Can solid-phase assays replace immunofluorescence for ANA screening?

The paper by Pisetsky *et al*[1] has stimulated a timely and interesting debate on a focal point in the diagnosis of autoimmune rheumatic diseases, that is, the accuracy of the antinuclear antibody (ANA) test and the reliability of the results provided by this test. The data produced by Pisetsky *et al* once more demonstrate the poor standardisation of the ANA assay when performed by the indirect immunofluorescence (IIF) method and the enormous difference that exists between different ANA-IIF kits. However, issues are related to intermethods variability and to the intrinsic limitations of the IIF method. Whatever title is chosen, it entails either a relevant loss of diagnostic specificity or sensitivity. This raises the question of whether IIF on HEp-2 cell substrates should be still considered the *gold standard* for ANA detection as stated almost 10 years ago by the American College of Rheumatology (ACR). The reasons that led the ACR to take this position were related to the insufficient diagnostic sensitivity of emerging alternative methods to IIF. These alternative methods, which were then almost exclusively made up of immunoenzymatic assays (ELISA), were spreading in clinical laboratories as substitutes of the manual IIF method to overcome known IIF limitations and for their higher throughput. At that time, however, studies comparing the results of the IIF with the ELISA methods had shown that the ANA-IIF provided, in most cases, better performance than the ELISA methods,[2] despite IIF having low specificity and being non-sensitive in the detection of certain antibodies (such as Ro52, Ro60, ribosomes and Jo1) that play an important role in the diagnosis and classification of some ANA-associated rheumatic diseases (AARD) such as Sjögren's syndrome, systemic lupus erythematosus (SLE) and autoimmune inflammatory myopathies (AIM).

Ten years later, we must ask, 'Is this assumption still true?' As pointed out by Meroni *et al*[3] in their comment, in recent years, technology has made considerable progress. ELISA methods are now being abandoned and replaced by more accurate immunometric solid-phase assays (SPA) such as chemiluminescence and fluoroimmunoenzymatic methods. Over the years, these new SPAs have demonstrated high diagnostic performance, so the suggestion has been advanced that they could replace IIF for the detection of ANA, yielding a more objective, rapid and quantitative result.[4] Moreover, the consolidation of clinical laboratories, widely occurring both in Europe and in North America, has meant that the volume of autoantibody tests that each laboratory must perform today is greatly increased, making both manual and automated IIF techniques increasingly difficult to apply, because of their long turnaround time compared with the fully automated and random access SPA methods, and because the ANA-IIF test must be personally read and interpreted (under a microscope or video) and is therefore exposed to a certain degree of subjectivity and to the operator's experience.[5]

Two solid-phase monotest methods, called CTD screen, are available today for ANA screening which include a mixture of 15–16 purified native or recombinant antigens among those most frequently recognised by autoantibodies in AARD. We reviewed all the studies that have compared the diagnostic accuracy (sensitivity, specificity and efficiency) of the CTD screen assays versus IIF both in selected cohorts of patients with AARD and in the daily workup[6–12] (table 1), reproducing the real-life ANA testing as recommended in their correspondence by Infantino *et al*.[13]

Taken together, these studies show that IIF has a higher sensitivity and a much lower specificity than SPA. However, when these data are analysed using receiver operating characteristic curves and compared with an equal specificity value, even sensitivity is higher for SPA. Overall, these data suggest that screening by SPA yields results that are at least comparable to—and probably better than—ANA-IIF results. However, it is important to note that, with regard to the individual AARD, diagnostic accuracy is different. From the cited studies, it is evident that IIF is slightly superior to SPA in detecting SLE and scleroderma, while SPA methods guarantee better results in Sjögren's syndrome and in AIM. So, if today we should have to choose between one of the two methods, neither would allow us to diagnose all patients with AARD.

Therefore, from a clinical point of view, the best diagnostic strategy seems to be the combined use of the two methods, according to an algorithm which requires the subsequent identification of individual antibodies only in cases that are positive by SPAs. Would this strategy also be economically sustainable? A recent cost analysis has shown that screening by IIF followed by analysis of antibody fine specificity by immunometric or immunoblot methods in all ANA-IIF positive samples provides, in most cases, negative or clinically irrelevant results; and that, by using the two methods in parallel and proceeding with testing to identify the fine antibody specificity only in SPA positive samples, the costs associated with the many false ANA-IIF positives would be reduced,[12] avoiding in addition unnecessary clinical referrals and test repetitions.

A final issue regards the fact that the ANA-IIF test allows for identification of patterns such as mitochondrial, multiple nuclear dots, and rim-like, which have diagnostic significance for autoimmune liver diseases such as primary biliary cholangitis. The available evidence, therefore, suggests that SPAs are not yet able

Table 1 (A) Diagnostic accuracy and overall efficiency (correct classification rate) of the ANA-immunofluorescence (IIF) method compared with solid-phase assays (SPA). (B) The best performing method in ANA-associated autoimmune rheumatic diseases according to the different studies

A							B			
	Sensitivity (%)		Specificity (%)		Efficiency (%)					
Author/year	ANA-IIF	SPA	ANA-IIF	SPA	ANA-IIF	SPA	SLE	SjS	SSc	AIM
Op De Beeck *et al* (2011)[6]	87.2	73.0	86.3	96.9	86.6	88.6	SPA	SPA	IIF	SPA
Robier *et al* (2016)[7]	98.7	82.7	85.8	98.2	81.1	90.7	IIF	SPA	IIF	–
Bentow *et al* (2015)[9]*	84.8	78.1	64.7	94.1	81.0	86.6	IIF	SPA	SPA	–
Otten *et al* (2017)[8]	81.7	78.9	88.6	95.1	72.8	77.1	IIF	SPA	Equal	IIF
van der Pol *et al* (2018)[10]†	90.0	95.1	76.0	80.0	78.9	83.4	SPA	SPA	Equal	Equal
Claessens *et al* (2018)[11]†*	95.2	83.1	61.0	92.9	69.8	89.1	IIF	SPA	Equal	SPA
Bizzaro *et al* (2018)[12]†	89.2	87.1	64.6	98.0	69.9	96.0	IIF	SPA	IIF	SPA

*In this study, ANA-IIF reading was performed with a computer-aided system (Nova View, Inova Diagnostics).
†These studies compared IIF with two SPA methods (chemiluminescence and fluoroimmunoenzymatic assay); data for SPA methods are combined.
AIM, autoimmune inflammatory myopathies; ANA, antinuclear antibody; IIF, indirect immunofluorescence; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; SjS, Sjögren's syndrome.

to completely replace IIF and that the IIF method should be used for ANA screening until solid-phase methods become available to detect a greater number of autoantibodies not yet present in the antigenic panel of CTD screen assays. Only then will it be possible to evaluate whether these new methods would actually be able to completely replace the ANA-IIF method.

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