Titre-specific positive predictive value of antinuclear antibody patterns

Damoiseaux *et al* recently presented the ICAP perspective on the clinical relevance of antinuclear antibody (ANA) patterns.¹ The authors acknowledge that in addition to the antibody pattern, the antibody titre (level) is clinically important as well. Indeed, the probability of identifying anti-dsDNA and antibodies to extractable nuclear antigens increases with increasing ANA titres.² Overall, the higher the antibody level, the higher the probability of an ANA-associated systemic rheumatic disease (AASRD).^{3–5}

There are only few studies that demonstrate the association of a particular ANA pattern and titre with AASRDs or other ANA-associated diseases. We previously studied the titre-dependent clinical association of rare ANA patterns.⁶ We here demonstrate how the positive predictive value (PPV) for an AASRD (including systemic lupus erythematosus (SLE), Sjögren's syndrome (SjS), systemic sclerosis (SSc), idiopathic inflammatory myopathy (IIM), mixed connective tissue disease and undifferentiated connective tissue disease (UCTD)) depends on the combination of antibody pattern and titre.

We evaluated the pattern-specific and titre-specific association of ANA for AASRDs, cutaneous lupus erythematosus (CLE), autoimmune hepatitis (AIH) and juvenile idiopathic arthritis (JIA) on (1) 9851 unique consecutive patients tested for ANA by HEp-2000 (ImmunoConcepts) (for description of the population, see Willems *et al*⁶) in a university hospital setting (University Hospitals Leuven, Belgium) and on (2) 529 unique consecutive patients tested for ANA by automated indirect immunofluorescence (IFA) (NOVAView) on human epithelial (HEp-2) cells from INOVA in the rheumatology department of a secondary hospital setting (OLV hospital Aalst, Belgium). The patients have been clinically defined by the respective classification criteria for each AASRD (as described in Willems *et al*⁶) or by expert clinical diagnosis (for patients who did not fulfil the classification criteria).

The analysis was performed for the most prevalent ANA patterns (speckled, homogeneous, nucleolar and centromere) and only monospecific nuclear patterns were included, except for the nucleolar and SSA pattern (ie, characteristic staining of the SSA-transfected cells in the HEp-2000 substrate) for which we also included combinations with other nuclear patterns. Samples with cytoplasmic patterns were excluded. EliA CTD Screen (which detects antibodies to dsDNA, Sm, SSA Ro60 and 52, SSB, CENPB, Scl-70, Mi-2, RNA-Pol III, PM/Scl, Jo-1, PCNA, Rib-P, U1-RNP, fibrillarin) (Thermo Fisher Scientific, Germany) was performed on an ImmunoCAP 250 Instrument (Phadia, Germany) and anti-dsDNA assay by Farr (Trinity Biotech, Ireland) were performed on all samples in the first cohort. For the second cohort (OLV hospital Aalst), only the speckled pattern was included for analysis, because of a lack of sufficient samples for other monospecific patterns in this specific study cohort. The titre-specific and pattern-specific PPV for AASRDs, CLE, AIH and JIA are presented in table 1.

For all patterns, the titre-specific PPV for AASRD was low for titre 1:80 ($\leq 4\%$) and increased with increasing antibody titres. For AIH, CLE and JIA, PPVs were low for all patterns at any titre.

The highest PPV for AASRD was found for the centromere pattern, with a PPV of 29%, 42%, 77% and 82% for a titre of 1:160, 1:320, 1:640 and 1:1280, respectively. The association was mainly with SSc. A subset of patients with a diagnosis of SjS or with a diagnosis of SLE also had a centromere pattern. These associations were previously noted and mentioned by ICAP [1 (online

supplemental data)].⁷ Of note, even at a high 1:1280 titre, the PPV for SSc was not 100% but 76%, making a centromere pattern at a high-titre highly suggestive but not pathognomonic for definite SSc in our population. Ninety-three per cent of patients with a centromere pattern had a positive CTD Screen: all of these were positive for anti-CENP (online supplementary table 1).

