Antinuclear antibody as entry criterion for classification of systemic lupus erythematosus: pitfalls and opportunities

Antinuclear antibodies (ANAs) are helpful to support the diagnosis of ANA-associated systemic rheumatic diseases (AASRD). Pisetsky *et al* recently reported on the variability of ANA detection, with differences observed between assay platforms (indirect immunofluorescence (IIF) vs solid phase) and kits in patients with established systemic lupus erythematosus (SLE).[1] Variation of ANA detection has also been shown for automated IIF systems.[2] Initiatives to better understand the variability of ANA tests are needed.[3] Pisetsky *et al*[1] also pointed out that ANA negativity occurs in established SLE, thereby complicating screening for patients for clinical trials.[1] Yet, an Italian study reported a high sensitivity of ANA for established SLE.[4]

Testing for ANAs is complex and accurate interpretation of test results might be difficult. A task force of the European League Against Rheumatism (EULAR) has recently been installed that will address these issues in conjunction with other international committees.[5] In this context and of particular interest is that new criteria for the classification of patients with SLE are being developed under the umbrellas of the EULAR and the American College of Rheumatology (ACR).[6] In these criteria, a history of ANAs ≥1:80 by HEp-2 IIF will be the entry criterion (ie, must be present to be considered for classification as SLE).[6] The ≥1:80 cut-off was chosen in order to ensure high sensitivity.[6]

We evaluated the performance of ANA for SLE diagnosis on 9851 unique consecutive patients tested for ANA (for description of the population, see Willems *et al*[7]). All patients were tested for ANA by IIF (HEp-2000; ImmunoConcepts) and by solid-phase assay (EliA CTD screen; Thermo Fisher).[7] The clinical diagnosis was documented for 2475 patients, including (1) all patients who tested positive for IIF (cut-off 1:80) and/or CTD screen (cut-off ratio 0.7) and (2) a selection of 500 patients who tested double negative (including 150 patients with IIF titre 1:40).[7] This allowed us to calculate the positive predictive value (PPV) of IIF for SLE. As all samples were also tested by CTD screen, we could document SLE cases that tested negative by IIF but positive by CTD screen. Patients with SLE were divided into newly diagnosed SLE, established SLE and patients who did not fulfil the classification criteria.[8]

The titre-specific PPV of IIF 1:80 for SLE fulfilling the ACR classification criteria[8] was 1%, which is low and comparable with the estimated prevalence of SLE in the entire population (0.9%). The estimated likelihood ratio (LR) associated with IIF 1:80 was 1.16, indicating almost no difference in pretest to post-test probability. Of note, IIF 1:80 accounted for 37% of all positive ANA IIF results. The titre-specific PPV for SLE increased with increasing antibody levels and was 3.5%, 5.8%, 8.7%, 11.8% and 16.8% for, respectively, IIF titre 1:160, 1:320, 1:640, ≥1:1280 and reactivity to overexpressed SSA on the HEp-2000 substrate. The estimated titre-specific LRs were, respectively, 4.1, 7.0, 10.8, 14.7 and 21.8. Newly diagnosed patients with SLE had IIF results ≥1:160, whereas 10% (8/83) of patients with established SLE were IIF negative. Of note, six of the eight IIF-negative patients with established SLE tested positive with CTD screen.

ANAs are also associated with cutaneous lupus, mixed connective tissue disease (MCTD), systemic sclerosis (SSc), Sjögren's syndrome (SS) and idiopathic inflammatory myopathy (IIM). The PPV for AASRD (SLE, SSc, SS, IIM, MCTD and cutaneous lupus) was 2%, 6.8%, 15%, 31.7%, 47.6% and 50% for, respectively, IIF 1:80, 1:160, 1:320, 1:640, ≥1:1280 and reactivity to the overexpressed SSA. Thus, SLE has to be distinguished from other AASRDs.

The PPV for SLE of IIF 1:80 combined with a positive CTD screen was 5.6% (estimated LR: 6.8) compared with 1% for IIF 1:80 alone. It was 0.4% for IIF 1:80 combined with a negative CTD screen (estimated LR: 0.4). Similar findings (ie, increased PPV for double positivity and decreased PPV for singly positivity) were found when CTD screen was combined with higher IIF titres (see table 1 for an overview of the PPVs). For AASRD, an analogous increase in PPV was observed when IIF was combined with solid phase assay (see table 1 and Willems *et al*[7]).

Taken together, we found (1) that the titre-specific PPV of low-titre ANA for SLE is low, (2) that the PPV for SLE increases with increasing IIF titre and (3) that combining IIF with solid-phase assay adds value.

