
<td>Nucleolar</td>
<td>Scl 70, RNA Polymerase 1, PM-Scl</td>
</tr>
<tr>
<td>Nucleolar</td>
<td>Speckled</td>
<td>Centromere</td>
</tr>
<tr>
<td>Anti-centromere</td>
<td>: CREST syndrome (highly)</td>
<td>SSc</td>
</tr>
</tbody>
</table>

With more specific auto antibodies tests available, these patterns are less helpful.

### Extractable Nuclear Antigens

**ENA**

- Detection of SIX (6) antibodies which better correlate with specific disease states
- Enzyme linked Immunosorbent Assay (ELISA)

### ELISA

**(Enzyme Linked Immunosorbent Assay)**

- Chromogen - Changes colour when cleaved by enzyme attached to the second antibody
- Anti-Human Immunoglobulin coupled to an enzyme binds to human antibodies
- Conjugate
- Human Antibodies Precipitate bind to antigen form Immune Complexes
- Patient Serum

### Extractable Nuclear Antigens

**Methods**

- Screen - Using plates coated with all SIX (6) antigens - If positive (>20) then

- Individual ELISA with plates coated with single antigens - Determines specifically which antibodies are present in patient’s serum
Anti-Nuclear Antibodies

- Negative ANA does not R/O SLE completely.
- Rarely people with anti-Ro or anti-single stranded DNA (anti ssDNA) have negative ANA test.
- Patients with anti-phospholipid syndrome may have a negative ANA.

Chromatin associated antibodies

- Anti-ssDNA [anti-single stranded antibodies]
- Anti-dsDNA (anti-double stranded DNA)
- Anti-histone

Anti-double stranded DNA (Anti-dsDNA)

- High tiers are highly specific for SLE.
- Only 60% of SLE patients have high titers.
- Titer absence does not R/O SLE.
- Low tiers can be present in:
  - Normal population
  - Sjogren’s syndrome
  - RA

Anti-double stranded DNA (Anti-dsDNA)

- Anti ds DNA level correlates with disease activity in SLE
- Its presence correlates with lupus nephritis
- Testing not recommended with negative ANA

Anti-Ribosome antibodies

- Highly specific for SLE.
- But present only in 10-20% of cases.
- Associated with lupus psychosis.

Scleroderma antibodies

- Anti-centromere
- Anti-topoisomerase 1 (anti Scl-70)
### Anti-centromere
- 20-40% of patients with scleroderma
- Presence associated with: Raynaud’s phenomenon, CREST syndrome, limited skin involvement
- Also present with primary biliary cirrhosis

### Anti-topoisomerase 1 (Anti Scl-70)
- Highly specific
- 20-40% of patients with scleroderma
- Its presence correlated with:
  - Diffuse cutaneous disease
  - Pulmonary fibrosis
  - Cardiac involvement
  - Longer disease duration

### Other Antibody Test
**Anti-Jo1** [histidyl-tRNA synthetase]
- 30% patients of polymyositis and dermatomyositis
- Associated with
  - Pulmonary fibrosis
  - Raynaud’s phenomenon

### APS: Anti-phospholipid Syndrome
- Presence in the serum of at least one type of autoantibody known as an antiphospholipid antibody (aPL).
- The occurrence of at least one clinical feature from a diverse list of potential disease manifestations
  - venous or arterial thromboses
  - recurrent fetal loss
  - thrombocytopenia.

### Anti-cardiolipin antibodies
- Anti-cardiolipin Ab if positive should be repeated after 3 – 6 months to diagnose anti-phospholipid syndrome
- Level cannot predict thrombosis as once suggested.
- IgG is more specific than IgM

### Lupus anticoagulant
**Precautions:**
- Not on heparin or oral anticoagulant
- Is not useful as a follow up test
Relationship of the LA and aCL

The test may be positive for one or both.

A Side note on Hypercoagulability...

