Algorithm for ANA testing and interpretation

Autoantibody Study Group Section - October 19, 2009
ACR Annual Scientific Meeting

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Conflict of interest

• Immunology consultant to Fleury Medicine & Health – private clinical laboratory
Points to be addressed

- Special features of autoantibodies and the different methods for autoantibody detection/identification
- Distinctive features of ANA-HEp-2 test in autoimmune patients and healthy subjects
- Algorithm for ANA testing – laboratory perspective
- Algorithm for ANA interpretation – clinician perspective
- How to provide the best help for the patient?
“Antibodies that are present in the serum of healthy individuals in the absence of deliberate immunization with any antigen, are referred to as natural antibodies. A vast majority of natural antibodies react with one or more self antigens and are termed as natural autoantibodies…It is now well established that auto-reactive antibodies and B cells, and auto-reactive T cells, are present in healthy individuals, and in virtually all vertebrate species.”

Sebastien Lacroix-Desmazes, Srini V. Kaveri, Luc Mouthon, Ahidjo Ayouba, Evelyne Malanchere, Antonio Coutinho, Michel D. Kazatchkine

Journal of Immunological Methods 216 1998 117-137
### FEATURES OF NATURAL AND PATHOLOGIC AUTOANTIBODIES

<table>
<thead>
<tr>
<th>NATURAL</th>
<th>PATHOLOGIC</th>
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<tbody>
<tr>
<td>IgG, IgM, IgA</td>
<td>MAINLY IgG</td>
</tr>
<tr>
<td>POLY-REACTIVITY</td>
<td>RESTRICTED REACTIVITY</td>
</tr>
<tr>
<td>LOW AFFINITY</td>
<td>HIGH AFFINITY</td>
</tr>
<tr>
<td>LOW TITER</td>
<td>HIGH TITER</td>
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<tr>
<td>DERIVED FROM GERM LINE</td>
<td>SOMATIC MUTATION</td>
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#### Pathologic autoantibodies

Threshold for clinical significance

Antibody concentration and avidity

**Natural autoantibodies**
Threshold for clinical significance

Methods with limited sensitivity

Threshold for analytical detection

Antibody concentration and avidity

Methods with limited sensitivity

Threshold for clinical significance

Threshold for analytical detection
Threshold for clinical significance

Methods with high sensitivity

Threshold for analytical detection

Antibody concentration and avidity
Special features of autoantibodies

- Not etiologic markers (compare to anti-HIV) ➔ markers for patterns of “immunogenetic disorders”
- Not abnormal elements (natural autoantibodies) ➔ consider qualitative and quantitative approach
- “Complex analytes” (contrast to simple analytes, e.g., glucose) ➔
  Polyclonal response with considerable inter-individual variability in
  - Concentration
  - Avidity
  - Epitope selection

 ➔ Consider peculiarity in the several methodological platforms

Different methodological platforms detect antibodies with different concentration and avidity profiles

- Double immunodiffusion
- HEp-2 ANA
- Hemagglutination
- Dot blot
- Line blot
- ELISA
- Luminex - ALBIA
Screening for antinuclear antibodies

- LE cells
- Animal tissue
- Human Cell culture

1948
Hargraves

1950
Coons & Kaplan

1980

Specificity Sensitivity

Screening for systemic lupus erythematosus

LE cell  Animal tissue  HEP-2

LE cell  Animal tissue  HEP-2

Specificity  Sensitivity
The case of HEp-2 ANA

- Highly sensitive test
- Normal population ➔ 5-15% positive tests ➔ NOT FALSE POSITIVE
- HEp-2 ANA test became very popular
  - Formerly ➔ Rheumatologists, nephrologists, dermatologists
  - Nowadays ➔ Gynecologists, hepatologists, neurologists, ophthalmologists, ENT, endocrinologists, pneumologists, general practitioners
- Low pre-test probability ➔ “Idiopathic Positive ANA Syndrome”
- Are there distinctive features in a positive HEp-2 ANA test from:
  - A patient with systemic autoimmune disease?
  - An individual with no evidence of autoimmune disease?

