Advance technology in autoimmunity tests

Dr. Chia-Ching Lin
Global marketing autoimmunity, Immunodiagnostics division
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Sweden

- **Uppsala** – Allergy
  - Global headquarters
  - PHC (Pharmaceutical and Healthcare Collaborations)
- **Helsingborg** – Allergy
  - Allergon – Allergen raw material

Germany

- **Freiburg** – Autoimmunity
The product range in autoimmunity

- Connective tissue diseases
- Rheumatoid arthritis
- Anti-phospholipid syndrome
- ANCA-associated vasculitis
- Celiac disease
- Inflammatory bowel diseases
- Thyroid diseases
- Autoimmune liver diseases
50 markers for > 20 different autoimmune diseases
Phadia Laboratory Systems

Phadia 100

Phadia 250

Phadia 2500

Phadia 5000
Pathogenesis of autoimmune diseases

- Mostly, T cells are the trigger.
- Autoantibodies are usually not triggers but useful markers.
- Genetic predisposition (specific HLA class II alleles)
- More frequent in women – female hormones increase the risk, disease often starts in times of hormonal changes.
- Possible triggers for AI diseases:
  - Viral or bacterial infections (cross reactivity with common epitopes)
  - Wrong expression of MHC class II antigens of normal tissue cells
  - Vaccination
  - Antibiotics
  - …
Modern technology used nowadays to help autoimmune disease diagnosis - ANCA-associated vasculitis
### Connective Tissue Diseases
- EliA CTD Screen
- EliA Symphony®
- EliA dsDNA
- EliA U1RNP
- EliA RNP70
- EliA SmD®
- EliA Ro
- EliA Ro52
- EliA Ro60
- EliA La

### Rheumatoid Arthritis
- EliA Sc1 70
- EliA Jo-1
- EliA CENP
- EliA Rb-P
- EliA PCNA
- EliA PM-Scl
- EliA Fibrillarin
- EliA Mi-2
- EliA ssDNA
- EliA RNA Pol III

### Anti-Phospholipid Syndrome
- EliA β2 Glycoprotein-1 IgG
- EliA β2 Glycoprotein-1 IgM
- EliA β2 Glycoprotein-1 IgA
- EliA Cardiolipin IgG
- EliA Cardiolipin IgM
- EliA Cardiolipin IgA

### Vasculitis
- EliA MPO®
- EliA PR3®
- EliA GBM

### Celiac Disease
- EliA Gliadin IgA
- EliA Gliadin IgG
- EliA Gliadin DP IgA
- EliA Gliadin DP IgG
- EliA Celikey IgA
- EliA Celikey IgG

### IBD
- EliA Calprotectin2
- EliA ASCA IgG
- EliA ASCA IgA

### Thyroid
- EliA anti-TG
- EliA anti-TPO
- EliA anti-TSH-R

### Miscellaneous
- EliA Anti-IgA
- EliA Intrinsic Factor
- EliA Parietal Cells
Antic-Neutrophil Cytoplasmic Antibodies on indirect immunofluorescence assay (IIF)

- cytoplasmic ANCA = c-ANCA
  - Antigen in most cases anti-proteinase 3 (PR3)
- perinuclear ANCA = p-ANCA
  - Antigen in most cases anti-myeloperoxidase (MPO)
  - sometimes other enzymes from granulocytes, but in these cases usually not related to vasculitis
- atypical ANCA:
  - Not identifiable as p- or c-ANCA
  - Different antigens, usually not specific for ANCA-associated vasculitis
IIF as first-line test

Recommendations from 1990:
IIF ANCA as first-line test, all positives measured on antigen-specific tests.

Multicenter study 2016:
IIF ANCA have a much lower likelihood ratio than antigen-specific tests.

Damoiseaux et al. 2016. Ann Rheum Dis 2016;0:1
“Consequently, dual IIF/antigen-specific immunoassay testing of each sample is not necessary for maximal diagnostic accuracy. These results indicate that the current international consensus on ANCA testing for AAV needs revision.”
Revised 2017 international consensus on testing of ANCAs in granulomatosis with polyangiitis and microscopic polyangiitis

Xavier Bossuyt¹, Jan-Willem Cohen Tervaert², Yoshihiro Arimura³, Daniel Blockmans⁴, Luis Felipe Flores-Suárez⁵, Loïc Guillevin⁶, Bernhard Helmich⁷, David Jayne⁸, J. Charles Jennette⁹, Cees G. M. Kallenberg¹⁰, Sergey Moiseev¹¹, Pavel Novikov¹¹, Antonella Radice¹², Judith Anne Savidge¹³, Renato Alberto Sinico¹⁴, Ulrich Specks¹⁵, Pieter van Paassen¹⁶, Ming-hui Zhao¹⁷, Niels Rasmussen¹⁸, Jan Damoiseaux¹⁹ and Elena Csernok⁷

New Consensus

New recommendations
In this Consensus Statement, we recommend the use of high-quality immunoassays as the preferred first screening method for GPA and MPA, and put forward a new testing algorithm (recommendations 1–6).

(a) 1999 consensus
(b) 2017 consensus

Ideal approach
- IIF
  - IIF- ANCA-
  - IIF- ANCA+
  - IIF+ ANCA-
  - IIF+ ANCA+

Recommended approach
- IIF
  - IIF-
    - PR3-ANCA and MPO-ANCA
    - Immunoassays
  - IIF+
    - PR3-ANCA and MPO-ANCA
    - Immunoassays

Recommended approach
- Suspected AAV
  - Gating strategy
    - Non AAV
    - Not covered by this consensus
- ANCA-
  - Second assay*
- ANCA+
  - Second assay*

Will ANCA IIF be obsolete?

• For autoimmune vasculitis, ANCA IIF is no longer deemed suitable as the first screening test\(^1\).

• However, for hepatitis and inflammatory bowel syndrome, ANCA IIF still might be of interest\(^1\).

• For these diseases, antigen-specific tests such as EliA PR3\(^S\) and EliA MPO\(^S\) are not of diagnostic use, as in most cases, other antigens are responsible for the ANCA pattern\(^1\).

How to interpret the test result?

• What is the meaning behind the numbers?

• Example: 10 IU/ml EliA MPO\textsuperscript{S} \textit{(Cutoff} = 5 IU/ml\textit{)}

• How high is the risk for the patient to have an autoimmune vasculitis? (Post-test probability)
• Likelihood Ratio in intervals of antibody titer.

• A patient with relatively low probability for vasculitis (eg. Radiographic presence of pulmonary infiltrates or nodules)

• How much information does a test result give? How much more probable is vasculitis?

