

CONCISE REPORT

Specific antinuclear antibodies are associated with clinical features in systemic lupus erythematosus

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Objectives: To study associations between antinuclear antibodies (ANA) and signs/symptoms in patients with systemic lupus erythematosus (SLE).

Methods: A consecutive cohort of 289 patients with SLE was included; 235 fulfilled ACR criteria for SLE and were further analysed. ANA profiles were determined by line immunoassay and by indirect immunofluorescence on *Critidia luciliae*. An extensive list of signs/symptoms was evaluated.

Results: Five clusters of antibodies were defined by cluster analysis: 1—antibodies to SmB, SmD, RNP-A, RNP-C, and RNP-70k; 2—antibodies to Ro52, Ro60, and SSB; 3, 4, and 5—antibodies to ribosomal P, histones and dsDNA, respectively. Significant associations ($p \leq 0.01$) were found between anti-RNP-70k, anti-RNP-A, anti-RNP-C and Raynaud's phenomenon, between anti-RNP-A, anti-RNP-70k and leucopenia, and between anti-RNP-A, anti-RNP-C and a lower prevalence of urine cellular casts. Anti-SSA, anti-SSB were associated with xerostomia, and anti-SSB with pericarditis. Antibodies to ribosomal P were associated with haemolytic anaemia, leucopenia, and alopecia. Patients with anti-dsDNA antibodies had a higher risk for cellular casts and a lower risk for photosensitivity. Antihistone antibodies were associated with arthritis.

Conclusions: In a large and consecutive cohort of patients with SLE, clusters of antibodies were identified. Previously reported associations of antibodies with symptoms were confirmed and new associations found.

The presence of autoantibodies is important for the diagnosis of systemic lupus erythematosus (SLE). Antinuclear antibodies (ANA), anti-Sm, or anti-dsDNA antibodies are part of the American College of Rheumatology criteria for SLE.^{1,2} Specific reactivities are associated with distinct clinical features of SLE.^{3–5} Known associations are anti-dsDNA antibodies with lupus nephritis,^{3,5} anti-SSA and anti-SSB antibodies with sicca symptoms,^{3,5} and anti-RNP antibodies with Raynaud's phenomenon.^{3,4,6} More associations have been described, but different studies yield conflicting results.^{3–6}

Earlier studies used counterimmunoelectrophoresis or immunodiffusion for the detection of specific ANA, but more sensitive techniques have now been developed—for instance, a line immunoassay (LIA).^{7–9}

We designed this study to evaluate associations of specific ANA detected by LIA, and anti-dsDNA detected by indirect immunofluorescence (IIF) on *Critidia luciliae*, and symptoms of SLE in a consecutive cohort of patients.

PATIENTS AND METHODS

Patients

Two hundred and eighty nine patients diagnosed as having SLE, were prospectively included in four European centres

(University Hospital Ghent, Belgium; University Hospital Leiden, The Netherlands; Research Institute for Rheumatic Diseases Piestany, Slovakia; University College London, United Kingdom). Of these patients, 235 (81.3%) met the ACR revised criteria for classification of SLE,^{1,2} and were further analysed. The male to female ratio was 29:206. The mean age was 40 years (range 16–77).

A questionnaire covering the clinical data was completed, based on anamnesis and available medical data. Data were listed as present during the past year or earlier during the disease. The questionnaire was an extension of the ACR criteria for SLE^{1,2} (table 1). The study was conducted after approval by the local ethics committees. Informed consent was obtained from all patients.

ANA

The results obtained in each centre at the time of sampling were used. All centres used IIF on HEp-2 or HEp-2000 cells.

LIA

Serum samples were analysed in a single laboratory by LIA (INNO-LIA ANA Update, Innogenetics NV, Zwijnaarde, Belgium) as described previously.⁷ This assay contains the following recombinant and natural antigens: SmB, SmD, RNP-A, RNP-C, RNP-70k, Ro52, Ro60, La/SSB, Cenp-B, Topo-I, Jo-1, ribosomal P, and histones.

Anti-dsDNA antibodies

Anti-dsDNA antibodies were detected by IIF on *Critidia luciliae* (Immunoconcepts, Sacramento, USA). Samples were analysed in a single laboratory.

Statistical analysis

Statistical analysis was performed using SPSS (Chicago, IL, USA). We performed cluster analysis using average linkage (between groups), based on the squared Euclidean distance. This is a hierarchical clustering method, aiming to group similar variables together. To determine associations between autoantibodies and symptoms, χ^2 or Fisher's exact tests were used. We computed odds ratios (OR) and their 95% confidence interval (95% CI). No correction for multiple testing was made, but only a p value ≤ 0.01 was taken to indicate significance.