The PPV of the characteristic SSA pattern (including combinations with other nuclear patterns) on the HEp-2000 substrate was high for AASRDs (47%). The association was mainly with SLE and SjS. Ninety-eight per cent of patients with a monospecific SSA pattern had a positive CTD Screen: all were positive for anti-Ro60, demonstrating the effectiveness of SSA-transfected cells in identifying patients with anti-Ro60. ICAP might consider to include SSA-transfected cells in their nomenclature, given the high clinical association with AASRD. Of note, SSA antibodies were found in a subset of patients with AIH.

The PPV of the speckled pattern for AASRD was 13%, 39%, 32%, and 71% for a titre of 1:160, 1:320, 1:640 and 1:1280, respectively. The speckled pattern was observed in all AASRDs and in CLE, JIA and AIH. U1-RNP and Ro60 were the main detected underlying antigens. In the setting of a secondary hospital (online supplementary table 2), the speckled pattern (excluding dense fine speckled) was mainly found at low titres, with a low PPV for AASRD (4% for titre 1:80).

The PPV of the nucleolar pattern (including combinations with other nuclear patterns) for AASRD was 19%, 31% and 61% for a titre of 1:160, 1:320 and 1:1280, respectively. The association was mainly with SSc, but also with SLE and IIM. A nucleolar pattern in SLE has been reported previously and has been mentioned by ICAP [1 (supplemental data)].⁸ Some patients with AIH had a nucleolar pattern as well. The CTD Screen, which includes the nucleolar antigens fibrillarin and PM-Scl, was only positive in 8.8% of patients with a monospecific nucleolar pattern (online supplementary table 1).

The PPV of the homogeneous pattern for AASRD was 6%, 9% (11%), 29% (32%) and 39% (40%) for a titre of 1:160, 1:320, 1:640 and 1:1280, respectively. The values in parentheses represent PPVs after exclusion of anti-DFS70 reactivity (see next paragraph). The association was mainly with SLE, but also with SSc. The homogeneous pattern was present in several patients with AIH, CLE or JIA. In patients with JIA, the homogeneous pattern (next to some patients with a speckled pattern) was the most prevalent pattern, but titres were low. It should be recognised that a homogeneous pattern can be found in a wide variety of other (non-AASRD) conditions. Consequently, the PPV of a homogeneous pattern was rather low. For example, the PPV of a homogeneous pattern with titre 1:640 for SLE was only 21%. Fifteen per cent of samples with a monospecific homogeneous pattern had a positive anti-dsDNA Farr assay and only 11% a positive CTD Screen. Some of these samples with a positive CTD Screen had detectable anti-Scl-70 autoantibodies. ICAP appreciated that autoantibodies to topoisomerase I (formerly Scl-70) may be reported as nuclear homogeneous, but more recently proposed the TOPOI-like pattern.

As we did not morphologically distinguish the DFS70 pattern from the homogeneous pattern in the first cohort (University Hospitals Leuven), all homogeneous/DFS70 patterns with titre $\geq 1:320$ that tested negative for dsDNA antibodies by Farr assay and negative for antibodies to extractable nuclear antigens by CTD Screen ($n=93$) were tested for anti-DFS70 antibodies. Thirty-seven per cent of these samples were anti-DFS70 positive. Of these, one patient had a diagnosis of possible SLE (but did not fulfil the classification criteria) and one patient had UCTD. Overall, exclusion of anti-DFS70-positive samples had a limited effect on PPV (online

Table 1 Titre-specific positive predictive value of nuclear patterns (University Hospitals Leuven)