<table>
<thead>
<tr>
<th>EliA MPOs and EliA PR3s</th>
<th>Positive Likelihood Ratio</th>
<th>Percentage of vasculitis patients in a multicenter study (n=1175)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 2.1 IU/ml</td>
<td>0.1</td>
<td>10%</td>
</tr>
<tr>
<td>2.1 – 4.9 IU/ml</td>
<td>3.35</td>
<td>8%</td>
</tr>
<tr>
<td>5.0 – 16.0 IU/ml</td>
<td>12</td>
<td>18%</td>
</tr>
<tr>
<td>16.0 – 142.0 IU/ml</td>
<td>59</td>
<td>57%</td>
</tr>
<tr>
<td>142.0 – 180.0 IU/ml</td>
<td>∞</td>
<td>7%</td>
</tr>
</tbody>
</table>

How to interpret test results? Example: 10% pre-test probability

10% pre-test probability
• radiographic evidence of mucosal thickening involving one or more sinuses
• radiographic presence of pulmonary infiltrates or nodules, or both

85 % pre-test probability

- radiographic evidence of mucosal thickening involving one or more sinuses
- radiographic presence of pulmonary infiltrates or nodules, or both
- urinalysis demonstrating hematuria and red blood cell casts

Modern technology used nowadays to help autoimmune disease diagnosis - Rheumatoid arthritis
<table>
<thead>
<tr>
<th>Connective Tissue Diseases</th>
<th>Rheumatoid Arthritis</th>
<th>Anti-Phospholipid Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>EliA CTD Screen</td>
<td>EliA CCP IgG</td>
<td>EliA β2 Glycoprotein-I IgG</td>
</tr>
<tr>
<td>EliA SymphonyS</td>
<td>EliA RF IgM</td>
<td>EliA β2 Glycoprotein-I IgM</td>
</tr>
<tr>
<td>EliA dsDNA</td>
<td>EliA RF IgA</td>
<td>EliA Cardiolipin IgG</td>
</tr>
<tr>
<td>EliA U1RNP</td>
<td>EliA RF IgG</td>
<td>EliA Cardiolipin IgM</td>
</tr>
<tr>
<td>EliA RNP70</td>
<td></td>
<td>EliA Cardiolipin IgA</td>
</tr>
<tr>
<td>EliA SmDp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EliA Ro</td>
<td>EliA MPOS</td>
<td></td>
</tr>
<tr>
<td>EliA Ro52</td>
<td>EliA PR3S</td>
<td></td>
</tr>
<tr>
<td>EliA Ro60</td>
<td>EliA ssDNA</td>
<td></td>
</tr>
<tr>
<td>EliA La</td>
<td>EliA RNA Pol III</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Celiac Disease</th>
<th>IBD</th>
<th>Thyroid</th>
<th>Miscellaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>EliA Gliadin IgA</td>
<td>EliA Calprotectin2</td>
<td>EliA anti-TG</td>
<td>EliA Anti-IgA</td>
</tr>
<tr>
<td>EliA Gliadin IgG</td>
<td>EliA ASCA IgG</td>
<td>EliA anti-TPO</td>
<td>EliA Intrinsic Factor</td>
</tr>
<tr>
<td>EliA Gliadin^{DP}_IgA</td>
<td>EliA ASCA IgA</td>
<td>EliA anti-TSH-R</td>
<td>EliA Parietal Cells</td>
</tr>
<tr>
<td>EliA Gliadin^{DP}_IgG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EliA Celikey IgA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EliA Celikey IgG</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Current ACR classification criteria

A score of at least 6/10 is needed for classification of a patient as having definite RA

**1. Joint involvement**

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1 large joint</td>
</tr>
<tr>
<td>1</td>
<td>2 – 10 large joints</td>
</tr>
<tr>
<td>2</td>
<td>1 – 3 small joints (with or without involvement of large joints)</td>
</tr>
<tr>
<td>3</td>
<td>4 – 10 small joints (with or without involvement of large joints)</td>
</tr>
<tr>
<td>5</td>
<td>&gt;10 joints (at least 1 small joint)</td>
</tr>
</tbody>
</table>

**2. Serology** (at least 1 test result is needed for classification)

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Negative RF and negative ACPA</td>
</tr>
<tr>
<td>2</td>
<td>Low-positive RF or low-positive ACPA</td>
</tr>
<tr>
<td>3</td>
<td>High-positive RF or high-positive ACPA</td>
</tr>
</tbody>
</table>

**3. Acute-phase reactants** (at least 1 test result is needed for classification)

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal CRP and normal ESR</td>
</tr>
<tr>
<td>1</td>
<td>Abnormal CRP or abnormal ESR</td>
</tr>
</tbody>
</table>

**4. Duration of symptoms**

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt;6 weeks</td>
</tr>
<tr>
<td>1</td>
<td>≥6 weeks</td>
</tr>
</tbody>
</table>

CCP antibodies appear in early stage of disease

- Anti-CCP may appear years before first symptoms occur.

The target: gain time

Joint damage and functional disability

window of opportunity for early efficient treatment opened by CCP!

Diagnosis

without treatment
c conventional treatment
treatment with biologicals
treatment with biologicals

time
## Antibody prevalence in associated disease(s)

<table>
<thead>
<tr>
<th>Associated Disease(s)</th>
<th>Antibody Prevalence [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatoid Arthritis</td>
<td>70-80</td>
</tr>
<tr>
<td></td>
<td>0-15</td>
</tr>
<tr>
<td>Psoriatic Arthritis</td>
<td>7-16</td>
</tr>
</tbody>
</table>

### EliA CCP Well

<table>
<thead>
<tr>
<th>Rheumatoid Arthritis</th>
<th>70-80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juvenile Idiopathic Arthritis (but associated with polyarticular manifestation)</td>
<td>0-15</td>
</tr>
<tr>
<td>Psoriatic Arthritis</td>
<td>7-16</td>
</tr>
</tbody>
</table>

### EliA RF IgM Well

<table>
<thead>
<tr>
<th>Rheumatoid Arthritis</th>
<th>70-80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sjögren's Syndrome</td>
<td>55-70</td>
</tr>
<tr>
<td>Systemic Lupus Erythematosus</td>
<td>15-35</td>
</tr>
<tr>
<td>Scleroderma</td>
<td>20-30</td>
</tr>
<tr>
<td>Mixed Connective Tissue Disease</td>
<td>50-60</td>
</tr>
<tr>
<td>Granulomatosis With Polyangiitis</td>
<td>5-20</td>
</tr>
<tr>
<td>Endocarditis Lenta</td>
<td>25-60</td>
</tr>
<tr>
<td>Chronic hepatitis Primary Biliary Cirrhosis</td>
<td>15-70</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>15</td>
</tr>
<tr>
<td>Bacterial Infections</td>
<td>5-60</td>
</tr>
<tr>
<td>Parasite infections</td>
<td>20-90</td>
</tr>
<tr>
<td>Viral Infections</td>
<td>15-65</td>
</tr>
</tbody>
</table>

Why is testing of RF still indicated?

- Combination of anti-CCP and RF IgM for a reliable diagnosis of RA according to the diagnostic criteria\(^1\).

- Individual RF isotype measurement for a better prognosis of RA to help the clinician in the treatment decision\(^2\).

- RF isotypes with high titer have a good specificity for RA to differentiate from other diseases\(^1,3\).

More reliable diagnosis of RA through the combination of EliA CCP and EliA RF IgM, IgA and IgG

<table>
<thead>
<tr>
<th>Test results</th>
<th>Interpretation</th>
<th>Probability for RA</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF IgM</td>
<td>RF IgA</td>
<td>RF IgG</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
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<tr>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Triple positivity of RF isotypes makes RA almost certain, even in CCP-negative patients!

„Measurement of all 3 isotypes of RF may increase by 7- to 21-fold the chance of making the serologic diagnosis of RA.“2010)

Juvenile idiopathic arthritis (JIA)

- JIA comprises a heterogeneous group of rheumatic joint disease with an onset in childhood (before 16th of age).

