

ROLE OF LABORATORY MARKERS IN THE DIAGNOSIS OF RHEUMATIC DISEASES

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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

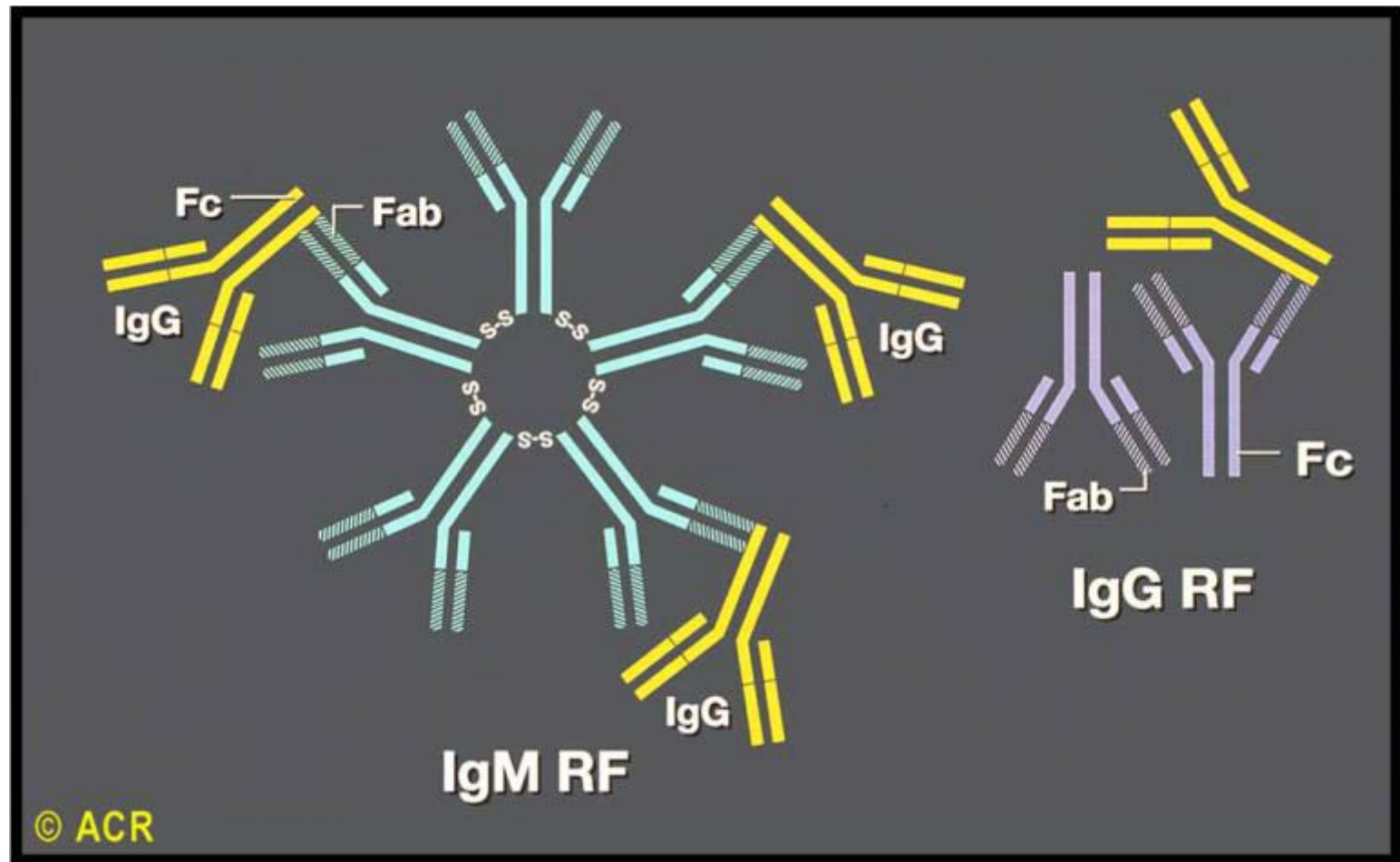
Outline

- 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



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 ($\geq 1:640$) almost always reflects underlying RA.