RESULTS

Presence of ANA

ANA were found by IIF in 280/291 (96.2%) patients. The frequencies of the specific reactivities were: anti-Ro52 or anti-Ro60 31.5%; anti-dsDNA 29.1%; anti-Ro60 28.9%; antihistones 28.5%; anti-SmB 28.1%; anti-RNP-C 24.3%;

Abbreviations: ANA, antinuclear antibodies; IIF, indirect immunofluorescence; LIA, line immunoassay; SLE, systemic lupus erythematosus

Table 1 Prevalence of the different symptoms during the year before sampling and during the entire course of the disease

Symptom	Prevalence past year (%)	Frequency ever (%)
Cutaneous symptoms	74.2	93.3
Butterfly rash	33.0	68.0
Photosensitivity	29.3	60.7
Alopecia	27.2	57.1
Oral ulcers	13.3	30.6
Localised discoid rash	10.6	26.1
Disseminated discoid rash	6.4	13.7
Generalised erythema	4.3	13.7
Genital ulcers	4.3	6.3
Subacute cutaneous LE	2.6	3.4
Chilblains	1.3	1.3
Lupus profundus	0.7	1.4
Sicca symptoms	25.0	28.0
Xerostomia	22.2	25.6
Xerophthalmia	19.8	22.7
General symptoms	76.3	92.3
Fatigue	67.0	85.5
Raynaud's phenomenon	40.3	47.8
Fever	16.7	66.2
Musculoskeletal symptoms	74.4	97.0
Arthralgia	69.1	91.7
Arthritis	36.4	76.4
Myalgia	36.3	54.1
Serositis	16.9	47.9
Pleuritis	12.1	39.2
Pericarditis	5.8	24.9
Renal symptoms	32.4	58.9
Proteinuria	29.7	57.2
Glomerulonephritis*	21.8	48.3
Cellular casts	15.6	45.1
Neurological symptoms	39.8	60.1
Headache	23.4	37.6
Concentration disorder	17.6	25.0
Depression	12.4	20.1
Encephalopathy	5.5	9.3
Seizures	5.4	12.7
Cerebrovascular accident	2.4	10.2
Psychosis	2.4	7.9
Peripheral neuropathy	1.8	6.7
Cranial neuropathy	1.2	1.2
Transverse myelitis	0.6	1.9
Haematological symptoms	58.6	84.3
Lymphopenia	41.7	65.9
Leucopenia	34.5	67.0
Thrombopenia	11.7	36.2
Haemolytic anaemia	8.6	22.6
Thrombosis	3.0	13.2
Recurrent abortion	0	6.3

*Glomerulonephritis is defined as the occurrence of at least one of the following: ever proteinuria >1.5 g/24 h, or >30% decline in renal function, or repeatedly active urine sediment, or uncontrolled hypertension, or biopsy proven glomerulonephritis.

anti-RNP-A 20.4%; anti-Ro52 18.7%; reactivity with at least two RNP determinants 18.3%; anti-SSB 14.5%; anti-SmD 13.6%; anti-RNP-70k 13.2%; antiribosomal P 12.3%; anti-Cenp-B 2.6%; anti-Topo-I 2.6%; anti-Jo1 1.3%. Antibodies to Cenp-B, Topo-I, and Jo-1 were not considered further, because of their low frequencies.

Presence of clinical symptoms

Table 1 lists the frequencies of clinical symptoms. Only symptoms occurring in more than 10% of the patients during the past year and in more than 15% of the patients ever were analysed.

Identification of clusters of antibodies

Using cluster analysis, we identified five clusters of antibodies. Cluster 1 consisted of antibodies to SmB, SmD, RNP-A, RNP-C, RNP-70k; cluster 2 consisted of antibodies to Ro52, Ro60, and SSB; clusters 3, 4, and 5 consisted of antibodies to ribosomal P, histones, and dsDNA, respectively (fig 1).

Associations of antibodies with symptoms

Table 2 lists the associations of antibodies with symptoms; corresponding OR and 95% CI are given. Only strong ($p < 0.001$) associations are discussed here. At least one antibody of cluster 1 was found in 36.5% of the patients; such a pattern was associated with Raynaud's phenomenon (past year or ever). The highest OR was found when anti-RNP-70k was present. At least one antibody of cluster 2 was found in 32.3% of the patients and this was associated with xerostomia ever. Furthermore, anti-RNP-A was associated with leucopenia past year, while antiribosomal P was associated with haemolytic anaemia and alopecia past year.

