

Pitfalls of antinuclear antibody detection in systemic lupus erythematosus: the positive experience of a national multicentre study

The recent paper by Pisetsky *et al*,¹ which already elicited some debate,^{2,3} reported data on the use of antinuclear antibodies (ANA) detection in patients with systemic lupus erythematosus (SLE) in the real life and raised concerns on the usefulness of the assays because of the significant percentage of samples tested negative for ANA in spite of the use of well-validated assays and the inclusion of patients with an established diagnosis. This finding may have negative implications both for the correct classification and the inclusion in clinical trials.

We report here a multicentre study carried out by an Italian interdisciplinary group (Forum Interdisciplinare per la Ricerca sulle Malattie Autoimmuni) for the validation of new automated reading systems for ANA detection by indirect immunofluorescence on HEp-2 cells (HEp-2 IFA). Ninety-one patients with well-established SLE (18/91 men, mean age 40±11 years)⁴ were tested for ANA, anti-extractable nuclear antigens (ENA) and anti-double stranded (ds)-DNA. Manual HEp-2 IFA (with different HEp-2 commercial preparations including HEp-2000) was performed at the recruiting centre and then sent to two core labs where three different automated ANA reading systems were used with the manufacturers' cell substrate (1:80 screening dilution). Moreover, all the samples were tested by two commercial connective tissue disease (CTD) screening solid phase arrays (SPA). We found almost a perfect agreement between manual and automated ANA reading. As expected, SPA displayed a lower sensitivity (table 1).⁵ By testing all the samples with both HEp-2 IFA and SPA we reached 100% sensitivity. One sample only tested positive for DFS70 but the positivity was associated with anti-ENA antibodies, a combination that can be found in systemic autoimmune rheumatic diseases.⁶ The detection of antibodies against ENA and dsDNA was performed at the recruiting centres by using different commercial kits. The percentages of patients positive for anti-ENA and anti-dsDNA were similar to those of the Pisetsky's series making the two cohorts comparable.

The results of our study show that: (1) ANA is a hallmark of established SLE; (2) ANA detection by different commercially available kits is reliable in a multicentre setting and the variability linked to the single operator does not seem to be a critical issue. The CTD screening SPAs display a lower sensitivity than HEp-2 IFA likely due to the limited number of autoantigens, however they can offer an additional diagnostic value when carried out together with IFA.⁵

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REFERENCES

- Pisetsky DS, Spencer DM, Lipsky PE, *et al*. Assay variation in the detection of antinuclear antibodies in the sera of patients with established SLE. *Ann Rheum Dis* 2018. doi: 10.1136/annrheumdis-2017-212599. [Epub ahead of print 9 Feb 2018].
- Mahler M. Lack of standardisation of ANA and implications for drug development and precision medicine. *Ann Rheum Dis* 2018. doi: 10.1136/annrheumdis-2018-213374. [Epub ahead of print 24 Mar 2018].
- Pisetsky DS, Spencer DM, Lipsky PE, *et al*. Response to: 'Lack of standardization of ANA and implications for drug development and precision medicine' by Mahler. *Ann Rheum Dis* 2018. doi: 10.1136/annrheumdis-2018-213399. [Epub ahead of print 28 Mar 2018].
- Petri M, Orbai AM, Alarcón GS, *et al*. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum* 2012;64:2677–86.
- Claessens J, Belmondo T, De Langhe E, *et al*. Solid phase assays versus automated indirect immunofluorescence for detection of antinuclear antibodies. *Autoimmun Rev* 2018. doi: 10.1016/j.autrev.2018.03.002. [Epub ahead of print 8 Mar 2018].
- Conrad K, Röber N, Andrade LE, *et al*. The clinical relevance of Anti-DFS70 autoantibodies. *Clin Rev Allergy Immunol* 2017;52:202–16.

Table 1 ANA detection

Test results	HEp-2 IFA				CTD SPA*			
	Manual	Auto 1†	Auto 2	Auto 3	a	b	Anti-ENA	Anti-dsDNA
Positive % (n/N)	100 (91/91)	100 (91/91)	98 (89/91)	99 (90/91)	88 (80/91)	91 (83/91)	45 (40/89)	59 (54/91)
Negative % (n/N)	0 (0/91)	0 (0/0)	2 (2/91)	1 (1/91)	12 (11/91)	9 (8/91)	55 (49/89)	41 (37/91)

*CTD SPA, connective tissue diseases screening solid phase assay; (a) Quanta Flash CTD screen plus, INOVA Diagnostics: recombinant Scl-70, Jo-1, SSA/Ro 52, SSA/Ro 60, SSB/La, centromere A and B, RNA Pol III, Mi-2, Ku, Th/To, PCNA, native Sm and RNP, synthetic Pm/Scl and ribosomal-P peptides and synthetic dsDNA; (b) ELiA CTD screen, Thermo Fisher: dsDNA, SSA/Ro 52, SSA/Ro 60, SSB/La, U1-RNP (RNP-70, A, C), Sm, centromere B, Jo-1, Scl-70, Rib-P, fibrillarin, RNA Pol III, PM-Scl, PCNA and Mi-2, all recombinant except native purified dsDNA.

†Automated reading systems: (1) AKLIDES, Medipan GMBH; (2) NOVA-View, INOVA Diagnostics; (3) G-Sight, Menarini Diagnostics.

ANA, anti-nuclear antibodies; ENA, extractable nuclear antigen; HEp-2 IFA, indirect immunofluorescence on HEp-2 cells.