Guidelines for interpretation of a positive HEp-2 ANA test

- Intrinsic features of the test can help
  - ANA titer
  - ANA immunofluorescence pattern
Survey for distinctive features in positive ANA test in healthy individuals and autoimmune patients

- 918 healthy individuals
  - 492 healthy female workers in an electric power plant
  - 426 blood donors
- Thorough clinical rheumatologic questionnaire and serology for common viral infectious diseases
- Screening at 1/80 in 2 different commercial HEp-2 slides
- Two independent experienced observers
- Positive results ➔ both observers & both slides

Mariz HA et al, submitted

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### ANA-HEp-2 titer in 918 healthy subjects and 153 patients with autoimmune rheumatic diseases

![Titer chart showing ANA-HEp-2 titers for healthy subjects and patients with autoimmune rheumatic diseases](chart.png)

**Healthy subjects ➔ 12.8% positive ANA x ARD ➔ 90.1% Positive ANA**

Mariz HA et al, submitted
ANA-HEp-2 titer in 918 healthy subjects and 153 patients with autoimmune rheumatic diseases

Healthy subjects ➔ 12.8% positive ANA x ARD ➔ 90.1% Positive ANA

Mariz HA et al, submitted
ANA-HEp-2 pattern in 918 healthy subjects and 153 patients with autoimmune rheumatic diseases

Healthy subjects ➔ 12.8% positive ANA x ARD ➔ 90.1% Positive ANA

Mariz HA et al, submitted
ANA-HEp-2 pattern in 918 healthy subjects and 153 patients with autoimmune rheumatic diseases

Healthy subjects ➔ 12.8% positive ANA x ARD ➔ 90.1% Positive ANA

Mariz HA et al, submitted
Simple fine speckled pattern
Healthy subjects show lower titer than patients with ARD

![Graph showing TITER distribution between Healthy and ARD groups with P<0.001](image)

Mariz HA et al, submitted

Microarray technology

Multiplex assay with orderly distributed analytes
ANA-HEp-2 test ➔ a natural microarray?

Danton H. O'Day and Michael A. Myre, University of Toronto.

Adapted from Danton H. O'Day and Michael A. Myre, University of Toronto.
Strong association of DFS pattern and autoantibodies to LEDGF/p75

81 samples sequentially selected for the Dense Fine Speckled pattern

80 reacted with the 75kDa band

LEDGF/p75 – Transcription co-factor stimulated by cellular stress

Dellavance A, Andrade LEC et al, J Rheumatol 2005; 32: 2144

Scl-70 pattern – specific for anti-topoisomerase I abs

Dellavance et al, Rheumatology 48: 632-9, 2009
**Relevant features of the ANA-HEp-2 test**

- The ANA-HEp-2 cell assay is a powerful tool for ANA screening
- It offers relevant information on the possible involved autoantibodies
- Careful analysis of ANA-HEp-2 pattern is helpful in distinguishing positive reactivity observed in patients with systemic autoimmune diseases and apparently healthy subjects

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**National Consensus for Standardization of ANA-HEp-2 Testing and Interpretation**

- ANA experts from all over the country
- Standardization of technical procedure
- Standardization of nomenclature (ANA patterns)
- Recommendations for test report
- Three editions performed
  - August 2000
  - September 2002 Goiânia, Brazil
  - April 2007
- Next edition on June 2010
Recommendations for ANA-HEp-2 pattern report

National Consensus for Standardization of ANA-HEp-2

Recommendations for performing the test

- Control for technical parameters
  - Low titer positive control and negative control
  - “Positive control for “strategic ANA patterns” (especially DFSp)
  - Lamp lifetime
  - Conjugate titer

- Consider using two or more HEp-2 ANA substrates for unusual patterns

- Internal and external quality control programs
Recommendations for test report

- To state the ANA pattern in five cell compartments
  - Nucleus, nucleolus, metaphase plate, mitotic apparatus, cytoplasm
- To state the titer
- To provide interpretation of the pattern in terms of possible involved autoantigens and autoantibodies

How to run a ANA-HEp-2 IIF routine in a large clinical lab?

1,200,000 tests/month

8,000-9,000 ANAs/month
Integrated autoantibody team
Step wise expertise
Screening ↔ titration
Quality control rounds
Clinical – ANA correlation rounds

Possible significance of a positive autoantibody test in an individual with no evidence of autoimmunity

- **Early manifestation of an incipient autoimmune condition?**
- Transient immune disturbance?
  - Infection?
  - Drugs?
  - Cancer?
- Familial autoimmunity trait?
- Minimal manifestation of a spectral autoimmune condition?
- Incidentaloma? Autoantibodies not associated to autoimmune diseases
Temporal dissociation between autoantibody serum levels and clinical manifestations

Evolution of autoantibody serum levels

Analytical detection threshold

Clinical threshold

Evolution of clinical manifestations

Time line

Presence of autoantibodies years before the development of clinical systemic lupus erythematosus