- Autoantibodies are not considered to be of diagnostic help but have relevance in differential diagnosis\(^1\).

- International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001\(^2\)
  - Systemic Arthritis
  - Oligoarthritis
  - Polyarthritis (RF-)
  - Polyarthritis (RF+)
  - Psoriatic arthritis
  - Enthesitis related arthritis
  - Undifferentiated arthritis

- Anti-CCP antibodies are associated with RF positive polyarticular course of JIA


**Table 2 RF positive poly and all JIA vs controls**

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>All JIA</th>
<th>RF + poly JIA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>Titer mean U</td>
<td>N (%)</td>
</tr>
<tr>
<td>ACPA (CCP IgG)</td>
<td>1 (2)</td>
<td>8</td>
<td>48 (14)</td>
</tr>
<tr>
<td>RF IgM</td>
<td>4 (8)</td>
<td>10</td>
<td>39 (12)</td>
</tr>
<tr>
<td>RF IgG</td>
<td>4 (8)</td>
<td>10</td>
<td>26 (8)</td>
</tr>
<tr>
<td>RF IgA</td>
<td>0 (0)</td>
<td>6</td>
<td>27 (8)</td>
</tr>
<tr>
<td>Anti RA33</td>
<td>3 (6)</td>
<td>18</td>
<td>21 (6)</td>
</tr>
<tr>
<td>ANA IFA</td>
<td>3 (6)</td>
<td>-</td>
<td>88 (26)</td>
</tr>
</tbody>
</table>

Controls = 50, cases = 334, and RF positive poly JIA cases 30. Means of ACPA, RF, RA33 values presented as Units/dL Numbers of subjects positive for ANA by immunofluorescence at titers ≥ 1:40 are indicated.

Tebo et al. Pediatric Rheumatology 2012, 10:29
Thank you!
Say goodbye to the last-generation technology

– How tests nowadays help autoimmune disease diagnosis?
## EliA test panel for autoimmune diseases

### Connective Tissue Diseases
- EliA CTD Screen
- EliA Symphony<sup>S</sup>
- EliA dsDNA
- EliA U1RNP
- EliA RNP70
- EliA SmD<sup>P</sup>
- EliA Ro
- EliA Ro52
- EliA Ro60
- EliA La
- EliA Scl 70
- EliA Jo-1
- EliA CENP
- EliA Rib-P
- EliA PCNA
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- EliA PR<sup>3</sup><sup>S</sup>
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### Miscellaneous
- EliA Anti-IgA
- EliA Intrinsic Factor
- EliA Parietal Cells
<table>
<thead>
<tr>
<th>autoimmune connective tissue diseases</th>
<th>Prevalence</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sjögren’s syndrome (SS)</td>
<td>0,5 -1: 100</td>
<td>6,0 : 100.000</td>
</tr>
<tr>
<td>systemic lupus erythematosus (SLE)</td>
<td>3 - 400: 100.000</td>
<td>5,1 : 100.000</td>
</tr>
<tr>
<td>Scleroderma</td>
<td>4 – 253: 1.000.000</td>
<td>21,0 : 1.000.000</td>
</tr>
<tr>
<td>Dermatomyositis / Polymyositis (DM/PM)</td>
<td>15: 1.000.000</td>
<td>6,0 : 1.000.000</td>
</tr>
<tr>
<td>Mixed connective tissue disease (MCTD)</td>
<td>50 : 100.000</td>
<td>2,0 : 1.000.000</td>
</tr>
</tbody>
</table>

• No single characteristic feature
• Common symptom - nonspecific fatigue
• A wide variety of symptoms may occur
  • fever
  • muscle and joint pain and stiffness,
  • weakness
  • many other symptoms
• specific and/or non-specific autoantibodies could present
• Multi-organs are affected, especially skin, joints, lungs.
• Diagnosis: mixture of the examination

laboratory results and image diagnostic aid the final diagnosis of connective tissue
diseases which will be made by the physicians

SLE- first symptoms

- Fatigue
- Hair fall
- Oral Ulcer
- Arthralgia
- Raynaud’s phenomenon
- Fevers
- And many many more

<table>
<thead>
<tr>
<th>9. Hematologic Disorder</th>
<th>1. Hemolytic anemia—with reticulocytosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1. OR</td>
</tr>
<tr>
<td></td>
<td>2. Leukopenia—$&lt;4,000/mm^3$ on $\geq 2$ occasions</td>
</tr>
<tr>
<td></td>
<td>1. OR</td>
</tr>
<tr>
<td></td>
<td>3. Lymphopenia—$&lt;1,500/mm^3$ on $\geq 2$ occasions</td>
</tr>
<tr>
<td></td>
<td>1. OR</td>
</tr>
<tr>
<td></td>
<td>4. Thrombocytopenia—$&lt;100,000/mm^3$ in the absence of offending drugs</td>
</tr>
<tr>
<td></td>
<td>1. OR</td>
</tr>
<tr>
<td>10. Immunologic Disorder</td>
<td>1. Anti-DNA: antibody to native DNA in abnormal titer</td>
</tr>
<tr>
<td></td>
<td>1. OR</td>
</tr>
<tr>
<td></td>
<td>2. Anti-Sm: presence of antibody to Sm nuclear antigen</td>
</tr>
<tr>
<td></td>
<td>1. OR</td>
</tr>
<tr>
<td></td>
<td>3. Positive finding of antiphospholipid antibodies on:</td>
</tr>
<tr>
<td></td>
<td>1. an abnormal serum level of IgG or IgM anticardiolipin antibodies,</td>
</tr>
<tr>
<td></td>
<td>2. a positive test result for lupus anticoagulant using a standard method, or</td>
</tr>
<tr>
<td></td>
<td>3. a false-positive test result for at least 6 months confirmed by Treponema pallidum immobilization or fluorescent treponemal antibody absorption test</td>
</tr>
<tr>
<td>11. Positive Antinuclear Antibody</td>
<td>An abnormal titer of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs</td>
</tr>
</tbody>
</table>

Antibody against Ro/La can cross the placenta and create a syndrome called Neonatal lupus\(^1\).

**Could occur up to**
- 1-2\% of infants from mothers with SLE\(^1,2\)
- 15-20\% of infants from mothers with SLE and anti-Ro Ab\(^1,2\).

**Auto-antibodies directed against Ro52 kDa are associated with a higher risk of congenital heart block (CHB)**\(^3\).

**CHB is believed to affect approximately 2\% of offspring exposed to anti-Ro Ab**\(^4\).

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| 9. Hematologic Disorder | 1. Hemolytic anemia--with reticulocytosis
2. Leukopenia--<4,000/mm³ on ≥ 2 occasions
3. Lymphopenia--<1,500/mm³ on ≥ 2 occasions
4. Thrombocytopenia--<100,000/mm³ in the absence of offending drugs |
|-------------------------|-----------------------------------------------------------------|
| 10. Immunologic Disorder | 1. Anti-DNA: antibody to native DNA in abnormal titer
2. Anti-Sm: presence of antibody to Sm nuclear antigen
3. Positive finding of antiphospholipid antibodies on:
   1. an abnormal serum level of IgG or IgM anticardiolipin antibodies,
   2. a positive test result for lupus anticoagulant using a standard method, or
   3. a false-positive test result for at least 6 months confirmed by Treponema pallidum immobilization or fluorescent treponemal antibody absorption test |
| 11. Positive Antinuclear Antibody | An abnormal titer of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs |

How are ANA detected?