Rheumatoid Factor

Diseases commonly associated with RF

Rheumatic diseases	Rheumatoid arthritis, systemic lupus erythematosus, scleroderma, mixed CTD Sjögren's syndrome
Viral infections	Acquired immunodeficiency syndrome, mononucleosis, hepatitis, influenza; after vaccination (may yield falsely elevated titers of antiviral antibodies)
Parasitic infections	Trypanosomiasis, kala-azar, malaria, schistosomiasis, filariasis
Chronic bacterial infections	Tuberculosis, leprosy, yaws, syphilis, brucellosis, subacute bacterial endocarditis, salmonellosis
Neoplasms	Lymphoproliferative diseases
Other hyperglobulinemic states	Hypergammaglobulinemic purpura, cryoglobulinemia, chronic liver disease, sarcoidosis, other chronic pulmonary diseases

Rheumatoid Factor

- 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.

ANA

- 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

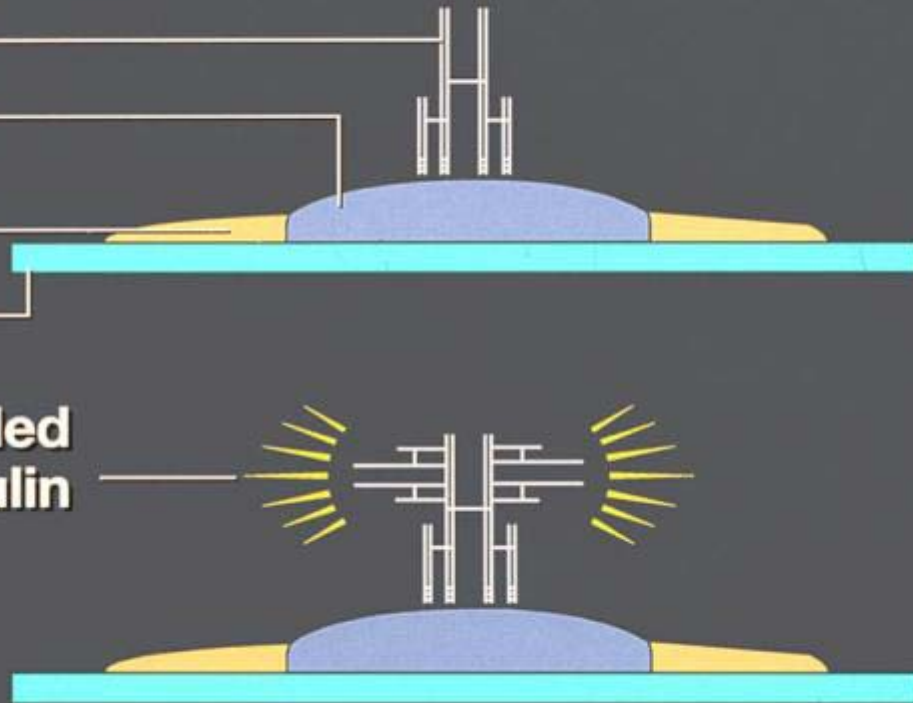
Serum Antibody

Nucleus

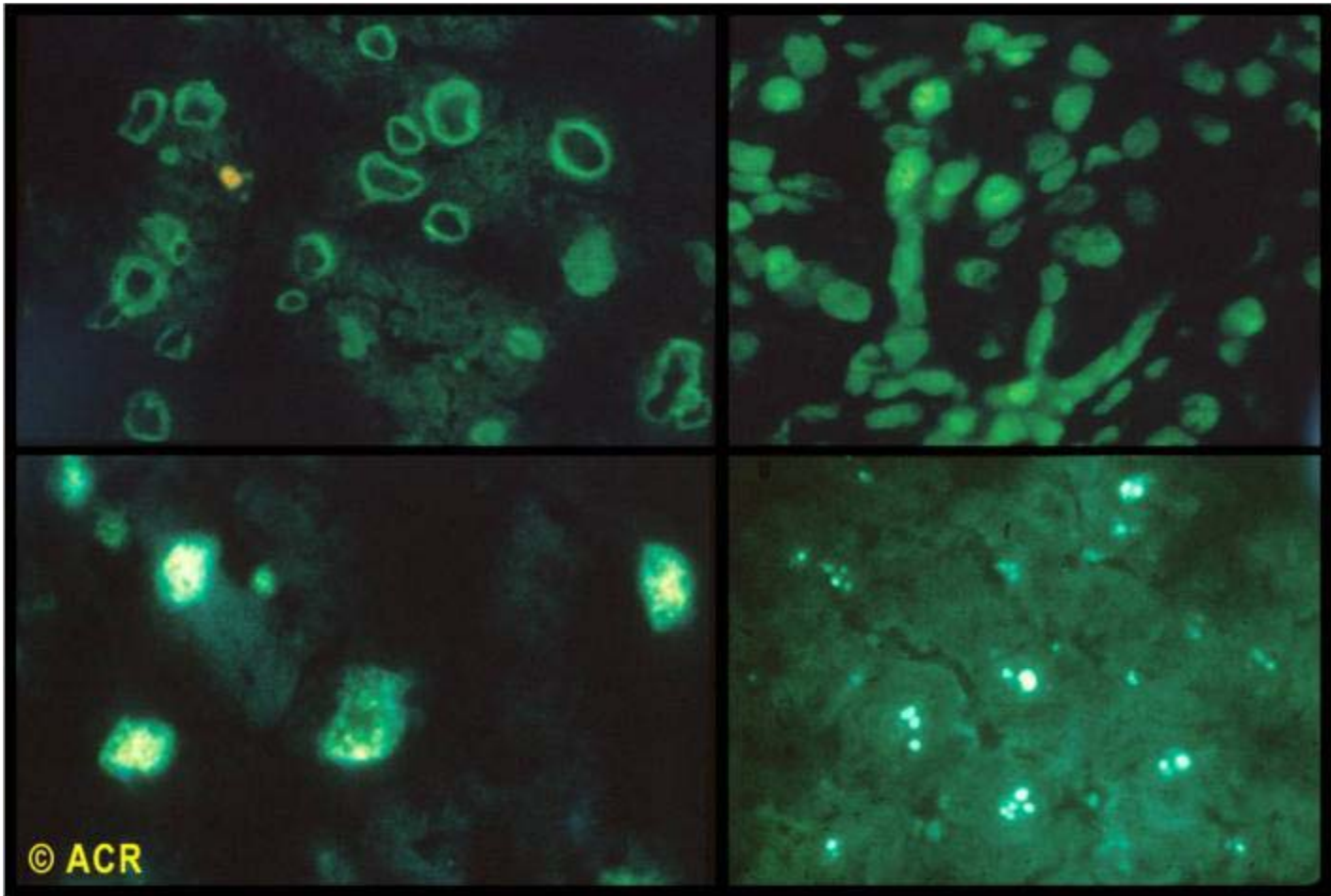
Cytoplasm

Slide

**Fluorescent Labelled
Anti-Immunoglobulin**



ANA



ANA in different rheumatic diseases

Condition	Patients with ANAs (%)
Diseases for Which ANA Testing Is Helpful for Diagnosis	