<table>
<thead>
<tr>
<th>Autoantibody</th>
<th>Positive Test before Diagnosis</th>
<th>Time from First Detection to Diagnosis</th>
<th>Positive Test in First Serum Sample</th>
<th>Total Patients with Positive Test and Diagnosis</th>
<th>Interval between Positive Test and Onset of Symptoms</th>
<th>Positive Test before Onset of Symptom</th>
<th>Interval between Positive Test and Onset of Symptom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antinuclear antibodies</td>
<td>101 (78)</td>
<td>9.2</td>
<td>50 (48)</td>
<td>102 (80)</td>
<td>3.04±0.25</td>
<td>82 (77)</td>
<td>3.25±0.22</td>
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<tr>
<td>Anti-Ro antibodies</td>
<td>61 (47)</td>
<td>9.4</td>
<td>64 (49)</td>
<td>64 (49)</td>
<td>3.08±0.14</td>
<td>55 (48)</td>
<td>2.97±0.59</td>
</tr>
<tr>
<td>Anti-La antibodies</td>
<td>44 (34)</td>
<td>9.2</td>
<td>61 (45)</td>
<td>45 (35)</td>
<td>3.63±0.38</td>
<td>39 (34)</td>
<td>2.33±0.43</td>
</tr>
<tr>
<td>Anti-nuclear phospholipid antibodies</td>
<td>24 (18)</td>
<td>7.6</td>
<td>67 (51)</td>
<td>67 (51)</td>
<td>2.94±0.50</td>
<td>39 (34)</td>
<td>2.29±0.56</td>
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<tr>
<td>Anti-double-stranded DNA antibodies</td>
<td>72 (55)</td>
<td>9.3</td>
<td>48 (36)</td>
<td>80 (60)</td>
<td>2.24±0.31</td>
<td>54 (47)</td>
<td>1.24±0.31</td>
</tr>
<tr>
<td>Anti-Sm antibodies</td>
<td>41 (32)</td>
<td>8.1</td>
<td>31 (24)</td>
<td>49 (38)</td>
<td>3.47±0.34</td>
<td>28 (24)</td>
<td>0.47±0.44</td>
</tr>
<tr>
<td>Anti-nuclear ribonucleoprotein antibodies</td>
<td>54 (41)</td>
<td>7.2</td>
<td>23 (18)</td>
<td>43 (33)</td>
<td>0.88±0.32</td>
<td>25 (20)</td>
<td>0.26±0.47</td>
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Possible significance of a positive autoantibody test in an individual with no evidence of autoimmunity

- Early manifestation of an incipient autoimmune condition?
- Transient immune disturbance?
  - Infection?
  - Drugs?
  - Cancer?
- Familial autoimmunity trait?
- Minimal manifestation of a spectral autoimmune condition?
- Incidentaloma? Autoantibodies not associated to autoimmune diseases

Transient ANA induced by Erythrovirus infection

1:320
March, 12, 2003

1:80
September 13, 2003

A.S. – Female, 32 years old
Fever, malaise, polyarthritis in small and large joints, and mild diffuse erythematous rash for two months.
Full recovery with no specific medication.
Erythrovirus (parvovirus) B19 serology ➔ IgM and IgG positive
Possible significance of a positive autoantibody test in an individual with no evidence of autoimmunity

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ANTI-CENTROMERE ANTIBODY IN AN OTHERWISE ASYMPTOMATIC INDIVIDUAL

SCLERODERMA SPECTRUM
Autoantibodies may be a familial affair...

Autoantibodies as a familial trait

Asymptomatic 4-years old girl
Asymptomatic 5-years old brother
Asymptomatic mother
Possible significance of a positive autoantibody test in an individual with no evidence of autoimmunity

- Early manifestation of an incipient autoimmune condition?
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- Familial autoimmunity trait?
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- **Incidentaloma? Autoantibodies not associated to autoimmune diseases**

Conclusions

- Autoantibodies are complex analytes and exhibit wide intra and inter-individual heterogeneity in avidity, serum concentration, and epitope targets
- Methodological platforms determine the spectrum of autoantibodies detected in any particular sample
- As a screening test, ANA-HEp-2 platform has the advantage of offering a “natural structured array” and therefore the ANA-HEp-2 pattern is highly informative on the nature of the autoantibody
Conclusions

- ANA-HEp-2 routine must be guided by strict quality control policies and the ANA-HEp-2 report should specify the fluorescence pattern and titer at the various cell compartments as well as an interpretation of the overall findings
- Clinical interpretation of ANA-HEp-2 tests must take into consideration the possibilities of subclinical disease, incipient autoimmunity, and transient immune imbalance due to infection, drugs and cancer

ACKNOWLEDGEMENTS

<table>
<thead>
<tr>
<th>ANA dream-team</th>
<th>ANA National Consensus</th>
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<tbody>
<tr>
<td>Alessandra Dellavance</td>
<td>Paulo Luiz C. Francescantonio</td>
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<td>Silvia Helena Rodrigues</td>
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<td>Marcia Pereto</td>
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Study comparing ANAs in healthy subjects and ARD patients

- Henrique Athayde Mariz
- Emilia Inoue Sato
- Silvia Helena Barbosa
- Alessandra Dellavance