Introduction

- Laboratory testing in rheumatology has a major role in clinical practice.
- Depending on the disease suspected and antibody used, laboratory testing can play a valuable role in:
  - screening for disease
  - confirming diagnoses
  - establishing disease activity
  - determining prognosis
  - following responses to therapy
Outline

- Rheumatoid factor
- Anti-CCP
- ANA
  - Anti-ds DNA
  - Anti-Sm
  - Anti-histone
  - Anti-RNP
  - Anti-SSA/SSB
  - Anti-centromere
  - Anti-Scl 70
- APA
- ANCA
Rheumatoid Factor

- RF is an autoantibody that binds to the Fc region of human IgG.
- IgM is the most common RF isotype (IgG and IgA RF may also be detected).
- Method of detection: latex fixation.
Rheumatoid Factor

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Rheumatoid Factor

- RF is present in 75-80% of patients with rheumatoid arthritis.
- Positive tests occur in a wide range of autoimmune, inflammatory and chronic infections. (specificity of test for RA 80%)
- Prevalence also increases with age (25% of persons above the age of 65 may be positive).
- Titer is important:
  - In absence of diseases it is usually low
  - High titer (≥1:640) almost always reflects underlying RA.
## Rheumatoid Factor

### Diseases commonly associated with RF

<table>
<thead>
<tr>
<th>Category</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatic diseases</td>
<td>Rheumatoid arthritis, systemic lupus erythematosus, scleroderma, mixed CTD Sjögren's syndrome</td>
</tr>
<tr>
<td>Viral infections</td>
<td>Acquired immunodeficiency syndrome, mononucleosis, hepatitis, influenza; after vaccination (may yield falsely elevated titers of antiviral antibodies)</td>
</tr>
<tr>
<td>Parasitic infections</td>
<td>Trypanosomiasis, kala-azar, malaria, schistosomiasis, filariasis</td>
</tr>
<tr>
<td>Chronic bacterial infections</td>
<td>Tuberculosis, leprosy, yaws, syphilis, brucellosis, subacute bacterial endocarditis, salmonellosis</td>
</tr>
<tr>
<td>Neoplasms</td>
<td>Lymphoproliferative diseases</td>
</tr>
<tr>
<td>Other hyperglobulinemic states</td>
<td>Hypergammaglobulinemic purpura, cryoglobulinemia, chronic liver disease, sarcoidosis, other chronic pulmonary diseases</td>
</tr>
</tbody>
</table>
Indication: Should be ordered when there is a clinical suspicion of rheumatoid arthritis.

Because of the larger number of disorders associated with RF, the value of a positive test depends on the pretest probability.

A negative test does not R/O rheumatoid arthritis.

Up to 30% of patients are seronegative.
Anti-CCP

- Anti-Cyclic Citrullinated Peptide (CCP) antibody
- Another relatively new & important marker in diagnosis of RA.
- Anti-CCP antibodies have a sensitivity for RA that is similar to that of RF, but are much more specific (95% specificity).

Considerable usefulness of anti-CCP antibodies in setting of:
- Seronegative patients suspected of having RA
- Patients with other forms of CTD who are RF positive
- Patients with hepatitis C or other infections that are often associated with RF positivity.
Anti-CCP

- Anti-CCP antibodies are often detectable in early RA.
- A negative test for anti-CCP does not exclude RA (at initial presentation, 50% of patients lack detectable anti-CCP antibodies).
- A positive anti-CCP combined with a positive RF IgM correlates strongly with radiographic progression.
- Anti-CCP levels are not useful in the longitudinal monitoring of disease activity.
Anti-nuclear Antibodies (ANA) are a diverse group of autoantibodies that react with antigens in the cell nucleus.

Indirect immunofluorescence assays (Hep-2 cells) report the titer of ANA and pattern of staining.

- Titer is of clinical significance (low titers can be seen in healthy individuals).
- Staining pattern correlates poorly with underlying disease (except centromeric pattern which is specific for limited scleroderma).
ANA

Serum Antibody

Nucleus

Cytoplasm

Slide

Fluorescent Labelled Anti-Immunoglobulin

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ANA
## ANA in different rheumatic diseases

<table>
<thead>
<tr>
<th>Condition</th>
<th>Patients with ANAs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diseases for Which ANA Testing Is Helpful for Diagnosis</strong></td>
<td></td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>99-100</td>
</tr>
<tr>
<td>Systemic sclerosis</td>
<td>97</td>
</tr>
<tr>
<td>Polymyositis/dermatomyositis</td>
<td>40-80</td>
</tr>
<tr>
<td>Sjögren's syndrome</td>
<td>48-96</td>
</tr>
<tr>
<td><strong>Diseases for Which ANA Is Required for Diagnosis</strong></td>
<td></td>
</tr>
<tr>
<td>Drug-induced lupus</td>
<td>100</td>
</tr>
<tr>
<td>Mixed connective tissue disease</td>
<td>100</td>
</tr>
<tr>
<td>Autoimmune hepatitis</td>
<td>100</td>
</tr>
<tr>
<td><strong>Diseases for Which ANA May Be Useful for Prognosis</strong></td>
<td></td>
</tr>
<tr>
<td>Juvenile rheumatoid arthritis</td>
<td>20-50</td>
</tr>
<tr>
<td>Antiphospholipid antibody syndrome</td>
<td>40-50</td>
</tr>
<tr>
<td>Raynaud's phenomenon</td>
<td>20-60</td>
</tr>
<tr>
<td><strong>Diseases for Which ANA Is Typically not Useful</strong></td>
<td></td>
</tr>
<tr>
<td>Discoid lupus erythematosus</td>
<td>5-25</td>
</tr>
<tr>
<td>Fibromyalgia</td>
<td>15-25</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>30-50</td>
</tr>
<tr>
<td>Relatives of patients with autoimmune diseases</td>
<td>5-25</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>25</td>
</tr>
<tr>
<td>ITP</td>
<td>10-30</td>
</tr>
<tr>
<td>Thyroid disease</td>
<td>30-50</td>
</tr>
<tr>
<td>Patients with silicone breast implants</td>
<td>15-25</td>
</tr>
<tr>
<td>Infectious diseases</td>
<td>Varies widely</td>
</tr>
<tr>
<td>Malignancies</td>
<td>Varies widely</td>
</tr>
</tbody>
</table>
ANA in healthy individuals