This implies that a low-positive ANA IIF titre (1:80) does not significantly increase the post-test probability for SLE (as the PPV is comparable with the PPV for the entire population tested for ANA). Thus, in those cases, classification will have to rely on clinical manifestations/characteristics. The downsides of the low PPV include potential false diagnoses and inappropriate treatment by clinicians not familiar with rheumatic diseases or inappropriate referrals to rheumatologists. It is important that clinicians are acquainted with the clinical manifestations/characteristics of SLE.

It is valuable to distinguish a low positive IIF titre (1:80) from a negative IIF result, as a negative result is useful to exclude SLE, whereas a low-positive result is not. It is also valuable to distinguish a low-positive IIF titre from a high-positive IIF titre, as a high titre has a higher PPV for SLE than a low titre. Therefore, an IIF result should not be seen as a dichotomous result (positive vs negative) but as a result with titre-specific LRs for disease. A potential danger of the new classification criteria is that clinicians not familiar with systemic rheumatic diseases will overestimate the PPV of a low-positive IIF ANA, as a cut-off of 1:80 is explicitly mentioned.

New classification criteria should recognise the high (but not absolute) negative predictive value of IIF and also that a low-positive IIF ANA has a lower PPV than a high-positive IIF ANA. Different weights could be assigned to an IIF result depending on the level of positivity. Furthermore, combining IIF with solid-phase assay can help to better stratify patients, especially in case of low-positive IIF titre.[9–11]

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Table 1 Clinical diagnoses in patients tested for antinuclear antibodies (ANAs)

Disease	IIF negative	IIF 1:80	IIF 1:160	IIF 1:320	IIF 1:640	IIF ≥1:1280	IIF SSA	Total
SLE (newly diagnosed)			3 (1,0,2)	1 (0,1,0)	2 (1,0,1)	6 (1,0,5)	3 (0,0,3)	15
SLE (established)	8 (2,0,6)	6 (2,1,3)	14 (8,0,6)	11 (4,2,5)	7 (2,0,5)	6 (0,0,6)	16 (0,0,16)	68
SLE (not fulfilling classification criteria)	6 (3,2,1)	2 (0,0,2)	2 (2,0,0)	1 (0,0,1)	1 (1,0,0)	3 (1,1,1)	5 (0,0,5)	20
Cutaneous lupus	4 (4,0,0)	3 (1,0,2)	4 (2,0,2)	1 (1,0,0)			10 (0,0,10)	22
Systemic sclerosis (newly diagnosed)			3 (1,0,2)	5 (0,0,5)	9 (2,0,7)	19 (3,0,16)		36
Systemic sclerosis (established)			5 (1,0,4)	5 (1,1,3)	11 (0,0,11)	6 (1,0,5)		27
Systemic sclerosis (not fulfilling classification criteria)	1 (0,1,0)	3 (3,0,0)	1 (0,0,1)	2 (0,0,2)	4 (0,0,4)	2 (1,0,1)	1 (0,0,1)	14
Polymyositis/dermatomyositis (newly diagnosed)	2 (0,0,2)		1 (0,0,1)	2 (1,1,0)	2 (1,0,1)	2 (0,0,2)		9
Polymyositis/dermatomyositis (established)			1 (1,0,0)	2 (1,1,0)	1 (0,0,1)	3 (1,1,1)	1 (0,0,1)	8
Polymyositis/dermatomyositis (not fulfilling classification criteria)	2 (0,0,2)	1 (0,1,0)	2 (2,0,0)	4 (3,0,1)	2 (0,0,2)		1 (0,0,1)	12
Sjögren's syndrome (newly diagnosed)	4 (0,0,4)	1 (0,0,1)				1 (0,0,1)	13 (0,0,13)	19
Sjögren (established)	2 (0,1,1)	2 (2,0,0)	1 (0,0,1)	3 (0,0,3)	1 (0,0,1)	1 (0,0,1)	16 (0,0,16)	26
Sjögren (not fulfilling classification criteria)	2 (1,1,0)	1 (1,0,0)					1 (0,0,1)	4
Mixed connective tissue disease (newly diagnosed)						4 (0,0,4)		4
Mixed connective tissue disease (established)			1 (0,0,1)	1 (0,0,1)		2 (0,0,2)		4
Mixed connective tissue disease (not fulfilling classification criteria)			1 (0,1,0)			3 (0,0,3)		4
Not differentiated (doubtful)	1 (1,0,0)		3 (3,0,0)	2 (1, 1)	1 (1,0,0)		1 (0,0,1)	8
Non-AASRD	47 (30,3,14)	30 (22,0,8)	18 (17,01)	8 (6,0,2)	2 (1,0,1)	2 (2,0,0)	3 (0,0,3)	110
Rheumatic disease	49 (25,11,13)	42 (37,1,4)	52 (48,0,4)	23 (20,1,2)	6 (5,1,0)	4 (4,0,0)	7 (0,0,7)	183
Inflammatory disease	76 (39,15,22)	64 (54,4,6)	44 (37,2,5)	27 (3,15,9)	12 (10,1,1)	12 (10, , 2)	6 (0,0,6)	241
No inflammatory disease	655 (395,96,164)	439 (399,10,30)	332 (296,13,23)	109 (92,9,8)	43 (33,4,6)	29 (18,1,10)	34 (4,2,28)	1641
Total	859 (500,130,229)	594 (521,17,56)	488 (419,16,53)	207 (145,19,43)	104 (56,5,43)	105 (42,4,59)	118 (4,2,112)	2475
Positive predictive values (PPVs)								
PPV of IIF for SLE	0.001	0.010	0.035	0.058	0.087	0.118	0.168	
PPV of IIF/EliA(–) SLE		0.004	0.022	0.028	0.055	0.024		
PPV of IIF/EliA(+)* SLE		0.056	0.116	0.131	0.125	0.180	0.174	
PPV of IIF for AASRD		0.020	0.069	0.157	0.344	0.515	0.541	
PPV of IIF/EliA(–) for AASRD		0.010	0.034	0.057	0.109	0.150		
PPV of IIF/EliA(+)* for AASRD	0.040	0.100	0.284	0.404	0.659	0.772	0.562	
PPV of IIF/EliA(+)† for AASRD	0.058	0.111	0.365	0.447	0.750	0.796	0.573	
Estimated likelihood ratios (LRs)								
Estimated LR of IIF for SLE	0.096	1.2	4.1	7.0	10.9	15.1	23.0	
Estimated LR of IIF/EliA(–) for SLE		0.44	2.5	3.2	6.6	2.8		
Estimated LR of IIF/EliA(+) for SLE		6.8	14.9	17.1	16.2	25.0	24.0	

