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# Recommendations of the ACR committee on the standardization of autoantibody detection

PL Meroni
University of Milan - Dept. Int. Medicine
Division of Rheumatology - Ist. G. Pini
IRCCS Ist Auxologico Italiano

# An old story

## Detection and quantification of autoantibodies are vital tests in the diagnosis and management of autoimmune diseases

Fritzler et al. *Arthritis Res Ther* 2003;5:192-201.
Wiik et al. *Lupus* 2006;15:391-96

# A new aspect

- Increased volume of investigations
- No more in specialized labs only
- More techniques (new techniques)
- Automated assays

the problem of their reproducibility is a burning question

# Additional troubles

- clinical manifestations in the initial phases of AID are often faint and uncharacteristic
- the early diagnosis is a prerequisite for a successful treatment
- a wrong assay may delay the diagnosis or
- may be responsible for additional costs due to the repetition of confirmatory tests and/or to consequent unnecessary diagnostic investigations.
- Ethical aspect (Bossuyt et al. *Ann Rheum Dis* 2008;67:1061-63).

# Anti-nuclear antibody (ANA) screening: a paradigmatic example of the problem

# ANA Task Force

- '08 increased number of false negative ANA results in the clinical practice in USA
- Specific concern by ACR Members
- Ad hoc Committee (ANA Task Force)

Peter Schur (chair),
Morris Reichlin,
Joe Craft,
Eng Tan,
Westley Reeves,
Dan Wallace,
John A. Goldman,
Donald Bloch,
Pier Luigi Meroni,
Eileen Moynihan

# The cause of the clash

- ANA screening by ELISA or coated beads as cost/time saving methods are becoming popular and are susbstituting the standard IIF
- A small but significant number of SLE or other CTD patients may display autoantibodies detectable by IIF but not by solid phase substrates that employ a limited number of autoantigens.

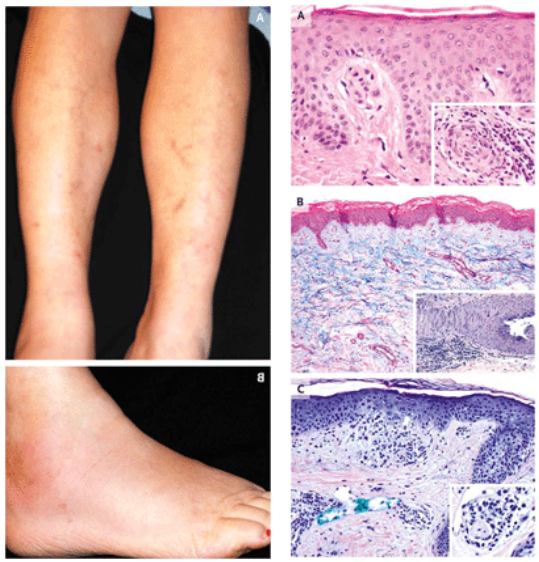
The NEW ENGLAND JOURNAL of MEDICINE

CASE RECORDS OF THE MASSACHUSETTS GENERAL HOSPITAL

Volume 360:711-720 February 12, 2009 Number 7

# Case 5-2009 — A 47-Year-Old Woman with a Rash and Numbness and Pain in the Legs

*Daniela Kroshinsky, M.D., John H. Stone, M.D., M.P.H., Donald B. Bloch, M.D., and Alireza Sepehr, M.D.*



The negative ANA performed at the beginning of the clinical evaluation delayed the right diagnosis (SLE) for months.

# The reason of the clash

- The solid phase methods are able to detect antibodies *only* against the molecules/antigens coated on the solid phase used.
- HEp-2 cells contain many more than the limited numbers of gens linked to the fluorescent beads.

- In case of beads coated with the so called Ep-2 nuclear extract it is not known whether all the antigens are available. Important epitopes may be altered or lost during the process of conjugation of the antigens to the beads.

| Reference | N. SLE pats | ANA IF<br>% SLE pat. Pos | ANA Solid Phase Assay % SLE pat Pos | ANA Solid Phase Assay Method |
|---|---|---|---|---|
| 9 | 55 | 91% (1:80)* | 87%<br>89%<br>78% | Radim SpA<br>EIA Zeus<br>EIA Varelisa<br>ReCombi (Pharmacia) |
| 11 | 53 | 91%(>1:50) | 49% | Athena Multilyte |
| 16 | 71 (inlcuding SLE, DLE, drug induced) | 98% (1:40) | 91% | RADIAS (Biorad) |
| 17 | 34 | 76%(>1:160) | 62% | ELIA Pharmacia |
| 19 | 202 | 87% | 75% | Varelisa ELISA |
| 30 | 50 | 84% at 1:50<br>80% at 1:100<br>76% at 1:200 | 40% Anti-Nucleosomes<br>56% Quanta Lite | GmbH<br>Inova |
| 32 | 38 | 92% | 79% | QUANTA Lite (INOVA) |
| 33 | 192 | 99 (81)%° | 75.5% | Bioplex |
| 34 | 35 | 97%(>1:160) | 100%<br>94%<br>100%<br>60%<br>62% | Quanta Life<br>Bio-Rad Relisa<br>VarElisa<br>UniCap |

# ANA Task Force Recommendations

- IFA ANA test should remain the gold standard for ANA testing.
- Hospital and commercial laboratories using bead-based multiplex platforms or other solid phase assays for detecting ANA must provide data to ordering physicians on request that their assay has the same or improved sensitivity and specificity compared to the IFA ANA.
- In-house assays for detecting ANA as well as anti-DNA, anti-Sm, anti-RNP, anti-Ro/SS-A, anti-La/SS-B, *etc.* should be standardized according to national (*e.g.*, CDC) and/or international (*e.g.*,WHO, IUIS) standards;
- Laboratories should specify the methods utilized for detecting ANA when reporting their results.

# Failure of Clinical Trials on SLE: an additional aspect

- The debate on the failure of SLE clinical trials include among the other problem the hetereogeneicity of patients and the lack of reliable biomarkers (Merrill & Buyon *Nat Rev Rheumatol.* 2009 ).
- In SLE classification autoantibody play an important role: 2 out of 4 ACR classification criteria needed to include patients in clinical trials and they are useful to identify the disease profile of patients

# Standardisation/harmonization of the diagnostic tests for autoimmune diseases

- evaluation of the variability in the methods used;
- interpretation of the tests and correct algorithms;
- characterization of the antigens used as reagents to bind to the auto antibodies in the patients samples;
- availability of reference material and assay standardisation;
- characterization of the affinity and avidity of the autoantibodies in the patient sample.