Autoimmune testing according to Berney

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1. **Nonspecific tests:**
   
   - Hematocrit
   - Platelet count
   - ESR
   - CRP
   - C3/C4/CH50

2. **Specific Antibody tests:**
   
   - RF
   - CCP
   - ANA
   - DS-DNA
   - ENA
   - Ro(SSA)/La(SSB)
   - Centromere
   - SCL-70
   - Antisynthetase antibodies
   - ANCA
   - Antiphospholipid antibodies

3. **Joint/Serosal fluid analysis**
35 year old female with a H/O bilateral MCP, PIP, wrist, knee, ankle and MTP swelling for 3 years. 6 hours of AM stiffness. Difficulty with ADL. Fatigue and 20 lb weight loss.

PE: extensive synovitis

Labs: RF+ (1:8),
- HCT=31 with MCV=78
- Platelet=578 (150-270)
- ESR=31 (<20)
- CRP=7 (<1)
Hematocrit

Anemia of chronic disease is presumably 2° to cytokine inhibition of the bone marrow (TNF-α, IL-1, IL-6, IL-8).

HCT = 28-35%
Borderline low-normal RBC MCV = 76-83
Fe- ↓
TIBC- ↓ or nl
Ferritin- nl or ↑

As inflammation ↓, HCT ↑
Platelet count

In inflammatory disease, the platelet count parallels inflammation.

Elevated platelet count reflects ongoing inflammation.

As the inflammation is adequately treated, the platelet count decreases.
ESR

Most widely used inflammatory measurement available.

Actually measures the repellence of RBC as they sediment in plasma and only indirectly reflects the production of acute phase reactant proteins which interfere with the RBC sedimentation.

2 methods- Westergren
Wintrobe
Normal

Inflammation

RBC

repel

RBC

fibrinogen
gamma globulin
alpha globulin
Westergren method:

2 ml of blood + anticoagulant (EDTA or NaCitrate)

1 hour

distance sedimented (mm)

sedimented RBC
Inflammation (and the resulting acute phase protein production) increases ESR by:

1. Neutralizing the RBC repulsion.

2. Producing heavier RBCs
ESR is very sensitive BUT not specific

ESR is influenced by:

- RBC shape
- HCT - anemia $\rightarrow$ $\uparrow$ ESR
- polycythemia $\rightarrow$ $\downarrow$ ESR
- Age - $\uparrow$ age $\rightarrow$ $\uparrow$ ESR
- Food - fatty meals $\rightarrow$ $\uparrow$ ESR
- Ig levels - $\uparrow$ Ig $\rightarrow$ $\uparrow$ ESR
- Gender - females have $>$ ESR than males
Arguments against measuring ESR:

1. ESR is only an indirect reflection of acute phase protein concentration.
2. Monoclonal Igs increase ESR.
3. It is impossible to correct for RBC size, shape, HCT.
4. Plasma viscosity influences ESR.
5. Gender/age influence ESR.
6. ESR may take up to 10 days to increase in inflammation (if it increases at all).

But the cost, ease and turn around time make it attractive.
Nonrheumatologic conditions with ↑ ESR:

1. Aging
2. Anemia
3. Cirrhosis
4. Heparin
5. Hyperlipidemia
6. Infection (Tuberculosis)
7. Macroglobulinemia (Myeloma)
8. Malignancy
9. Pregnancy
C-Reactive Protein (CRP)

Predates dinosaurs (isolated from horseshoe crab).

Produced in liver, probably by hepatic macrophages. (? 2° to IL-1)

Its exact role is unknown however:

1. Activates classic complement pathway.
2. Activates neutrophils.
3. Enhances phagocytosis.
4. May interfere with platelet activation by inhibiting platelet activating factor.
CRP

Rises within a few hours of inflammation onset or tissue injury.

Peaks 2-3 days after an acute stimulus.

Remains elevated in infection and chronic inflammation and falls rapidly with adequate therapy.