Inherited thrombophilia
- Factor V Leiden mutation
- Prothrombin gene
- Protein S deficiency
- Protein C deficiency
- Antithrombin deficiency
- Dysfibrinogenemia (rare)

Anti-Nuclear Antibodies

Clinical Associations in SLE

<table>
<thead>
<tr>
<th>Antigen specificity</th>
<th>Clinical associations</th>
<th>Prevalence, percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>dsDNA</td>
<td>Marker for active disease, titers fluctuates with disease activity, correlates best with renal disease</td>
<td>40-60</td>
</tr>
<tr>
<td>ssDNA</td>
<td>Nonspecific, no clinical utility</td>
<td>7%</td>
</tr>
<tr>
<td>Ro/SSA</td>
<td>Subacute cutaneous lupus (75 percent), photosensitivity, neonatal lupus, complement deficiencies</td>
<td>40</td>
</tr>
<tr>
<td>RNP (U1-RNP)</td>
<td>SLE generally in conjunction with Sm; in MCTD, required for diagnosis</td>
<td>30-40</td>
</tr>
<tr>
<td>La/SSB</td>
<td>With La, low prevalence of renal disease Neonatal lupus (75 percent)</td>
<td>10-15</td>
</tr>
</tbody>
</table>

Anti-Nuclear Antibodies

Clinical Assoc with SLE

<table>
<thead>
<tr>
<th>Antigen specificity</th>
<th>Clinical associations</th>
<th>Prevalence, percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sm</td>
<td>Marker for disease, not generally useful in management; May be associated with CNS disease</td>
<td>About 20</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>Hypercoagulable state in some patients. No clinical significance in others. Thrombocytopenia, later trimester abortions</td>
<td>30</td>
</tr>
<tr>
<td>Histones</td>
<td>&gt;95 percent in drug-related lupus. Also present in RA, SLE, reported in systemic sclerosis with pulmonary fibrosis</td>
<td></td>
</tr>
<tr>
<td>Ribosomal P</td>
<td>Initially associated with psychosis in SLE, more recently with depression</td>
<td>10-40</td>
</tr>
<tr>
<td>Ku</td>
<td>SLE, MCTD (European, American population) Scleroderma/myositis overlap (Japanese population)</td>
<td>19, 39</td>
</tr>
</tbody>
</table>

Rheumatoid Factor (s) (RF)

- Auto-antibodies directed against antigenic determinants on the Fc portion of immunoglobulin (Ig) G molecules
- Can be IgM, IgG, IgA, or IgE antibodies
- IgM only one routinely tested

Rheumatoid Factor

by Latex Agglutination

- Latex particles coated with human IgG - Patient serum added - read under a lamp 2 minutes later.
- Dilution of 1/40 generally considered as positive
Rheumatologic Testing in Primary Care

October 4, 2008

Fernando Vega, M.D.

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**Rheumatoid Factor by Latex Agglutination**

Latex Particles with IgG

---

**Rheumatoid Factor by Latex Agglutination**

IgM Rheumatoid Factor

Latex Particles with IgG

---

**Rheumatoid Factor by Nephelometry**

- More sensitive screening method than latex agglutination
- Amount of scattered light ∝ concentration of Ab-Ag complexes

---

**Rheumatoid Factor**

- **NOT USED AS A SCREEN**
- **NOT USED TO MAKE DIAGNOSIS**
  - Not specific to Rheumatoid Arthritis: seen in other diseases
  - USEFUL FOR FULLFILLING CRITERIA to help make a diagnosis
  - USEFUL FOR PROGNOSIS-RF +ve patients may have more aggressive disease
  - Present in 70-85% of people with RA

---

**Rheumatoid Factor**

Positive in:

- **Rheumatic Diseases**
  - Sjogren’s syndrome
  - Rheumatoid Arthritis
  - SLE
  - MCTD
  - Myositis
  - Cryoglobulinemia

- **Non-Rheumatic Diseases**
  - Normal Aging
  - Infection
    - Hepatitis B & C
    - MMR, influenza
    - SBE
    - Tb
    - HIV
  - Parasitic Diseases
  - Sarcoidosis
  - Idiopathic Pulmonary Fibrosis
  - Primary Biliary Cirrhosis
  - Malignancies (leukemia, colon)

---

**Rheumatological Conditions**

- Rheumatoid arthritis 50-90%
- Sjogren’s syndrome 75-95%
- Cryoglobulinemia 40-100%
- MCTD 50-60%
- SLE 15-35%
- Systemic sclerosis 20-30%
Rheumatologic Testing in Primary Care

October 4, 2008

Fernando Vega, M.D.