- The most popular screening test for ANA is the indirect immunofluorescence assay (IIF) using HEp-2 cells as substrate.
- IIF detects all ANA with high sensitivity (except for: Ro52\(^1\), Ro60\(^1\), Jo-1\(^2,3\) and Rib-P\(^4\) antibodies).
- What you get as result is a certain pattern

How can you differentiate ANA in IIF?

A homogeneous
B quasihomogeneous
C fine speckled
D coarse speckled
E dense fine speckled
F centromeric

Which Antibodies are responsible for these patterns?

<table>
<thead>
<tr>
<th>Nuclear patterns</th>
<th>Synonyms</th>
<th>Antigen associations</th>
<th>Disease association</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Homogeneous (AC-1)</strong></td>
<td>Diffuse</td>
<td>dsDNA, nucleosomes, histones</td>
<td>SLE, drug-induced lupus, juvenile idiopathic arthritis</td>
</tr>
<tr>
<td><strong>Speckled (AC-2,4,5)</strong></td>
<td>Granular</td>
<td>hnRNP, U1RNP, Sm, SS-A/Ro (Ro60), SS-B/La, RNA polymerase III, Mi-2, Ku DFS70/LEDGF</td>
<td>MCTD, SLE, SjS, DM, SSc/PM overlap</td>
</tr>
<tr>
<td><strong>Dense fine speckled (AC-2)</strong></td>
<td>None</td>
<td>Rare in SLE, SjS, SSc</td>
<td></td>
</tr>
<tr>
<td><strong>Fine speckled (AC-4)</strong></td>
<td>Fine granular</td>
<td>SS-A/Ro (Ro60), SS-B/La, Mi-2, TIF1γ, TIF1β, Ku, RNA helicase A, Replication protein A</td>
<td>SjS, SLE, DM, SSc/PM overlap</td>
</tr>
<tr>
<td><strong>Large/coarse speckled (AC-5)</strong></td>
<td>Spliceosome/nuclear matrix</td>
<td>hnRNP, U1RNP, Sm, RNA polymerase III</td>
<td>MCTD, SLE, SSc</td>
</tr>
<tr>
<td><strong>Discrete nuclear dots</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Centromere (AC-3)</strong></td>
<td>Kinetochores</td>
<td>CENP-A/B (C)</td>
<td>Limited cutaneous SSc, PBC</td>
</tr>
<tr>
<td><strong>Multiple nuclear dots (AC-6)</strong></td>
<td>6–20 nuclear dots, NSpl, PML bodies</td>
<td>Sp100, PML proteins, MJ/NXP-2</td>
<td>PBC, SARD, PM/DM</td>
</tr>
<tr>
<td><strong>Few nuclear dots (AC-7)</strong></td>
<td>1–6 nuclear dots, Cajal bodies (coiled body)</td>
<td>p80-coilin, SMN</td>
<td>SjS, SLE, SSc, PM, asymptomatic individuals</td>
</tr>
<tr>
<td><strong>Nucleolar (AC-8,9,10)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Homogeneous (AC-8)</strong></td>
<td>None</td>
<td>PM/Scl-75, PM/Scl-100, Th/To, B23/nucleophosmin, nucleolin, No55/SC65</td>
<td>SSc, SSc/PM overlap</td>
</tr>
<tr>
<td><strong>Clumpy (AC-9)</strong></td>
<td>None</td>
<td>U3-snoRNP/fibrillarin</td>
<td>SSc</td>
</tr>
<tr>
<td><strong>Punctate (AC-10)</strong></td>
<td>Nucleolar speckled</td>
<td>RNA polymerase I, hUBF/NOR-90</td>
<td>SSc, SjS</td>
</tr>
<tr>
<td><strong>Nuclear envelope (AC-11,12)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Smooth nuclear envelope (AC-11)</strong></td>
<td>Nuclear rim, nuclear membrane, membranous</td>
<td>Lamin A,B,C, or lamin-associated proteins</td>
<td>SLE, SjS, seronegative arthritis</td>
</tr>
<tr>
<td><strong>Punctate nuclear envelope (AC-12)</strong></td>
<td>Nuclear membrane pores</td>
<td>Nuclear pore complex proteins (i.e., gp22)</td>
<td>PBC</td>
</tr>
<tr>
<td><strong>Pleomorphic (AC-13,14)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PCNA-like (AC-13)</strong></td>
<td>None</td>
<td>PCNA</td>
<td>SLE, other conditions</td>
</tr>
<tr>
<td><strong>CENP-F-like (AC-14)</strong></td>
<td>MSA-3, NSp-Ii</td>
<td>CENP-F</td>
<td>Cancer, other conditions</td>
</tr>
</tbody>
</table>

Chan. et al. 2015. Front Immunol. 20;6:412
The relevance of ANA-IIF

- Antinuclear antibodies occur
  - in various autoimmune diseases
    - Connective tissue diseases (CTD)
    - Autoimmune hepatitis
    - Primary biliary cirrhosis
    - Rheumatoid arthritis
    - Addison’s disease
    - Hashimoto thyroiditis
    - Type 1 diabetes mellitus

- as well as in non-autoimmune diseases
  - Cancer
  - Gastrointestinal diseases
  - Lung diseases
  - Skin diseases
  - Infections

- ANA are positive in a considerable proportion of the healthy population

→ **ANA-IIF are not very specific for certain diseases**

→ **BUT ANA are mainly used to support diagnosis of CTDs**

1. Malleson. et al. 2010. Pediatric Rheumatology. 8:27
What is the effect

- The overall prevalence of ANA in the US population was 13.8%, 32.3 million people, while the prevalence of CTD is <0.5%, or 1.5 million

- There is a high degree of false positive in the general population

A sign of low test specificity

Which test provides higher diagnosis accuracy?

Lab performs 1000 screen incidence for CTD is 10% so 900 patients have no CTD and 100 of them have CTD

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANA-IIF (1:20)</td>
<td>89%</td>
<td>77%</td>
</tr>
<tr>
<td>EliA CTD Screen</td>
<td>74%</td>
<td>95%</td>
</tr>
</tbody>
</table>

Test sensitivity 100% = 100 CTD patients identified
Test specificity 100% = 900 healthy individual excluded

Which test provides higher diagnosis accuracy?

Lab performs 1000 screen incidence for CTD is 10% so 900 patients have no CTD and 100 of them have CTD

<table>
<thead>
<tr>
<th></th>
<th>ANA-IIF</th>
<th>EliA CTD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sen. 89%</td>
<td>Sen. 74%</td>
</tr>
<tr>
<td></td>
<td>Spe. 77%</td>
<td>Spe. 95%</td>
</tr>
<tr>
<td>test POS</td>
<td>test POS</td>
<td>test POS</td>
</tr>
<tr>
<td>CTD</td>
<td>89</td>
<td>74</td>
</tr>
<tr>
<td>none-CTD</td>
<td>207</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>296</td>
<td>119</td>
</tr>
<tr>
<td></td>
<td>704</td>
<td>881</td>
</tr>
<tr>
<td>PPV: 30%</td>
<td>NPV: 98%</td>
<td>PPV: 62%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NPV: 97%</td>
</tr>
</tbody>
</table>

Does IIF detect all antibodies?