80-85% situations that CRP > 10 the patient has a bacterial infection.
<table>
<thead>
<tr>
<th>Rheumatic diseases with elevated CRP:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ankylosing spondylitis</td>
</tr>
<tr>
<td>Behcet’s</td>
</tr>
<tr>
<td>Chronic juvenile arthritis</td>
</tr>
<tr>
<td>Gout</td>
</tr>
<tr>
<td>Polymyalgia rheumatica</td>
</tr>
<tr>
<td>Psoriatic arthritis</td>
</tr>
<tr>
<td>RA</td>
</tr>
<tr>
<td>Reiter’s syndrome</td>
</tr>
<tr>
<td>SLE</td>
</tr>
<tr>
<td>Vasculitis</td>
</tr>
<tr>
<td>Wegener’s granulomatosis</td>
</tr>
<tr>
<td>Normal or minimal elevation (&lt;1 mg/dl)</td>
</tr>
<tr>
<td>----------------------------------------</td>
</tr>
<tr>
<td>Angina</td>
</tr>
<tr>
<td>Common cold</td>
</tr>
<tr>
<td>CVA</td>
</tr>
<tr>
<td>Gingivitis</td>
</tr>
<tr>
<td>Pregnancy</td>
</tr>
<tr>
<td>Seizure</td>
</tr>
<tr>
<td>Vigorous exercise</td>
</tr>
</tbody>
</table>
C3/C4/CH50(CH100)

Acts as an acute phase reactant.

Acute inflammation, infection, or tissue injury cause increased hepatic complement production.

C4 may be more sensitive than C3 in detecting complement activation/consumption.

↓ C4 may even precede a SLE exacerbation in individual patients.
<table>
<thead>
<tr>
<th>C3</th>
<th>C4</th>
<th>CH50</th>
<th>Disease state</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>Acute inflammation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Acute infection</td>
</tr>
<tr>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>Active SLE</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Active serum sickness</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Post streptococcal glomerulonephritis</td>
</tr>
<tr>
<td>↓</td>
<td>nl</td>
<td>↓</td>
<td>Chronic SLE</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C3 deficiency</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Membranoproliferative GN</td>
</tr>
<tr>
<td>nl</td>
<td>↓</td>
<td>↓</td>
<td>Acute SLE</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C4 deficiency</td>
</tr>
<tr>
<td>nl</td>
<td>nl</td>
<td>↓</td>
<td>Complement deficiency</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(other than C3,C4)</td>
</tr>
<tr>
<td>nl</td>
<td>↓</td>
<td>nl</td>
<td>C1 esterase inhibitor deficiency</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Hereditary Angioedema)</td>
</tr>
</tbody>
</table>
**Hx:** 35 year old female with a H/O bilateral MCP, PIP, wrist, knee, ankle and MTP swelling for 3 years. 6 hours of AM stiffness. Difficulty with ADL. Fatigue and 20 lb weight loss.

**PE:** extensive synovitis

**Labs:** RF+ (1:8), HCT = 31 with MCV = 78, Platelet = 578K, ESR = 31, CRP = 7

**Response:** After 8 months of Arava and Sulfasalazine the patient has minimal synovitis, 30 minutes AM stiffness and HCT = 38, Plt = 280K, ESR = 31, CRP = 2.
Specific antibody tests:

a. Rheumatoid factor (RF)
b. CCP
c. ANA and subsets:
   DS DNA (native DNA)
   ENA (SM and RNP)
   Ro (SSA)/ La(SSB)
   Centromere
   SCL-70
d. Antisynthetase antibodies (Jo-1, PM)
e. ANCA
f. Antiphospholipid antibodies
Autoantibody directed against the Fc portion of IgG. Classic RF is an IgM isotype, however RF also occurs as IgG, IgA, IgE.

Test is either done by latex agglutination or by nephelometry.

LSU uses the agglutination method.
If IgM RF is present, the beads will clump. Patient serum with IgM RF

If IgM RF is absent, the beads will not clump.

