Unending story of the indirect immunofluorescence assay on HEp-2 cells: old problems and new solutions?

The paper by Pisetsky *et al* raised some critical points on the indirect immunofluorescence assay (IFA) on HEp-2 cells (HEp-2 IFA): (1) the low antinuclear antibodies (ANA) pretest and post-test probability, and (2) the variability of the IFA ANA result depending on the method type and reagent source.[1] One consequence of these points is the heterogeneity in the classification criteria for systemic lupus erythematosus and/or the inclusion criteria in clinical trials or in specific treatment protocols.

Unfortunately, the paper by Pisetsky *et al* conveys no practical suggestions on how to minimise these issues, and the impact on the readers could be one of confusion rather than resolution.

Several international committees are joining their efforts in order to avoid misdiagnosis and to develop approaches to the correct interpretation of the IFA ANA results depending on the technique used for detecting a given autoantibody.[2] Screening tests for ANA represent the best example. After the initial recommendation by the American College Rheumatology (ACR) Task Force in 2010,[3] several papers have addressed this issue in the last years. While IFA ANA offered advantages in comparison with the solid phase assays (SPA) available at the time of the ACR position paper, the performance of the newer SPA has recently improved. Advantages and disadvantages of the two methodological platforms have been reviewed and discussed, and none of the two immunoassays appears to satisfy completely the required demands.[4] However, the combination of HEp-2 IFA and SPA including the most relevant nuclear and cytoplasmic antigens for the diagnosis of systemic rheumatic autoimmune diseases (SARD) has been reported to display higher specificity and post-test probability than the use of the respective single tests.[5] In addition, new tests that employ a panel of autoantigens relevant for a given subset of SARD (eg, lupus-like, systemic sclerosis, myopathies, antiphospholipid syndrome) are now available or are going to be launched soon, increasing their specificity/post-test probability in a significant manner. For example, the combination of HEp-2 IFA and SPA for autoantibody screening could decrease 'false positive or false negative' results, while the use of one screening assay and the new antigen-specific multiplex immunoassays in the context of a specific clinical setting might increase the diagnostic power.

In parallel, the International Consensus on ANA Patterns initiative has defined 30 HEp-2 IFA patterns (www.ANApatterns.org) that provide clues for the autoantibodies most likely to be present in a given sample, thereby adding value to the test and directing the investigation towards specific autoantibody assays.[6] Since the several HEp-2 IFA patterns have diverse immunological and clinical implications, disease classification criteria and inclusion criteria for clinical trials referent to HEp-2 IFA should define which patterns are to be included.

In this interim, the strategy for autoantibody testing has been under reassessment, and it might be advantageous to consider the combination of the new serological tools for better understanding of the meaning of a given positive (or negative) result. The correct use and interpretation of autoantibody testing is mandatory, and a specific European League Against Rheumatism Task Force has been planned to address the issue in conjunction with the other international committees.

This paper was already the focus of some correspondence addressing the issue of ANA testing[7] and others that will also be published on the electronic pages (references to be added by typesetter).

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Handling editor Josef S Smolen

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Contributors All the authors contributed to the letter.

Competing interests None declared.

Patient consent Not required.

Provenance and peer review Not commissioned; internally peer reviewed.

To cite Meroni PL, Chan EK, Damoiseaux J, *et al*. *Ann Rheum Dis* 2019;**78**:e46.

Received 19 March 2018
Revised 3 April 2018
Accepted 6 April 2018
Published Online First 17 April 2019

Linked

► http://dx.doi.org/10.1136/annrheumdis-2018-213537

Ann Rheum Dis 2019;**78**:e46. doi:10.1136/annrheumdis-2018-213440

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