Systemic lupus erythematosus	99-100
Systemic sclerosis	97
Polymyositis/dermatomyositis	40-80
Sjögren's syndrome	48-96
Diseases for Which ANA Is Required for Diagnosis	
Drug-induced lupus	100
Mixed connective tissue disease	100
Autoimmune hepatitis	100
Diseases for Which ANA May Be Useful for Prognosis	
Juvenile rheumatoid arthritis	20-50
Antiphospholipid antibody syndrome	40-50
Raynaud's phenomenon	20-60

Condition	Patients with ANAs (%)
Diseases for Which ANA Is Typically not Useful	
Discoid lupus erythematosus	5-25
Fibromyalgia	15-25
Rheumatoid arthritis	30-50
Relatives of patients with autoimmune diseases	5-25
Multiple sclerosis	25
ITP	10-30
Thyroid disease	30-50
Patients with silicone breast implants	15-25
Infectious diseases	Varies widely
Malignancies	Varies widely

ANA in healthy individuals

Normal Individuals

$\geq 1:40$	20-30%
$\geq 1:80$	10-12%
$\geq 1:160$	5%
$\geq 1:320$	3%

➔ Significance of positive ANA depends on pretest probability

ANA- Indications for Testing

- When there is clinical suspicion of:
 - SLE
 - Drug-induced Lupus
 - MCTD
 - Scleroderma
 - Sjogren
- Prognostic information for patients with Raynaud phenomena identifying those at risk to develop rheumatic diseases.