Latex bead coated with human IgG

Positive RF

If IgM RF is present, the beads will clump.

Patient serum with IgM RF

Negative RF

Patient serum with IgG RF

If IgM RF is absent, the beads will not clump.
The amount of light scattered by a suspension of small particles is proportional to its concentration within a solution. The reflected light is measured and compared to the amount of scatter from known mixtures.

Patient sera (containing RF) is mixed with a RF nephelometry reagent (containing human IgG). The resulting immune complexes reflect the light. The computer which interprets the scattered light rays measures all RF isotypes.
75-80% of RA patients are RF+ (sero+).

Sensitivity of RF for RA is 75% but the specificity is much less.

RA patients with high titers of RF have a worse prognosis with more severe joint disease and extraarticular manifestations.

Serial RF are not very helpful in following RA disease activity.
Rheumatologic/Autoimmune disease with RF

Cryoglobulinemia
Dermatomyositis
Hashimoto’s thyroiditis
Polymyositis
RA
Sarcoidosis
Scleroderma
Sjogren’s syndrome
SLE
Wegener’s Granulomatosis
Infectious disease with RF

Viral-
- CMV
- EBV
- Hepatitis
- HIV
- Influenza
- Rubella

Bacterial-
- Leprosy
- SBE
- Syphilis
- TB

Parasitic-
- Filiariasis
- Malaria
- Schistosomiasis
- Trypanosomiasis
Other conditions with RF

Chronic liver disease
Interstitial pulmonary fibrosis
Malignancy
Periodontal disease
Waldenstrom’s macroglobulinemia

Aging- >20% individuals 90+ years old.

Normal individuals- 5-10% of the normal population
Anti-cyclic citrullinated peptide antibody (anti-CCP) is an antibody against the filament-aggregating protein, filaggrin (which contains citrulline).

Anti-CCP antibodies may predict disease persistence and radiographic joint damage in early arthritis.

Anti-CCP antibody is supposed to be VERY specific but not sensitive, which may identify patients with early RA and distinguish their disease from other types of arthritis.

It may be a key serologic marker in the near future.
Antinuclear antibody (ANA)

ANA is directed against nuclear components.

Significant titer is $\geq 1:80$

Found in 95% SLE patients but also-
- Interstitial pulmonary fibrosis
- MCTD
- PSS
- Polymyositis
- Procainamide/Hydralazine/Dilantin
- RA
- Sjogrens syndrome

Normal population
<table>
<thead>
<tr>
<th>Condition</th>
<th>Rodent tissue</th>
<th>Hep-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE</td>
<td>90-95</td>
<td>98</td>
</tr>
<tr>
<td>Scleroderma</td>
<td>50-70</td>
<td>95</td>
</tr>
<tr>
<td>Rheumatoid Arthritis</td>
<td>30-50</td>
<td>50-75</td>
</tr>
<tr>
<td>1&lt;sup&gt;o&lt;/sup&gt; Sjogrens Syndrome</td>
<td>50</td>
<td>75</td>
</tr>
<tr>
<td>Polymyositis</td>
<td>30</td>
<td>90</td>
</tr>
<tr>
<td>Chronic Juvenile Arthritis</td>
<td>25</td>
<td>?</td>
</tr>
<tr>
<td>Chronic Active Hepatitis</td>
<td>50</td>
<td>?</td>
</tr>
<tr>
<td>Infectious Mononucleosis</td>
<td>5-20</td>
<td>?</td>
</tr>
<tr>
<td>Lepromatous Leprosy</td>
<td>5-20</td>
<td>?</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>5-20</td>
<td>?</td>
</tr>
<tr>
<td>Subacute Bacterial Endocarditis</td>
<td>5-20</td>
<td>?</td>
</tr>
</tbody>
</table>
4 common ANA patterns:

1. Homogeneous/diffuse- seen in all CTD diseases.

2. Speckled- seen in patients with ab to Sm, RNP, centromere, and Scl-70. Seen in Scleroderma, overlap syndromes, RA and Sjogren’s syndrome.