• HEp-2 and even HEp-2000 (only spiked with Ro60 antigen) has a problem to detect Ro52 and even Ro60 antibodies¹

• Jo-1 is difficult to detect by IIF²,³.

• Rib-P is difficult to detect by IIF⁴.

¹ Mahler et al. 2014. J Immunol Res. 315179
³ López-Hoyos et al. 2007. Ann N Y Acad Sci.1109:322
How about ANA-IIF in pediatric rheumatology?

• Pediatric rheumatologists have pointed out in the literatures that the ANA is a poor screening test and is being used inappropriately\(^1,2,3,4\).

• the ANA test has such a high false-positivity rate that a positive test is of little, if any, clinical utility as a screening test and should not be ordered routinely to screen children with musculoskeletal complaints\(^5\).

• Its use should be limited to the diagnosis of SLE, MCTD, and similar systemic illnesses\(^5\).

  - ANA-IIF has a problem in detecting some autoantibodies
  - ANA-IIF is not very specific

→ EliA CTD screen has higher clinical utility

5. Malleson et al. Pediatric Rheumatology 2010, 8:27
EliA CTD Screen can help detect specific CTDs

EliA CTD Screen has a high sensitivity for Sjögren’s syndrome and systemic sclerosis

Confirmed antibodies (n=223) and their detection

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>dsDNA</th>
<th>Ro</th>
<th>La</th>
<th>Sm</th>
<th>CENP-B</th>
<th>U1RNP</th>
<th>SCL-70</th>
<th>Jo-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>EliA CTD Screen&lt;sup&gt;positive&lt;/sup&gt;</td>
<td>43</td>
<td>75</td>
<td>26</td>
<td>7</td>
<td>18</td>
<td>9</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>EliA CTD Screen&lt;sup&gt;borderline&lt;/sup&gt;</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ANA-IIF&lt;sup&gt;positive&lt;/sup&gt; 1:160</td>
<td>33</td>
<td>65</td>
<td>25</td>
<td>5</td>
<td>19</td>
<td>7</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

Test result positive → single test should be ordered according to clinical symptoms

EliA CTD Screen identifies the most common connective tissue diseases

<table>
<thead>
<tr>
<th>More common</th>
<th>Less common</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sjögren’s syndrome</strong></td>
<td><strong>Systemic lupus erythematosus</strong></td>
</tr>
<tr>
<td>Ro52 kDa, 70-100%1,2</td>
<td>dsDNA, 90% (active)15</td>
</tr>
<tr>
<td>Ro60 kDa, 70-100%1,2</td>
<td>Ro52 kDa, 40-50%3,4</td>
</tr>
<tr>
<td>La, 35-70%1,2</td>
<td>Ro60 kDa, 40-50%3,4</td>
</tr>
<tr>
<td>U1RNP, 30-40%7,8</td>
<td>Ro52 kDa, 20%6</td>
</tr>
<tr>
<td>SmD, 20-30%7,8</td>
<td>U1RNP (A,C,70), 8-14%11-14</td>
</tr>
<tr>
<td>Rib-P, 15-20%16</td>
<td>Ro60 kDa, 6%6</td>
</tr>
<tr>
<td>La, 6-15%5</td>
<td>Fibrillarin, 6-8%20</td>
</tr>
<tr>
<td>PCNA, &lt;5%18</td>
<td>Pm-Scl, 3%21,22</td>
</tr>
<tr>
<td>Polymyositis/scleroderma (overlap syndrome)</td>
<td></td>
</tr>
<tr>
<td>Pm-Scl, 24%21,22</td>
<td></td>
</tr>
</tbody>
</table>

Summary

- CTDs are rare and diagnosis is complicated\(^1,2\)
- At low titres the chance of false positives with ANA-IIF increases\(^3\)
- Incorrect diagnosis can cause patients emotional and physical harm\(^4,5\)
- EliA CTD Screen offers equivalent sensitivity and superior specificity to ANA-IIF and can help detect specific CTDs\(^6,7\)
- EliA CTD Screen has been successful as a first-line test in the real world\(^8\)

Thank you!
**Organ Specific Autoimmune Diseases**

- Diabetes mellitus Typ I (juvenile diabetes)
- Hashimoto Thyroiditis
- Basedow
- Celiac Disease
- Goodpasture-Syndrome
- Ulcerative Colitis, Crohn’s Disease
- Primary Biliary Cirrhosis
- Myasthenia Gravis
- Sjögren’s Syndrome
- Dermato-/Polymyositis
- Vasculitis
- Rheumatoid Arthritis
- MCTD
- Scleroderma
- Systemic Lupus Erythematosus, SLE

**Systemic Autoimmune Diseases**
Conclusion

- In more than 95% of all ANA requests the physician wants to know if CTD plays a role in these patients
- 4 of 5 ANA positive results cannot be traced back to antigens with known clinical relevance.
- IIF results have only a limited clinical usefulness for the doctors
- Other test methods can be used, according the ACR

⇒ Is IIF still the first test to use?
What do the experts say?

Prof. Marvin J Fritzler Calgary Canada

Speciality(ies): Rheumatology, Cell and Molecular Biology

Affiliation(s): Faculty of Medicine, University of Calgary Dr. Fritzler did his Bachelor in Biological Sciences at the University of Alberta in 1968. He then continued on to obtain his Ph.D. in Cell Biology in 1971 and his Medical Degree in 1974, both at the University of Calgary. Dr. Fritzler’s work relating to the CSRG includes autoantibody analysis, metabolomics and biomarker detection. He is both the director of the Mitogen Advanced Diagnostics Laboratory and the Chair of the Alberta Science and Research Authority Board (ASRA). Dr. Fritzler’s other professional interests include researching new diagnostic technologies and platforms to detect human autoantibodies. In his spare time, he enjoys singing in choral societies and gardening.

His statements during 10th Dresden Symposium on Autoantibodies

If you take a random SLE cohort you will find that 14% - 25% is ANA-IIF negative

ANA-IIF has never been the “golden standard” it is just the test we know the best, because it is one of the oldest.
Is there a clinical usefulness of IIF results?

- Even high titres (1:640) have only a positive predictive value of 35% for connective tissue diseases (CTDs).
- ANA in IFA have a predictive value of 11% for SLE and 11% for other CTDs.
- 4 of 5 ANA positive results cannot be traced back to antigens with known clinical relevance.