DISCUSSION

The generation of autoantibodies is an important feature of SLE. Several studies have demonstrated that specific ANA are associated with different symptoms of SLE.^{3-5, 10} In this study, we used a new multiparameter assay (LIA) for the detection of specific ANA. This is a more sensitive technique than immunodiffusion, and also allows for detection of fine reactivities to the different determinants of Sm (SmB and SmD), RNP (RNP-A, RNP-C, and RNP-70k), and SSA (Ro52 and Ro60).^{7, 9} It has already been suggested that autoantibody profiles might be a valuable alternative for individual autoantibodies. However, earlier studies used profiles generated only on the basis of pathophysiological considerations.⁵ Using cluster analysis, we defined five clusters of autoantibodies. Antibodies to the different determinants of the Sm/RNP system clustered together early. A second cluster was formed by Ro52, Ro60, and SSB, which is in accordance with studies using classical methods.^{10, 11} The other clusters consisted of individual antibodies—namely, antiribosomal P, antihistone, and anti-dsDNA antibodies. The frequency of anti-dsDNA antibodies in our cohort is low compared with that in other studies.⁴ This may be explained by the cross sectional character of

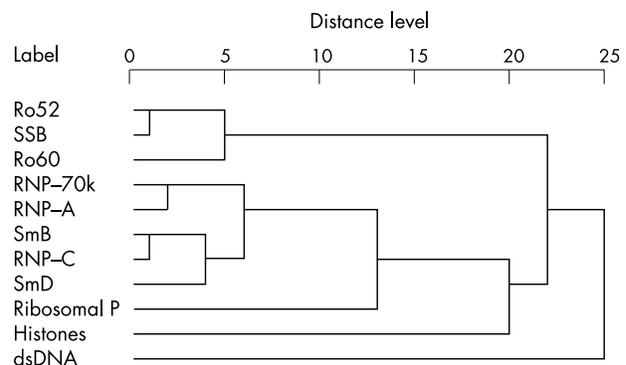


Figure 1 Cluster analysis, identifying clusters of autoantibodies (dendrogram). The scale indicates the distance level between the different variables. Two clusters of autoantibodies are formed early: cluster 1 is formed by RNP-70, RNP-A, SmB, RNP-C, and SmD; cluster 2 is formed by Ro52, SSB, and Ro60; while the other antibodies do not cluster early and are therefore considered to be a cluster by themselves: cluster 3 consists of ribosomal P, cluster 4 consists of histones, and cluster 5 consists of dsDNA.

Table 2 Associations between autoantibodies and clinical symptoms

Antibody	Associated symptom	OR	95% CI for OR
<i>p Value</i> ≤ 0.001			
RNP-70k	Raynaud's phenomenon past year	19.5	4.4 to 86.2
RNP-70k	Raynaud's phenomenon ever	15.3	3.5 to 67.1
Ribosomal P	Haemolytic anaemia past year	8.6	2.5 to 28.8
RNP-A	Raynaud's phenomenon ever	7.3	3.1 to 17.6
RNP-A	Raynaud's phenomenon past year	7.0	3.1 to 15.8
At least 2 RNP reactivities	Raynaud's phenomenon past year	5.6	2.4 to 12.7
At least 2 RNP reactivities	Raynaud's phenomenon ever	5.0	2.1 to 11.6
Ribosomal P	Alopecia past year	4.4	1.7 to 11.2
At least 2 RNP reactivities	Fever past year	3.8	1.6 to 8.8
At least 1 antibody of cluster 1	Raynaud's phenomenon past year	3.6	2.0 to 6.7
At least 1 antibody of cluster 2	Xerostomia ever	3.4	1.6 to 7.1
RNP-A	Leucopenia past year	3.2	1.5 to 6.5
At least 1 antibody of cluster 1	Raynaud's phenomenon ever	2.8	1.5 to 5.0
<i>p Value</i> ≤ 0.01			
SSB	Pericarditis past year	7.1	1.9 to 26.7
Ribosomal P	Haemolytic anaemia ever	4.2	1.5 to 11.6
SSB	Xerostomia ever	4.0	1.6 to 10.0
SSB	Xerostomia past year	3.6	1.4 to 9.3
RNP-A	Arthralgia past year	3.4	1.4 to 8.6
RNP-70k	Leucopenia past year	3.4	1.4 to 8.0
Ribosomal P	Leucopenia past year	3.4	1.4 to 8.0
At least 2 RNP reactivities	Leucopenia past year	3.2	1.5 to 6.6
At least 1 antibody of cluster 2	Xerostomia past year	3.2	1.5 to 6.8
Ro52 or Ro60	Xerostomia ever	3.1	1.5 to 6.4
Ro60	Xerostomia ever	3.1	1.5 to 6.4
dsDNA	Cellular casts past year	3.1	1.4 to 7.1
At least 2 RNP reactivities	Arthritis past year	3.1	1.4 to 6.6
Ro52 or Ro60	Xerostomia past year	2.9	1.3 to 6.1
At least 1 antibody of cluster 2	Xerophthalmia ever	2.7	1.3 to 5.8
Ro60	Xerostomia past year	2.7	1.3 to 5.8
At least 1 antibody of cluster 2	Pericarditis ever	2.5	1.2 to 5.2
RNP-C	Raynaud's phenomenon past year	2.5	1.3 to 4.9
At least 1 antibody of cluster 1	Butterfly rash ever	2.4	1.2 to 4.5
Histones	Arthritis past year	2.3	1.2 to 4.4
RNP-70k	Fatigue ever	1.2	1.1 to 1.3
dsDNA	Photosensitivity ever*	0.4	0.2 to 0.7
RNP-A	Cellular casts ever*	0.3	0.1 to 0.7
RNP-C	Cellular casts ever*	0.3	0.1 to 0.7