	1/80 (PPV, (n))					1/160 (PPV, (n))					1/320 (PPV, (n))					1/640 (PPV, (n))					≥1/1280 (PPV, (n))					SSA* (PPV, n)
	Homogeneous		Nucleolar*	Centromere	Speckled	Homogeneous		Nucleolar*	Centromere	Speckled	Homogeneous		Nucleolar*	Centromere	Speckled	Homogeneous		Nucleolar*	Centromere	Speckled						
AASRDs	0.02 (8)	0.04 (5)	0.02 (1)	–	0.06 (14)	0.13 (10)	0.06 (5)	0.19 (8)	0.29 (2)	0.11 (8)	0.39 (12)	0.31 (8)	0.42 (5)	0.32 (9)	0.32 (7)	–	0.77 (17)	0.40 (12)	0.71 (20)	0.61 (11)	0.82 (14)	0.47 (53)				
SLE	0.01 (4)	0.01 (2)	0.02 (1)	–	0.04 (10)	0.06 (5)	0.06 (5)	0.07 (3)	–	0.09 (6)	0.10 (3)	0.08 (2)	0.08 (1)	0.21 (6)	0.14 (3)	–	–	0.23 (7)	0.29 (8)	–	–	0.20 (22)				
SJS	0.01 (3)	0.01 (1)	–	–	–	–	–	–	–	–	0.06 (2)	–	0.08 (1)	0.04 (1)	–	–	–	–	–	–	0.06 (1)	0.24 (27)				
SSc	0.00 (1)	0.01 (1)	–	–	–	0.03 (2)	0.03 (2)	0.12 (5)	0.29 (2)	0.03 (2)	0.06 (2)	0.15 (4)	0.25 (3)	0.04 (1)	0.09 (2)	–	0.77 (17)	0.10 (3)	0.04 (1)	0.56 (10)	0.76 (13)	0.01 (1)				
IMM	–	0.01 (1)	–	–	0.00 (1)	0.04 (3)	–	–	–	–	0.10 (3)	0.08 (2)	–	–	0.09 (2)	–	–	0.07 (2)	0.07 (2)	0.06 (1)	–	0.02 (2)				
MCTD	–	–	–	–	0.00 (1)	–	–	–	–	–	0.03 (1)	–	–	–	–	–	–	–	0.32 (9)	–	–	–				
UCTD	–	–	–	–	0.01 (2)	–	–	–	–	0.01 (1)	0.03 (1)	–	0.03 (1)	–	–	–	–	–	–	–	–	0.01 (1)				
AIH	0.00 (1)	0.01 (1)	0.02 (1)	–	0.00 (1)	–	–	0.02 (1)	–	0.02 (2)	–	0.04 (1)	–	–	–	0.13 (1)	0.13 (1)	0.07 (2)	–	–	–	0.02 (2)				
CLE	0.00 (1)	0.01 (2)	–	–	0.01 (2)	–	–	–	–	0.01 (1)	–	–	–	–	–	–	–	–	–	–	–	0.08 (9)				
JIA	0.02 (5)	0.01 (2)	–	–	0.03 (8)	–	–	–	–	0.03 (3)	0.03 (1)	–	–	0.04 (1)	–	–	–	–	0.04 (1)	–	–	0.01 (1)				
Other (n)	255	124	56	1	218	68	78	34	5	56	18	17	7	18	15	7	5	16	7	7	3	45				
Total (n)	270	134	58	1	243	78	78	43	7	70	31	26	12	28	22	8	22	30	28	18	17	110				

*Conclusions with other nuclear patterns included.
 †Including possible and DFS70 positive samples.
 ‡Anti-DFS70 positive (monospecific) samples included; only samples with monospecific, homogeneous pattern with titre ≥ 1/320, negative dsDNA for assay and negative CTD screen were tested for anti-DFS70 antibodies.

AASRD, ANA-associated systemic rheumatic disease; AIH, autoimmune hepatitis; CLE, cutaneous lupus erythematosus; IM, idiopathic inflammatory myopathy; JIA, juvenile idiopathic arthritis; MCTD, mixed connective tissue disease; PPV, positive predictive value; SJS, Sjögren's syndrome; SLE, systemic lupus erythematosus; SSA, systemic sclerosis; UCTD, undifferentiated connective tissue disease.

supplementary table 3). Anti-DFS70 was also measured in a random selection of 20 samples with a homogeneous pattern with titre 1:160. Anti-DFS70 antibodies were found in 2/20 samples, among which was a sample from a patient with AIH (titre 1:160). Anti-DFS70 antibodies have been detected in sera with high ANA titres,⁹ a condition we also observed.