⇒ IIF results have only a limited clinical usefulness for the doctors
EliA CTD Screen offers equivalent sensitivity and superior specificity to ANA-IIF\textsuperscript{1,2}

Confirmed antibodies (n=223) and their detection

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>ANA-IIF 1:100</th>
<th>EliA CTD Screen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive, n (%)</td>
<td>Sensitivity (%)</td>
</tr>
<tr>
<td>SLE</td>
<td>28</td>
<td>28 (100)</td>
<td>100</td>
</tr>
<tr>
<td>SS</td>
<td>17</td>
<td>16 (94)</td>
<td>94</td>
</tr>
<tr>
<td>SSc limited</td>
<td>9</td>
<td>9 (100)</td>
<td>100</td>
</tr>
<tr>
<td>SSc</td>
<td>2</td>
<td>2 (100)</td>
<td>100</td>
</tr>
<tr>
<td>MCTD</td>
<td>4</td>
<td>4 (100)</td>
<td>100</td>
</tr>
</tbody>
</table>

EliA CTD Screen has a high sensitivity for Sjögren’s syndrome, systemic sclerosis and mixed connective tissue disease\textsuperscript{2}

Clinical features of SLE

**Definition:** Inflammatory rheumatic systemic disease with a potential involvement of all organs

**Sex Ratio:** male : female = 1 : 9

**Age:** Every age, peaks at 15-25 and 40-50 years

**Critical manifestations:** kidneys, CNS

**Most frequent cause of death:** Infections

**Diagnosis:** 4 of 11 ACR-criteria have to be fulfilled
Clinical features of SLE

Criteria of SLE

1. Malar Rash
2. Discoid rash
3. Photosensitivity
4. Oral Ulcer
5. Arthritis
6. Serotitis (pleurisy pericarditis)
7. Renal Disorder (Persistent proteinuria Cellular casts)
8. Neurologic Disorder (Psychosis, Seizures)
9. Hematologic disorder (Hemolytic anemia, leukopenia, Lymphopenia, Thrombocytopenia)
10. Immunologic disorder (anti dsDNA, anti Sm anti APS)
11. Antinuclear antibody
   (four or more criteria for SLE)
SLE: early skin lesions and butterfly rash
SLE: Skin manifestations
SLE manifestations neuropsychiatric

- anything possible
- mild concentration disorder, personality change
- epilepsy, depression, psychosis
- behaviour disturbances
- stroke, movement disorders
**Scleroderma**

**Definition:** Fibrosing systemic disease with lesions of the vessels, leading to atrophy and fibrosis of almost all organs. (Fibrosis = proliferation of connective tissue)

**Sex Ratio:** male:female = 1:2

**Age:** mostly adults, peak at 40-50 years

**Diagnosis:** ARA-criteria: 1 main criterium (= symmetric, sclerodermal lesions of joints) and at least 2 of 3 minor criteria
Clinical features of systemic sclerosis

- Raynaud's phenomenon
- Honeycomb lung
- Diffuse skin systemic sclerosis with pigmentation
- Systemic sclerosis: telangiectasia, (rat bites) small mouth
Clinical features of systemic sclerosis

- Digital tip ulcers
  - Picture reference: https://www.studyblue.com/notes/note/n/scleroderma/deck/4903313

- Fingertip pitting scars

- Puffy fingers
  - Picture reference: https://pictures.doccheck.com/com/photo/18450-scleroderma-hands-1

- Systemic sclerosis: trying to make fists
Scleroderma

First symptoms:

- Fatigue
- Raynaud’s phenomenon
- Swollen face and hands in the morning

Further course:

- Calcium deposits in the skin
- Ulcerations of the fingers
- Telangiectasis (small dilated blood vessels near the surface)
- Involvement of the lung in >60%
# 2013 ACR / EULAR Criteria For The Classification Of Systemic Sclerosis (Scleroderma)*

<table>
<thead>
<tr>
<th>Item</th>
<th>Sub-items(s)</th>
<th>Weight/score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin thickening of the fingers of both hands extending proximal to the metacarpophalangeal joints <em>(sufficient criterion)</em></td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td>Skin thickening of the fingers <em>(only count the higher score)</em></td>
<td>Puffy fingers</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Sclerodactyly of the fingers (distal to the metacarpophalangeal joints but proximal to the proximal interphalangeal joints)</td>
<td>4</td>
</tr>
<tr>
<td>Fingertip lesions <em>(only count the higher score)</em></td>
<td>Digital tip ulcers</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Fingertip pitting scars</td>
<td>3</td>
</tr>
<tr>
<td>Telangiectasia</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Abnormal nailfold capillaries</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Pulmonary arterial hypertension and/or interstitial lung disease <em>(maximum score is 2)</em></td>
<td>Pulmonary arterial hypertension</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Interstitial lung disease</td>
<td>2</td>
</tr>
<tr>
<td>Raynaud’s phenomenon</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>SSc-related autoantibodies <em>(anticentromere, anti-topoisomerase I [anti-Scl-70], anti-RNA polymerase III)</em> <em>(maximum score is 3)</em></td>
<td>Anticentromere 3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Anti-topoisomerase I</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anti-RNA polymerase III</td>
<td></td>
</tr>
</tbody>
</table>

* The criteria are not applicable to patients with skin thickening sparing the fingers or to patients who have a scleroderma-like disorder that better explains their manifestations (e.g., nephrogenic sclerosing fibrosis, generalized morphea, eosinophilic fascitis, scleredema diabeticorum, scleromyxedema, erythromyalgia, porphyria, lichen sclerosis, graft-versus-host disease, diabetic cheiroarthropathy).

† The total score is determined by adding the maximum weight (score) in each category. **Patients with a total score of ≥ 9 are classified as having definite scleroderma.**

Sensitivity 91%  Specificity 92%

Definition: acute or chronic inflammatory disease of muscle and skin.

Sex Ratio: male:female = 1:3

Age: every age

Diagnosis: 5 Criteria (5.: manifestation of the skin, dermatomyositis) according to Bohan and Peter. The more criteria are fulfilled, the clearer the diagnosis.
Dermatomyositis / Polymyositis

First symptoms:
- Fatigue
- Muscle weakness in shoulders, pelvis or thighs

Further course:
- Symmetric pain
- When skin is involved: redness and swelling
- Pain in joints
- Difficulties with speech and swallowing

Prognosis:
Depending on severity, but often curable with steroids and immunosuppression
Dermatomyositis / Polymyositis

Criteria Dermatomyositis

• Myopathic muscle weakness (Yes)
• Serum skeletal muscle enzymes (High or Normal)
• Electromyographic findings (Myopathic)
• Muscle enzymes High (up to 50 fold normal)
• Muscle-biopsy findings (Perifascicular, perimysial or perivascular infiltrates; perifascicular artophy)
• Rash of Calcinosis (present)

Criteria Polymyositis

• Myopathic muscle weakness (Yes)
• Muscle enzymes High (up to 50 fold normal)
• Electromyographic findings (Myopathic)
• Muscle-biopsy findings (primary inflammation with CD8/MCH-1 complexes and no vacuoles)
• Rash of Calcinosis (absent)

No auto-antibody results required

**Definition**: a chronic inflammatory disease of unknown cause characterized by diminished lacrimal and salivary gland secretion resulting in keratoconjunctivitis sicca and xerostomia.

**Sex Ratio**: male:female = 1:9

**Age**: 30-40 Years

**Diagnosis**: ACR-EULAR Classification Criteria for primary Sjögren’s syndrome (pSS)
Sjögren‘s syndrome

First symptoms
- Fatigue
- Dry eyes

Due to Less/no lacrimal fluid saliva fluid the consequences are:
- Frequent eye infection, even up to blindness
- Difficulty with speech to swallow (Aphasia and dysphagia)
- Intense caries
- Involvement of other organs, esp. polyarthritis is possible

Secondary Sjögren‘s syndrome:
- Complication of rheumatoid arthritis (10-15%), less frequent of SLE (1-3%)
Sjogren’s Syndrome - criteria

- **Ocular symptoms**
  - Have you had daily, persistent troublesome dry eyes more than 3 months?
  - Do you have a recurrent sensation of sand or gravel in the eyes
  - Do you use tears substitutes more than 3 times a day?