OR, odds ratio; 95% CI, 95% confidence interval.

*Inverse associations (OR < 1.0).

our study: a consecutive cohort of patients with SLE seen at the participating clinics was included, regardless of disease activity or disease duration.

We then looked for associations of antibodies and clusters of antibodies with clinical signs/symptoms of SLE. Because we did not correct for multiple comparisons, the *p* values obtained should be interpreted with caution.

Previous studies found that anti-Sm antibodies were associated with oral ulcers, a lower prevalence of sicca symptoms, Raynaud's phenomenon, thrombopenia, and leucopenia.^{4-6,10} We could not confirm these associations.

We confirmed the previously reported association between anti-RNP antibodies and Raynaud's phenomenon (past year and ever).⁴⁻⁶ Also, significance was maintained when antibodies towards the individual RNP subparticles were considered. Consistent with earlier findings,⁵ we found an association between anti-RNP antibodies and arthritis (past year). Associations of renal disease with anti-RNP antibodies have been reported but remain controversial.¹²⁻¹³ In the present study, both anti-RNP-A and RNP-C antibodies were associated with a lower risk for urine cellular casts ever. Associations between antibodies to individual RNP subparticles and renal disease have not yet been reported. Other new associations concerning anti-RNP antibodies are the associations with fever and leucopenia during the past year and with malar rash ever. When considering the cluster Sm/RNP, patients with at least one antibody of the cluster had a higher risk for Raynaud's phenomenon (past year and ever) and butterfly rash (ever).

We confirmed the well known associations of anti-SSA antibodies (defined as antibodies to Ro52 or Ro60⁷) with xerostomia (past year and ever),³⁻⁵ but associations with xerophthalmia were not statistically significant. Looking at the individual antibodies we found a significant association between xerostomia and anti-Ro60 antibodies. We could not demonstrate the associations with subacute cutaneous lesions previously described.⁴ This is probably because of the low frequency of subacute cutaneous lesions in our patients. The association between anti-SSA and photosensitivity, which remains controversial,^{4,14} could not be confirmed. We confirmed the association between anti-SSB antibodies and xerostomia (past year and ever), while again the association with xerophthalmia was not significant.^{3,5} Consistent with earlier reports,⁴ we found an association between anti-SSB antibodies and pericarditis (past year). The presence of one of the three antibodies of the SSA/SSB cluster was associated with xerostomia (past year and ever), with xerophthalmia (ever), and with pericarditis (ever).

We confirmed associations of antiribosomal P antibodies and haematological disorders,¹⁵ more precisely with haemolytic anaemia (past year and ever) and leucopenia (past year). The association between antiribosomal P and alopecia (past year and ever) has not been described previously, and needs further confirmation. As psychosis was rare in our patients, we could not evaluate this association. Considering anti-histone antibodies, we found an association with arthritis (past year), which needs to be confirmed. Several studies have demonstrated an association between anti-dsDNA

antibodies and lupus nephropathy.^{3,4} We found significant associations with the presence of cellular casts in the urine during the past year. Furthermore, we found an inverse association with photosensitivity ever, as described by Podrebarac *et al.*¹⁴

In conclusion, using a sensitive and specific multiparameter assay for identifying antinuclear reactivities, we found clusters of antibodies and could confirm previously reported associations of antibodies with clinical symptoms of SLE. Most striking are the associations of anti-RNP antibodies with Raynaud's phenomenon, of anti-SSA and anti-SSB antibodies with sicca symptoms, and of antiribosomal P antibodies with haematological symptoms. We also found several new associations meriting further study. In our view, the most important finding is the lower occurrence of cellular casts in patients with antibodies to the individual RNP-A and RNP-C subparticles.

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