4. Peripheral/Rim- seen in patients with ab to DS-DNA. Very specific for SLE.
anti-DS-DNA antibody

Also known as anti-native DNA antibody.

Antibodies to DS-DNA are not sensitive but are very specific for SLE.

They indicate patients at increased risk for significant SLE renal disease.

DS-DNA levels may parallel lupus activity in individual patients.

Any titer is significant.
Smith (Sm) and Ribonuclear protein (RNP) are the extractable nuclear antigens (ENA).

Antibodies to Sm are not sensitive but are as specific as DS-DNA for SLE.

Antibodies to RNP- seen in SLE, MCTD, RA, PSS.
anti-Ro (SSA)/La (SSB) antibodies

Sjogren’s antibodies.

Ro and La are found in both the cell’s cytoplasm and the nucleus.

Ro and La are present in:
- Sjogren’s syndrome
- SLE
- RA
- PSS
Antibodies to Ro (SSA) are also found in

1. Subacute cutaneous lupus erythematosus- 63% patients have Ro.

2. Neonatal SLE- the vast majority have Ro ± La especially if neonate has heartblock.
Anticentromere antibody

Found in the 80-90% of patients with the subset of scleroderma patients now called limited or localized scleroderma (formerly known as CREST).

Only 10-15% of generalized scleroderma have this antibody.
Antibody to topoisomerase 1.

Found in 20-40% generalized scleroderma patients.

60-70% of patients with anti-SCL-70 ab have PSS.

Only 20% of patients with limited PSS have SCL-70.

Its presence predicts a worse prognosis.
anti-synthetase antibodies

Histidyl-tRNA synthetase (Jo-1)
Threonyl-tRNA synthetase (PL-7)
Alanyl-tRNA synthetase (PL-12)
Glycyl-tRNA synthetase (EJ)
Isoleucyl-tRNA synthetase (OJ)
anti-synthetase antibody syndrome:

1. Polymyositis/Dermatomyositis with relatively acute onset
   interstitial lung disease
   fever
   arthritis
   Raynaud’s phenomenon
   “Mechanic’s” hands (darkened or dirty-appearing cracking and fissuring of the lateral and palmar aspects of the fingers as seen on an auto-mechanic).
   AND

Antineutrophil Cytoplasmic Antibody (ANCA)

Antibodies directed against proteolytic enzymes in the alpha granule of the neutrophil.

May activate neutrophils thus contributing to disease OR May only be marker of disease.

2 staining patterns by immunofluorescence:
  perinuclear staining (pANCA)
  diffuse cytoplasmic staining (cANCA)
ANCA patterns by Immunofluorescence:

1. perinuclear staining pattern (pANCA).
   >70% of pANCA is directed against myeloperoxidase (MPO). Other pANCA antigens include cathepsin G, human leukocyte elastase and lactoferrin. Associated with PAN and Churg-Strauss.

2. diffuse cytoplasmic staining pattern (cANCA).
   >90% of cANCA is directed against Proteinase 3 (PR3). Associated with Wegener’s Granulomatosis.
## ANCA in Connective Tissue Disease:

<table>
<thead>
<tr>
<th>Condition</th>
<th>cANCA</th>
<th>pANCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ank Spond</td>
<td>0/50</td>
<td>0/50</td>
</tr>
<tr>
<td>Felty’s</td>
<td>0/14</td>
<td>3/14</td>
</tr>
<tr>
<td>MCTD</td>
<td>0/32</td>
<td>0/32</td>
</tr>
<tr>
<td>Polymyositis</td>
<td>0/10</td>
<td>1/10</td>
</tr>
<tr>
<td>Psoriatic arthritis</td>
<td>0/32</td>
<td>5/32</td>
</tr>
<tr>
<td>PSS</td>
<td>0/43</td>
<td>0/43</td>
</tr>
<tr>
<td>RA</td>
<td>0/241</td>
<td>6/241</td>
</tr>
<tr>
<td>Reiter’s</td>
<td>0/29</td>
<td>0/29</td>
</tr>
<tr>
<td>SLE</td>
<td>0/109</td>
<td>4/109</td>
</tr>
<tr>
<td>SLE vasculitis</td>
<td>0/21</td>
<td>0/21</td>
</tr>
</tbody>
</table>
ANCA in Vasculitis:

<table>
<thead>
<tr>
<th>Disease</th>
<th>cANCA</th>
<th>pANCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behcet’s</td>
<td>0/21</td>
<td>0/21</td>
</tr>
<tr>
<td>Churg-Strauss</td>
<td>4/13</td>
<td>1/13</td>
</tr>
<tr>
<td>Henoch-Schonlein</td>
<td>0/18</td>
<td>0/18</td>
</tr>
<tr>
<td>PAN</td>
<td>14/49</td>
<td>2/49</td>
</tr>
<tr>
<td>PMR</td>
<td>0/62</td>
<td>5/62</td>
</tr>
<tr>
<td>Takayasu’s</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Temporal arteritis</td>
<td>0/24</td>
<td>2/24</td>
</tr>
<tr>
<td>Unclassified vasculitis</td>
<td>8/110</td>
<td>9/110</td>
</tr>
<tr>
<td>Wegener’s</td>
<td>295/383</td>
<td>20/383</td>
</tr>
</tbody>
</table>
Other diseases with ANCA:

Bronchial carcinoma
Colon carcinoma
Endocarditis
Glomerular basement membrane disease (Goodpasture’s)
HIV
Mucoviscidosis
Sclerosing cholangitis (pANCA)
Ulcerative colitis (pANCA)
Antiphospholipid antibody

Directed against phospholipids in the body.

Occurs as IgG, IgA or IgM isotypes.

Clinically associated with:
1. Recurrent vascular thrombosis (IgG).
2. Recurrent fetal loss (IgM).
3. False + VDRL.
4. Falsely prolonged PTT.
5. Lupus anticoagulant.
6. Libman-Sachs endocarditis of SLE.
7. Livedo Reticularis.
8. Thrombocytopenia
### Classification of synovial effusions

<table>
<thead>
<tr>
<th>Type of fluid</th>
<th>Color</th>
<th>WBC/mm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Clear (colorless)</td>
<td>&lt;200 (&lt;25% PMN)</td>
</tr>
<tr>
<td>Noninflammatory</td>
<td>Clear-Yellow</td>
<td>200-2,000 (&lt;25% PMN)</td>
</tr>
<tr>
<td>Inflammatory</td>
<td>Coudy Yellow</td>
<td>2,000-100,000 (&gt;50% PMN)</td>
</tr>
<tr>
<td>Septic</td>
<td>Purulent</td>
<td>&gt;50,000 (&gt;75% PMN)</td>
</tr>
</tbody>
</table>
Joint fluid tests

Cell count with differential
Crystal analysis
Gram stain with culture and sensitivity

NO Protein, Glucose, LDH

Most important are:

crystals and culture
<table>
<thead>
<tr>
<th></th>
<th>SLE</th>
<th>RA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protein (g/dl)</strong></td>
<td>&gt;3.0</td>
<td>&gt;3.0</td>
</tr>
<tr>
<td><strong>LDH (IU)</strong></td>
<td>(\leq 500)</td>
<td>(\geq 700)</td>
</tr>
<tr>
<td><strong>Glucose (mg/dl)</strong></td>
<td>&gt;80</td>
<td>&lt;30</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>(\geq 7.35)</td>
<td>(\leq 7.2)</td>
</tr>
</tbody>
</table>
Pleural fluid ANA and complement levels are not specific for SLE and have been found in patients with empyema and malignancy but have not been extensively tested in other autoimmune diseases. **As a result** we don’t routinely measure ANA or C3/C4.

Pleural RF is found in TB, malignancy and other infections and as a result is also unhelpful.