- **Oral Symptoms**
  - Have you had a daily feeling of dry mouth for more than 3 months
  - Have you had recurrently or persistently swollen salivary glands as an adult
  - Do you frequently drink liquids to aid in swallowing dry food

- **Ocular signs**
  - Schirmer test
  - Rose bengal score

- **Salivary gland involvement**
  - Unstimulated whole salivary flow (~1.5 ml in 15 min)
  - Parotid sialography showing the presence of diffuse sialectasias
  - Salivary scintigraphy showing delayed uptake

- **Auto antibodies (Ro/SS-A or La/SS-B)**
The classification of SS applies to any individual who meets the inclusion criteria, does not have any condition listed as exclusion criteria, and who has a score $\geq 4$ when summing the weights from the following items.

<table>
<thead>
<tr>
<th>Item</th>
<th>Weight / Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labial salivary gland with focal lymphocytic sialadenitis and focus score $\geq 1.3$</td>
<td>3</td>
</tr>
<tr>
<td>Anti-SSA (Ro) +</td>
<td>3</td>
</tr>
<tr>
<td>Ocular staining score $\geq 5$ (or van Bijsterfeld score $\geq 4$) on at least one eye</td>
<td>1</td>
</tr>
<tr>
<td>Schirmer $\leq 5$ mm/5min on at least one eye</td>
<td>1</td>
</tr>
<tr>
<td>Unstimulated whole saliva flow rate $\leq 0.1$ ml/min</td>
<td>1</td>
</tr>
</tbody>
</table>

**Definition**: Overlap syndrome with unclear characterisation, a syndrome with features of scleroderma, rheumatoid arthritis, SLE, and polymyositis-dermatomyositis and characteristic high titre of U1RNP antibodies.

**Sex Ratio**: male:female = 1:3

**Age**: every age

**Diagnosis**: 1 of 2 general symptoms, antibodies to U1RNP, 2 of 3 mixed symptoms (according to Kasukawa)
First symptoms:

- Fatigue
- Raynaud's phenomenon (often many years in advance)
- Muscle weakness
- Swollen hands and general swelling of the skin

Further course:

- At least 50% of patients develop a classical connective tissue disease in the course of 10 years
MCTD - criteria

Clinical
- Edema of the hands
- Synovitis
- Myositis
- Raynaud’s phenomenon
- Acrosclerosis (Skin changes at the extremities typical of scleroderma)

Serologic
- Anti-U1RNP high concentrations

(Alacron-Segovia criteria for MCTD)
Summary

1. **SLE**: systemic!!! All organs can be involved.
   - Most frequent: joints, general symptoms, skin
   - Critical manifestation: kidney, CNS

2. **Systemic sclerosis**: calcium deposits in the skin and other organs

3. **Dermatomyositis/Polymyositis**: muscle weakness, often curable.

4. **Sjögren‘s syndrome**: Exocrine glands, especially lacrimal and salivary glands. Most often occurs as secondary disease.

ANA-IIF is mentioned but not mandatory often specific antibodies are mentioned Sm, dsDNA RNP70, SS-A, SS-B, Scl-70 CENP, RNA Pol III
## Screening

<table>
<thead>
<tr>
<th>Single Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symphony</td>
</tr>
<tr>
<td>U1RNP (RNP70, A, C)</td>
</tr>
<tr>
<td>Sm</td>
</tr>
<tr>
<td>SS-A/Ro (60 kDa, 52 kDa)</td>
</tr>
<tr>
<td>SS-B/La</td>
</tr>
<tr>
<td>Centromere B</td>
</tr>
<tr>
<td>Scl-70</td>
</tr>
<tr>
<td>Jo-1</td>
</tr>
</tbody>
</table>

---

## dsDNA

- Fibrillarin
- *RNA Pol III*
- Rib-P
- PM-Scl
- PCNA
- Mi-2

---

CTD Screen
## Advantages/Disadvantages of Different Methods

<table>
<thead>
<tr>
<th>dsDNA Abs Method</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLIFT</td>
<td>🙁😀</td>
<td>😊😊</td>
</tr>
<tr>
<td>FARR RIA</td>
<td>😊</td>
<td>😊</td>
</tr>
<tr>
<td>ELISA</td>
<td>😊😀</td>
<td>😞</td>
</tr>
</tbody>
</table>
Why this?

Usage of graph kindly allowed by Prof. O.P. Rekvig, Tromso, Norway
## Advantages/Disadvantages of Different Methods

<table>
<thead>
<tr>
<th>dsDNA Abs Method</th>
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<tbody>
<tr>
<td>CLIFT</td>
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<td>FARR RIA</td>
<td>😊</td>
<td>😊</td>
</tr>
<tr>
<td>ELISA</td>
<td>😊😊</td>
<td>😞</td>
</tr>
<tr>
<td>ELIA</td>
<td>😊</td>
<td>😊</td>
</tr>
</tbody>
</table>
Data from ´internal´ evaluation

EliA dsDNA in IU/ml

SLE
<table>
<thead>
<tr>
<th>activity Index (SLEDAI)</th>
<th>Activity Group</th>
<th>total</th>
<th>neg</th>
<th>pos</th>
<th>% pos in activity group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>I</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>I</td>
<td>28</td>
<td>19</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>I</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>29.0%</td>
</tr>
<tr>
<td>4</td>
<td>II</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>II</td>
<td>11</td>
<td>2</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>II</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>II</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>85.0%</td>
</tr>
<tr>
<td>12</td>
<td>III</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>III</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>III</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>III</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>100.0%</td>
</tr>
<tr>
<td>Total:</td>
<td></td>
<td>64</td>
<td>25</td>
<td>39</td>
<td>60.9%</td>
</tr>
</tbody>
</table>
The Importance of Specificity

### Prevalence 2%, Sens 74%, spec 98.5% (EliA CCP)

Bizzaro N et al. 2007

<table>
<thead>
<tr>
<th></th>
<th>Test positive</th>
<th>Test negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>148</td>
<td>52</td>
<td>200</td>
</tr>
<tr>
<td>Non-RA</td>
<td>147</td>
<td>9,653</td>
<td>9,800</td>
</tr>
<tr>
<td>Total</td>
<td>295</td>
<td>9,705</td>
<td>10,000</td>
</tr>
</tbody>
</table>

147 false positives, potentially referred on to specialists and/or treatment. PPV = 50%
Prevalence 2%, Sens 73%, spec 96% (Inova CCP3)

<table>
<thead>
<tr>
<th></th>
<th>Test positive</th>
<th>Test negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>146</td>
<td>54</td>
<td>200</td>
</tr>
<tr>
<td>Non-RA</td>
<td>392</td>
<td>9,408</td>
<td>9,800</td>
</tr>
<tr>
<td>Total</td>
<td>538</td>
<td>9,558</td>
<td>10,000</td>
</tr>
</tbody>
</table>