Rheumatoid Factor

- Prevalence of RF in healthy elderly could be 10%
- But titers are low, 1:40 or lower
- 20% of patients with RA are RF negative
- 40% may have negative RF in early stages of disease
- Not helpful in low clinical suspicion (i.e. Absence of joint inflammation)

Anti CCP antibodies

- Recently discovered antibodies to cyclic citrullinated peptide
- Not specific to patients with Rheumatoid arthritis but if present in a RF +ve patient connotation is that likely to have more aggressive disease prompting aggressive early treatment

Anti CCP antibodies

- Anti CCP antibodies can be detected years before appearance of the first symptoms of RA

<table>
<thead>
<tr>
<th></th>
<th>Specificity</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF +</td>
<td>75%</td>
<td>60%</td>
</tr>
<tr>
<td>Anti CCP +</td>
<td>96%</td>
<td>75%</td>
</tr>
<tr>
<td>Anti CCP+</td>
<td>99%</td>
<td>80%</td>
</tr>
</tbody>
</table>

Anti CCP antibodies

Citrulination of Araginine in Proteins

Ketone group substituted for NH3

Ketone group substituted for NH3
Short Delay of Therapy Affected Radiographic Outcome

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Anti-Nuclear Antibodies
- Chronic Active Hepatitis 100 %
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- Diabetes 25 %
- Normal < 5%
Human Leukocyte Antigen B27 (HLA-B27)
- Associated with spondyloarthropathies especially in ankylosing spondylitis
- 95% sensitive for AS
- 80% for Reiter’s syndrome
- 70% for spondylitis with psoriasis
- Only present in 6-10% Caucasian population, order only if suspicion is high.

Human Leukocyte Antigen System
- The human leukocyte antigen (HLA) system is synonymous with the human major histocompatibility complex (MHC).
- These terms describe a group of genes on chromosome 6 that encode a variety of cell surface markers, antigen-presenting molecules, and other proteins involved in immune function.

Erythrocyte Sedimentation Rate (ESR)
- Initially developed as a serologic test for pregnancy
- Later found to be a useful but non-specific marker of inflammation

Erythrocyte Sedimentation Rate
- Forces affecting sedimentation of RBC:
  - Size of RBC
  - Viscosity of Plasma
  - Repellent forces between negatively charged RBC membrane
  - The presence of asymmetric proteins (fibrinogen) affects quality of repellent force and allows formation of Rouleaux causing the RBC to settle more rapidly

Erythrocyte Sedimentation Rate
- Principle
- 200 mm long X 2.5 mm diameter vertically aligned anticoagulated tube of blood
- Look at the distance the column of blood falls in one hour (mm/hr)
- Normal ESR
  - Men: Age/2
  - Women: (Age+10)/2

NORMAL RBCs

RBCs & APPs

Forces affecting sedimentation of RBC:
- Size of RBC
- Viscosity of Plasma
- Repellent forces between negatively charged RBC membrane
- The presence of asymmetric proteins (fibrinogen) affects quality of repellent force and allows formation of Rouleaux causing the RBC to settle more rapidly
Erythrocyte Sedimentation Rate

- **ESR**
  - Inflammatory Diseases
  - Hypoalbuminemia (-)
  - Hypergammaglobulinemia
  - Tissue Necrosis (MI, trauma)
  - Pregnancy
  - Anemia
  - Age
  - Heparinized Blood

Acute phase reactants

- Coagulation proteins:
  - Fibrinogen
  - Prothrombin
- Transport proteins:
  - Hepatoglobins
  - Transferrin
  - Ceruloplasmin

Acute phase reactants

- Complement components:
  - C3 and C4
  - Protease inhibitor
- Miscellaneous:
  - Albumin
  - Fibronectin
  - CRP
  - ESR
  - Serum amyloid-A related proteins

Acute phase reactants

Most commonly used:

- **CRP and ESR**
- CRP responds more rapidly than ESR
- However ESR takes an hour and CRP takes a day.

**ESR**

- Measures ht of RBCs that settle in one hour.
- Upper limits:
  - Less than 50 yrs.
    - Men < 15 mm
    - Women < 20 mm
  - More than 50 yrs.
    - Men < 20
    - Women < 30
- Male- age/2 and Women [age+10] /2

Diagnostic criteria for:

- Temporal arteritis
- Polymyalgia rheumatica

Tends to correlates with clinical activity of disease

Used as a mean to stage RA.