9851 consecutive patients were tested for ANA by indirect immunofluorescence (IIF) (HEp-2000; ImmunoConcepts) and by solid phase (EliA CTD screen, detecting antibodies to dsDNA, SSA/Ro 52, SSA/Ro 60, SSB/La, U1-RNP (RNP-70, A, C), Sm, Jo-1, Scl-70, CENP, fibrillarin, RNA Pol III, PM-Scl, Mi-2, Rib-P and PCNA). The table gives an overview of the clinical diagnoses and the test results in consecutive patients who tested positive for ANA by IIF and/or CTD screen and in a selection on 500 patients who tested negative by both assays (total n=2475; 325 patients were excluded because there were insufficient data for proper clinical categorisation). The values indicate the number of patients with a particular IIF result. The values in parentheses indicate the number of patients who tested negative (first number), equivocal (second number) or positive (third number) with CTD screen. The population has been described in Willems *et al*.[7] Non-AASRD (non-ANA-associated systemic rheumatic disease) includes, for example, different types of vasculitis, polymyalgia rheumatica and sarcoidosis. Rheumatic diseases include, for example, rheumatoid arthritis and psoriatic arthritis. Inflammatory diseases include, for example, colitis ulcerosa, Crohn's disease, autoimmune hepatitis, autoimmune thyroiditis, psoriasis and immune thrombocytopenic purpura.

Newly diagnosed: tested on a diagnostic sample (ie, at the time of diagnosis).

Established: tested on a follow-up sample (most of these patients had received immunosuppressive therapy and had been diagnosed in another centre).

Not fulfilling classification criteria: the clinician strongly considered the presence of an AASRD and initiated immunosuppressive therapy, but the patient did not fulfil the classification criteria. For description of the classification criteria, see Willems *et al*.[7]

For the estimation of the PPV of a negative IIF result for systemic lupus erythematosus (SLE), we only took into account the SLE cases documented (1) in the patients who were single positive for CTD screen and (2) in a selection of 500 patients who were negative for IIF and CTD screen. This probably is an underestimation as we did not check the medical records of all double-negative patients. Patients with AASRD were checked whether they fulfilled the classification criteria of Sjögren's syndrome, systemic sclerosis, dermatomyositis/polymyositis, mixed connective tissue disease and SLE as described in Willems *et al*.[7] For estimation of the PPV, we excluded patients who did not fulfil the classification criteria. For estimation of the PPV for AASRD, we included cutaneous lupus as an AASRD.

*Including equivocal results.

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with clinical classification of the patients. XB wrote a draft of the manuscript. All authors reviewed the draft and approved the submission of the manuscript.

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