The CTD Screen was positive in 93% and 98% of samples with a monospecific centromere pattern and characteristic SSA pattern, respectively, but in only 6%, 11% and 18% of samples with a monospecific nucleolar, homogeneous and speckled pattern, respectively (online supplementary table 1). Overall, the CTD positivity rate was higher in samples with a high IFA antibody titre compared with samples with a low antibody titre. In samples with a negative CTD screen, higher titres were still associated with higher PPVs in samples with a monospecific nucleolar or speckled pattern (online supplementary table 4).

In conclusion, an integrated interpretation of the pattern and the titre allows a better appreciation of the clinical relevance of the Hep-2 IFA test. Recognising that the PPV for a particular disease is titre-specific and pattern-specific allows the physician to put the results of an individual patient in a real-life context and avoid over-diagnosis based on the Hep-2 IFA results. The low titre-specific PPVs underscore the need for confirmatory antigen-specific immunoassays in all cases. Combining Hep-2 IFA with solid phase assays adds clinical value.^{10 11} Standardisation of the nomenclature of patterns and further harmonisation of the IFA test, including estimation of antibody levels and combinations with specific assays, will contribute to a better interpretation of ANA testing.

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REFERENCES

- 1 Damoiseaux J, Andrade LEC, Carballo OG, *et al.* Clinical relevance of HEp-2 indirect immunofluorescent patterns: the International consensus on ANA patterns (ICAP) perspective. *Ann Rheum Dis* 2019;78:879–89.
- 2 Bossuyt X, Hendrickx A, Frans J. Antinuclear antibody titer and antibodies to extractable nuclear antigens. *Arthritis Rheum* 2005;53:987–8.
- 3 Op De Beeck K, Vermeersch P, Verschueren P, *et al.* Detection of antinuclear antibodies by indirect immunofluorescence and by solid phase assay. *Autoimmun Rev* 2011;10:801–8.
- 4 Claessens J, Belmondo T, De Langhe E, *et al.* Solid phase assays versus automated indirect immunofluorescence for detection of antinuclear antibodies. *Autoimmun Rev* 2018;17:533–40.
- 5 Willems P, De Langhe E, Claessens J, *et al.* Screening for connective tissue disease-associated antibodies by automated immunoassay. *Clin Chem Lab Med* 2018;56:909–18.
- 6 Vermeersch P, Bossuyt X. Prevalence and clinical significance of rare antinuclear antibody patterns. *Autoimmun Rev* 2013;12:998–1003.
- 7 Lee K-E, Kang J-H, Lee J-W, *et al.* Anti-Centromere antibody-positive Sjögren's syndrome: a distinct clinical subgroup? *Int J Rheum Dis* 2015;18:776–82.
- 8 Frodlund M, Dahlström Ö, Kastbom A, *et al.* Associations between antinuclear antibody staining patterns and clinical features of systemic lupus erythematosus: analysis of a regional Swedish register. *BMJ Open* 2013;3:e003608.
- 9 Carbone T, Pafundi V, Tramontano G, *et al.* Prevalence and serological profile of anti-DFS70 positive subjects from a routine ANA cohort. *Sci Rep* 2019;9:2177.
- 10 Bossuyt X, Claessens J, De Langhe E, *et al.* Antinuclear antibodies by indirect immunofluorescence and solid phase assays. *Ann Rheum Dis* 2019;annrheumdis-2019-215443.
- 11 Willems P, De Langhe E, Westhovens R, *et al.* Antinuclear antibody as entry criterion for classification of systemic lupus erythematosus: pitfalls and opportunities. *Ann Rheum Dis* 2019;78:e76.