Sensitivity 50% in RA but specificity is very low.
**ESR**

- Factors increasing ESR:
  - Old age
  - Female
  - Pregnancy
  - Anemia
  - Macrocytosis
  - Elevated fibrinogen levels:
    - Infections, Inflammation, Malignancy
  - Technical factors:
    - Dilution, temp., tilted tube

- Decreased ESR:
  - Polycythemia
  - Leukocytosis
  - Sickle cell disease
  - Protein abnormalities:
    - Hypofibrinogenemia
    - Hypogammaglobulinemia
    - Dysproteinemia
  - Microcytosis, Spherocytosis, Anisocytosis

**Anti-Neutrophil Cytoplasmic Antibody (ANCA)**

- A collection of antibodies directed against components of granules inside the neutrophil
- Detected in the laboratory by Immunofluorescence Assay and by ELISA methods for specific antibodies

**Anti-Neutrophil Cytoplasmic Antibody Immunofluorescence**

- Same manner as ANA
- Slide with wells coated with ethanol fixed neutrophils
- Add patient serum and incubate
- Add anti-human immunoglobulin with a fluorescent tag and incubate
- View under the microscope

**Anti-Neutrophil Cytoplasmic Antibody Immunofluorescence**

- Fluorescein conjugated anti-human immunoglobulin
- Patient Serum
- Monolayer of Human Neutrophils
- ANCA Ab in Patient Serum
- Glass Slide
- Microscope

**Anti-Neutrophil Cytoplasmic Antibody Immunofluorescence**

- ANCA
- pANCA
Anti-Neutrophil Cytoplasmic Antibody Immunofluorescence

- 2 patterns possible
  - Cytoplasmic
  - Perinuclear - artifact of the laboratory test

ELISA (Enzyme Linked Immunosorbent Assay)

Chromogen - Changes colour when cleaved by enzyme attached to the second antibody
Anti-Human Immunoglobulin coupled to an enzyme binds to human antibodies
Conjugate
Human Antibodies Precipitate bind to antigen form Immune Complexes
Cuvette Coated with Antigen
Patient Serum

Anti-Neutrophil Cytoplasmic Antibody Immunofluorescence - Disease Associations

- Wegener's Granulomatosis
  - c-ANCA = 75-80%
- Microscopic Polyangiitis (MPA)
  - p-ANCA = 50-60%
  - Ulcerative colitis
  - Crohn's

What are the Serological Markers in Inflammatory Bowel Disease?

- pANCA (perinuclear staining pattern)
- Loss of perinuclear pattern after DNAase
- Differentiate from the "other pANCA's"
  - Antibody against myeloperoxidase
  - Antibody against cathepsin G, elastase, lysozyme, and lactoferrin
- ASCA (anti-Saccharomyces cerevisiae)
  - Both IgG and IgA
  - Recognize mannose in the cell wall mannan of Saccharomyces cerevisiae

Anti-Neutrophil Cytoplasmic Antibody Immunofluorescence - Disease Associations

- pANCA and ASCA are specific for UC and CD respectively
- Neither pANCA nor ASCA are sensitive enough to exclude IBD
- In patients with Intermediate Colitis, available serological markers do not accurately predict the subsequent disease course
- Antibody profiles can predict disease behavior in IBD
Why Use Serological Markers in Clinical Practice?

- Differentiate IBD from functional bowel disorders
- Accurately diagnose Crohn’s or UC in a patient with:
  - Severe colitis
  - Indeterminate colitis
- Predict disease course or complications in IBD
  - CD phenotype
  - Severity of disease
  - Risk of pouchitis

Frequency of pANCA in UC Patients and Controls in a Referral Center


Prevalence of ASCA in Patients with CD and UC and Controls in the Different Assays


Can Serological Markers Differentiate IBD from Non-IBD?

- pANCA and ASCA are specific for and have high positive predictive value for UC and CD respectively
  - Rule in disease
- The low sensitivity and negative predictive value preclude them as a screening test
  - Cannot rule out disease
- Potential application may avoid invasive work up
**Anti-Neutrophil Cytoplasmic Antibody Immunofluorescence & Antibodies**

- c-ANCAs
- Anti-Proteinase 3 (PR3)
- p-ANCA
- Anti-Myeloperoxidase (MPO)
- Elastase
- Capthescin G
- Lactoferrin
- Lysozyme
- Azurocidin
- Histone 1

**Anti-Neutrophil Cytoplasmic Antibody ELISA**

- Same manner as ds-DNA
- Wells coated with Proteinase 3 (PR3) or myeloperoxidase (MPO)
- Add patient’s serum and incubate
- Add anti-human immunoglobulin with an enzyme tag and incubate
- Add chromagen – TURNS COLOUR!