245 patients more with a false positive result. PPV = 27%

Bizzaro N et al. 2007
## The Importance of Specificity

### Prevalence 2%, Sens 54%, spec 86% (RF)

Bizzaro N et al. 2007

<table>
<thead>
<tr>
<th></th>
<th>Test positive</th>
<th>Test negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>108</td>
<td>92</td>
<td>200</td>
</tr>
<tr>
<td>Non-RA</td>
<td>1,372</td>
<td>8,428</td>
<td>9,800</td>
</tr>
<tr>
<td>Total</td>
<td>1,480</td>
<td>8,520</td>
<td>10,000</td>
</tr>
</tbody>
</table>

1,225 patients more with a false positive result. PPV = 7%
CCP is much more specific than RF

<table>
<thead>
<tr>
<th>Disease</th>
<th>n</th>
<th>CCP n / in %</th>
<th>RF n / in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE</td>
<td>77</td>
<td>8 / 10</td>
<td>19 / 25</td>
</tr>
<tr>
<td>Sjögren’s syndrome</td>
<td>156</td>
<td>22 / 14</td>
<td>80 / 51</td>
</tr>
<tr>
<td>scleroderma</td>
<td>148</td>
<td>6 / 4</td>
<td>22 / 15</td>
</tr>
<tr>
<td>myositis</td>
<td>11</td>
<td>3 / 27</td>
<td>1 / 9</td>
</tr>
<tr>
<td>ankylosing spondylitis</td>
<td>43</td>
<td>6 / 14</td>
<td>4 / 9</td>
</tr>
<tr>
<td>psoriatic arthritis</td>
<td>34</td>
<td>2 / 6</td>
<td>3 / 9</td>
</tr>
<tr>
<td>non-classified arthritis</td>
<td>103</td>
<td>11 / 11</td>
<td>5 / 5</td>
</tr>
<tr>
<td>osteoarthritis</td>
<td>15</td>
<td>1 / 7</td>
<td>3 / 20</td>
</tr>
<tr>
<td>fibromyalgia</td>
<td>22</td>
<td>3 / 14</td>
<td>4 / 18</td>
</tr>
<tr>
<td>total</td>
<td>609</td>
<td>62 / 10,2</td>
<td>150 / 24,6</td>
</tr>
</tbody>
</table>

Fabien et al, Clin Rev Allerg Immunol 2008; 34:40-44
1997 Update of the 1982 American College of Rheumatology Revised Criteria for Classification of Systemic Lupus Erythematosus

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Malar Rash</td>
<td>Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds</td>
</tr>
<tr>
<td>2. Discoid rash</td>
<td>Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur in older lesions</td>
</tr>
<tr>
<td>3. Photosensitivity</td>
<td>Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation</td>
</tr>
<tr>
<td>4. Oral ulcers</td>
<td>Oral or nasopharyngeal ulceration, usually painless, observed by physician</td>
</tr>
<tr>
<td>5. Nonerosive Arthritis</td>
<td>Involving 2 or more peripheral joints, characterized by tenderness, swelling or effusion</td>
</tr>
<tr>
<td>6. Pleuritis or Pericarditis</td>
<td>1. Pleuritis—continuing history of pleural pain or rubbing heard by physician or evidence of pleural effusion</td>
</tr>
<tr>
<td>7. Renal Disorder</td>
<td>Persistent proteinuria &gt; 0.5 grams per day or &gt; 3+ if quantitated not performed</td>
</tr>
<tr>
<td>8. Neurologic Disorder</td>
<td>1. Seizures—in the absence of offending drugs or known metabolic derangements; e.g., uric acid, ketoadiposis or electrolyte imbalance</td>
</tr>
<tr>
<td>9. Hematologic Disorder</td>
<td>1. Hemolytic anemia—with reticulocytosis</td>
</tr>
<tr>
<td>10. Immunologic Disorder</td>
<td>1. Anti-DNA: antibody to native DNA in abnormal titer</td>
</tr>
<tr>
<td>11. Positive Antinuclear Antibody</td>
<td>An abnormal titer of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs</td>
</tr>
</tbody>
</table>

1. Hemolytic anemia—with reticulocytosis
2. Leukopenia—<4,000/mm³ on ≥ 2 occasions
3. Lymphopenia—<1,500/mm³ on ≥ 2 occasions
4. Thrombocytopenia—<100,000/mm³ in the absence of offending drugs
5. Anti-DNA: presence of antibody to native DNA in abnormal titer
6. Anti-Sm: presence of antibody to Sm-nuclear antigen
7. Positive finding of antiphospholipid antibodies on:
   1. an abnormal serum level of IgG or IgM antiphospholipid antibodies,
   2. a positive test result for lupus anticoagulant using a standard method, or
   3. a false-positive test result for at least 6 months confirmed by Treponema pallidum immobilization or fluorescent treponemal antibody absorption test
New Consensus

**New recommendations**

In this Consensus Statement, we recommend the use of high-quality immunoassays as the preferred first screening method for GPA and MPA, and put forward a new testing algorithm (recommendations 1–6). These

* A second PR3-MPO-ANCA or IIF can be considered for negative results in patients with a high clinical suspicion (to increase sensitivity) or in case of low antibody levels (to increase specificity). Take antibody level into account.

Source: see previous page
RF IgA has high prognostic value

- Patients presenting with raised RF IgA developed more severe, erosive disease:
  - They developed a greater number of erosions
  - These patients required much more pharmaceutical treatment
- The presence of RF IgA could justify more aggressive treatment at an early stage
  but may predict a poor response to TNF inhibitors

Anti-Neutrophil Cytoplasmic Antibodies on indirect immunofluorescence assay (IIF)

- Slides have very different qualities and high lot-to-lot variation

- Classical c-ANCA pattern has a c-ANCA pattern in both fixations, formalin and ethanol (mostly anti-PR3)

- Classical p-ANCA pattern is seen only on ethanol-fixed cells, gives a c-ANCA pattern on formalin (mostly anti-MPO)

- Most frequent atypical ANCA: formalin negative, ethanol p-ANCA (seldom anti-MPO)
How do interpret test result? Example: 30% pre-test probability

30 % pre-test probability
• radiographic presence of pulmonary infiltrates or nodules, or both
• urinalysis demonstrating hematuria and red blood cell casts

Source: Bossuyt X et al, 2017, Rheumatology 56(9): 1533-41
How do interprete test result? Example: 50% pre-test probability

50 % pre-test probability
• Rapidly progressive glomerulonephritis

Source: Bossuyt X et al, 2017, Rheumatology 56(9): 1533-41
<table>
<thead>
<tr>
<th>Disease</th>
<th>Anti-MPO</th>
<th>Anti-PR3</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPA (Wegener)</td>
<td>5-60 %</td>
<td>40-95 %</td>
</tr>
<tr>
<td>MPA</td>
<td>50-70 %</td>
<td>25-30 %</td>
</tr>
<tr>
<td>EGPA (Churg-Strauß)</td>
<td>30-40 %</td>
<td>9-30 %</td>
</tr>
<tr>
<td>Renal limited vasculitis (e.g. NCGN)</td>
<td>50-70 %</td>
<td>25-30 %</td>
</tr>
</tbody>
</table>

- PR3 antibodies are quite specific for GPA (Wegener’s granulomatosis) but may occur in other ANCA-associated vasculitides.
- MPO antibodies occur in all ANCA-associated vasculitides and in vasculitis of the kidney but almost never in other diseases such as infections, non-ANCA-associated vasculitides or connective tissue diseases.