ANA- Sensitivity

- 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.
 - 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%)

Anti-SSA/SSB

- 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- β 2 glycoprotein-I (IgM and IgG)

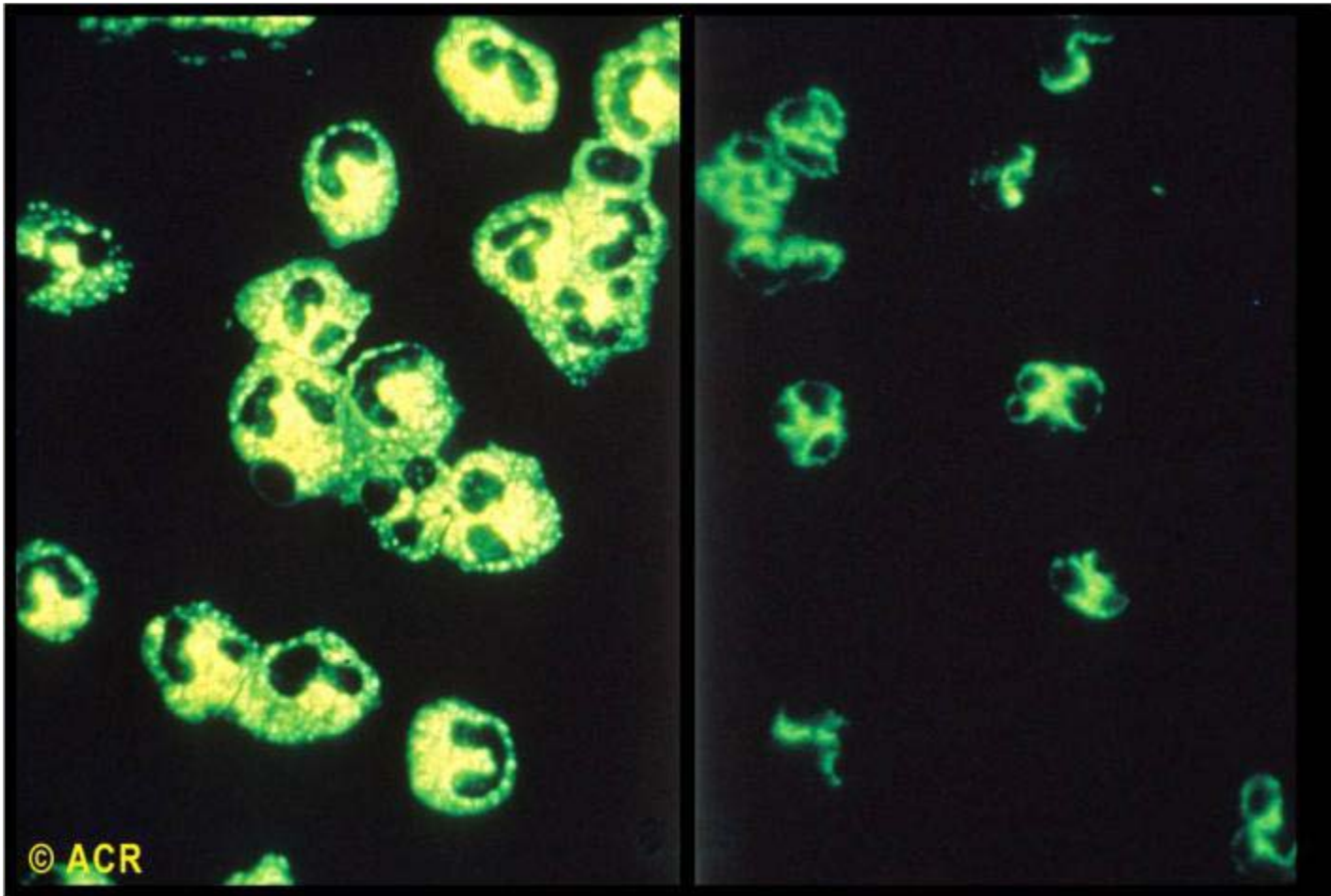
Screen in case of:

- 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

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Thank You