---

### In Summary: Autoantibodies Detected in Patients with Connective Tissue Disease

<table>
<thead>
<tr>
<th>Autoantibody</th>
<th>Disease (Frequency of autoantibody)</th>
<th>Cost</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF</td>
<td>RA (80%), other Connective tissue diseases</td>
<td>$15.00</td>
<td>Sensitive but not specific for rheumatoid arthritis; correlates with prognosis of disease severity (not disease activity)</td>
</tr>
<tr>
<td>ANA</td>
<td>SLE (99%), drug induced lupus (100%), other connective tissue diseases</td>
<td>$30.00</td>
<td>Sensitive but not specific for connective tissue diseases; correlates poorly with disease activity</td>
</tr>
</tbody>
</table>

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<th>Cost</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-dsDNA</td>
<td>Systemic lupus erythematosus (80%)</td>
<td>$30.00</td>
<td>Specific but not sensitive for SLE; correlates with lupus nephritis and disease activity</td>
</tr>
<tr>
<td>Anti-ssDNA</td>
<td>Infrequent</td>
<td>$200.00</td>
<td>Nonspecific and of little clinical utility</td>
</tr>
<tr>
<td>Anti-histone</td>
<td>Drug induced lupus (90%) SLE (50%)</td>
<td>$50.00</td>
<td>Sensitive but not specific for drug induced lupus</td>
</tr>
</tbody>
</table>

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<tbody>
<tr>
<td>Anti-Sm</td>
<td>Systemic lupus erythematosus (20-30%)</td>
<td>$50</td>
<td>Specific but not sensitive for SLE</td>
</tr>
<tr>
<td>Anti-U1 snRNP</td>
<td>Mixed connective tissue disease (100%)</td>
<td>$60</td>
<td>Associated with disease activity in SLE</td>
</tr>
<tr>
<td>Anti-RO (anti-SS-A)</td>
<td>Sjögren’s syndrome (75%), SLE (40%)</td>
<td>$50</td>
<td>Associated with photosensitive skin rash, pulmonary disease and lymphopenia in SLE</td>
</tr>
</tbody>
</table>

<table>
<thead>
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<th>Cost</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-La (anti-SS-B)</td>
<td>Sjögren’s syndrome (40%), SLE (10-15%)</td>
<td>$70</td>
<td>Associated with late onset SLE; secondary Sjögren’s syndrome and neonatal lupus syndrome</td>
</tr>
<tr>
<td>Anti-ribosome</td>
<td>SLE (10-20%)</td>
<td>$30.00</td>
<td>Highly specific but not sensitive for SLE; associated with lupus psychosis</td>
</tr>
<tr>
<td>Anti-centromere</td>
<td>Scleroderma (22-36%)</td>
<td>$30.00</td>
<td>Associated with CREST syndrome and Raynaud’s phenomenon</td>
</tr>
<tr>
<td>Autoantibody</td>
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</tr>
<tr>
<td>------------------------------</td>
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<td>----------------------------------------------------</td>
</tr>
<tr>
<td>Anti-topoisomerase 1</td>
<td>Scleroderma (22-40%)</td>
<td>$40.00</td>
<td>Highly specific but not sensitive for scleroderma</td>
</tr>
<tr>
<td>Anti-J01</td>
<td>Polymyositis and dermatomyositis (30%)</td>
<td>$40.00</td>
<td>Associated with pulmonary fibrosis and Raynaud’s phenomenon</td>
</tr>
<tr>
<td>c-ANCA</td>
<td>Wegener’s granulomatosis (&gt;90%)</td>
<td>$30.00</td>
<td>Highly specific and sensitive for Wegener’s granulomatosis; correlates with disease activity</td>
</tr>
</tbody>
</table>

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<th>Cost</th>
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</tr>
</thead>
<tbody>
<tr>
<td>p-ANCA</td>
<td>Wegener’s granulomatosis (10%), microscopic polyangiitis, glomerulonephritis</td>
<td>$30.00</td>
<td>Sensitivity and specificity quite low in Wegener’s granulomatosis</td>
</tr>
</tbody>
</table>