<table>
<thead>
<tr>
<th>Normal Individuals</th>
<th></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>

Significance of positive ANA depends on pretest probability
ANA- Indications for Testing

- When there is clinical suspicion of:
  - SLE
  - Drug-induced Lupus
  - MCTD
  - Sclerderma
  - Sjogren

- Prognostic information for patients with Raynaud phenomena identifying those at risk to develop rheumatic diseases.
The sensitivity of ANA for different rheumatic diseases:

- SLE → 95-97% (negative ANA almost excludes diagnosis)
- Scleroderma → 85%
- MCTD → 93%
- Polymyositis/dermatomyositis → 61%
- Rheumatoid arthritis → 41%
- Rheumatoid vasculitis → 33%
- Sjögren's syndrome → 48%
- Drug-induced lupus → 100%
- Discoid lupus → 15%
Anti-ds DNA

- Anti-ds DNA occur mainly in SLE.
- They are rare in other diseases and healthy persons (if found, titer is low).
- Absent in most forms of drug-induced lupus.
Anti-ds DNA

- Test when there is clinical suspicion of SLE and ANA is positive.
- Occurs in 60-80% of SLE patients with specificity of 97%.
- Reflect disease activity in patients with known SLE.
- Level correlates with risk of developing lupus nephritis and lupus vasculitis.
Anti-Sm

- Test when there is clinical suspicion for SLE.
- Occur in only 10-40% of SLE patients.
- Highly specific.
- Not useful for monitoring disease activity.
Anti-histone Antibody

- Almost always present in drug-induced lupus.
- Also common in SLE (50-70%) 
- Occur at low frequency in other rheumatic disorders.
- Absence of antibody is a strong evidence against the diagnosis of drug-induced lupus.
Anti-RNP

- Test when there is clinical suspicion of MCTD.
- Occur in 30-40% of lupus patients.
- The diagnosis of MCTD requires the presence of antibodies to RNP.

\[\text{100\% of patients with MCTD have anti-RNP}\]

- Not useful for monitoring disease activity.
Anti-SSA/SSB

- Also known as anti Ro/anti La.
- Uncommon in the normal population and in rheumatic diseases other than Sjogren and SLE.
- Anti-SSA present in:
  - 75% of primary Sjogren
  - 10-15% of RA with secondary Sjogren.
  - 50% of SLE and is associated with photosensitivity, subacute cutaneous lupus, and interstitial lung disease, neonatal lupus, and congenital complete heart block.
Anti-SSA/SSB

- Anti-SSB occur in almost always in association with anti-SSA.
  - Primary Sjogren (40-50%)
  - SLE (10-15%)
  - *Congential complete heart block (90%)*
  - Neonatal cutaneous lupus (70%)
Antibodies should be measured when in case of:
- Clinical suspicion of primary Sjogren or SLE.
- Suspected subacute cutaneous lupus (even with negative ANA).
- Mothers of children with neonatal cutaneous lupus or complete congenital heart block (even if mothers are asymptomatic).
- SLE patients who are pregnant or planning to become pregnant.
Anticentromere Antibody

- Occur in 60% of patients with *limited scleroderma (CREST)* and in 15% of diffuse scleroderma patients with very high specificity (>98%).
- Very rare in other rheumatic conditions and healthy persons.
- A positive test is a very strong argument for the presence of CREST syndrome or diffuse scleroderma.
- Presence of antibodies early in the course of disease predicts limited cutaneous involvement and a decreased likelihood of interstitial lung disease.
Anti Scl-70 antibody

- Also known as anti-topoisomerase I.
- Should be measured when there is clinical suspicion of diffuse scleroderma.
- Occur in 40% of patients with scleroderma.
- Specificity approaches 100%.
- Has prognostic value in scleroderma and carries an increased likelihood of diffuse skin involvement and interstitial lung disease.
- Not useful for monitoring disease activity.
Antiphospholipid Antibodies

3 main antibodies:
- Lupus Anticoagulant
- Anticardiolipin Antibody (IgM and IgG)
- Anti-\(\beta2\) glycoprotein-I (IgM and IgG)

Screen in case of:
- Recurrent venous or arterial thromboses
- Pregnancy morbidity (recurrent fetal loss or preeclampsia)
- Thrombocytopenia or prolonged PTT.
ANCA

- Aid in the diagnosis of the vasculitic diseases.
- 2 patterns on immunofluorescent testing: perinuclear (p-ANCA) and cytoplasmic (c-ANCA).
- 2 antigens on ELISA testing: PR3 and MPO
- PR3 is usually associated with c-ANCA
- MPO is usually associated with p-ANCA
ANCA
c-ANCA Clinical Significance

- c-ANCA + PR3:
  - Wegener’s Granulomatosis (sensitivity up to 60% and specificity 90%).
  - The likelihood of c-ANCA positivity increases with more severe disease.
p-ANCA Clinical Significance

- p-ANCA + MPO: (>98% specificity)
  - Churg-Strauss Vasculitis
  - Idiopathic GN
  - MPA
References


Thank You