Longitudinal anti-nuclear antibody (ANA) seroconversion in systemic lupus erythematosus: a prospective study of Swedish cases with recent-onset disease

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Original publication available at:
https://doi.org/10.1111/cei.13402

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Type of article: Original Article

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**SUMMARY**

Serum-IgG anti-nuclear antibodies (ANA) detected by indirect immunofluorescence (IF) microscopy remain a hallmark of systemic lupus erythematosus (SLE). Since it is controversial whether or not IF-ANA status varies over time, we designed a prospective study with longitudinal follow-up of patients with recent-onset SLE. The study population consisted of 54 recently diagnosed SLE cases, all meeting the 1982 ACR and/or the 2012 SLICC criteria. Clinical follow-up data, including disease activity, organ damage, and sera were collected from clinical onset of SLE and onwards, in most cases yearly (0–96 months). IF-ANA on HEp-2 cells was analysed and categorized regarding staining patterns. Using an addressable laser bead assay (FIDIS™ Connective profile), we measured IgG-ANA fine specificities against Ro52/SSA, Ro60/SSA, La/SSB, Sm, Sm/RNP, U1RNP, dsDNA, ribosomal-P protein and histone. At baseline, all patients were judged ANA-positive at an abnormal titre corresponding to the 95th percentile of healthy blood donors, but 7 of 54 patients (13%) lost ANA-positivity over time. Homogenous (AC-1; 46%) and speckled (AC-4 or 5; 31%) were the most frequently observed patterns at inclusion, whereas 7% switched pattern at least once during follow-up. Established associations between ANA fine specificities and clinical data were confirmed. Levels of anti-Sm/RNP, but not of anti-dsDNA, correlated with clinical disease activity (mSLEDAI-2K). Our data indicate that a considerable proportion of Swedish patients with SLE lose ANA-positivity over time, whereas consistent staining patterns were frequent. The clinical and mechanistic relevance of ANA seroconversion remains uncertain. Further prospective evaluations in larger SLE populations with more diverse ethnicities are warranted.
Introduction

Although patients with systemic lupus erythematosus (SLE) have heterogeneous clinical presentations, and a wide variation of disease severity, the presence of serum antinuclear antibodies (ANA) detected by immunofluorescence (IF) microscopy has long been regarded as a serologic hallmark and a common denominator of the disease (1-3). ANA was first included in the 1982 revised American College of Rheumatology (ACR) SLE criteria, stipulating “an abnormal titre of antinuclear antibody by IF, or an equivalent assay, at any point” (4). However, already in 1975, ANA was central in the applicable diagnostic principle for SLE introduced by Fries & Holman (5). More recently, lupus-related autoantibodies (≥1 immunological criterion needed) were ascribed increased importance in the 2012 ‘Systemic Lupus International Collaborating Clinics’ (SLICC) criteria set, where also the presence of ANA and/or antibodies to double-stranded (ds) DNA, combined with biopsy-proven lupus nephritis, was enough to classify as SLE (6). In the 2019 SLE classification criteria from the European League Against Rheumatism (EULAR) and ACR, the presence of IF-ANA has a pivotal role and serves as an entry criterion with a stipulated titre of ≥1:80 (7, 8).

The diagnostic sensitivity of IF-ANA in SLE has been estimated to >95% (9). However, this sensitivity is likely related to the time-point of SLE onset or “ever ANA positive” (10). The concept of “ANA negative SLE” was introduced in 1976, and has been linked to skin manifestations such as photosensitivity and the presence of anti-Ro/SSA antibodies (11, 12). Later, the existence of “ANA negative SLE” has been questioned, using modern microscopes and HEp-2 cells as the source of nuclear antigens (13). In the randomised controlled phase-2 trial of belimumab therapy in SLE, the investigators were surprised that a substantial proportion of the included (previously ANA positive) patients tested negative for ANA at inclusion. As a matter of fact, ANA seroconversion was discussed as a possible reason to why the trial did not meet its primary endpoints (14). Since then, seroconversion of IF-ANA in SLE has also been reported by other groups (10, 15, 16). In a study from the SLICC group, 298 incident SLE patients were recruited. At enrolment, 93.6% were ANA positive; and ten of the previously ANA negative cases seroconverted over 5 years (17). In a more recent study from SLICC which included 1137 recent-onset cases, 6.2% were judged ANA negative (18). Pisetsky and co-workers have highlighted that part of the divergent ANA results from different laboratories could be due to differences in assays and laboratory routines, resulting in
variations in the detection of ANA (19, 20). In addition, ANA fine specificities have not yet been standardised with regard to diagnostic specificity.

Circulating anti-dsDNA antibodies are not exclusively used to diagnose and classify SLE, but they also serve as a biomarker indicating renal involvement and increased disease activity (21). Anti-dsDNA levels are, however, expected to be lower in cases with properly treated or inactive SLE, regardless of renal disease (21-23). Autoantibodies to ribosomal P protein appear to be quite specific for SLE and have been suggested to associate with nephritis and hepatitis, whereas the association with neuropsychiatric SLE manifestations remains more controversial (24). In some studies, fluctuations and seroconversions of several ANA fine specificities have been evaluated, but their clinical significances are still unclear (25, 26). Ethnicity, sex, cigarette smoking, hypocomplementaemia and certain disease manifestations, have all been proposed to affect, or directly associate with specific ANA fine specificity levels in SLE (27-31).

In 2008, we demonstrated that abnormal ANA titres were less common than generally assumed in established SLE. Our retrospective data indicated that approximately 25% of SLE cases may lose their ANA positivity over time (10). The study population originated from Lund University hospital, a Swedish SLICC centre, and consisted solely of patients who were judged ANA positive at SLE onset. However, our results were questioned as patients at SLICC centres were claimed not to “lose ANA over time” (25). Thus, we decided to investigate this further, and designed a prospective study with longitudinal clinical and serological follow-up of cases with recent-onset SLE.
Materials and Methods

Patients
We included 54 newly diagnosed SLE cases (≤6 months of symptoms) meeting the 1982 ACR criteria (4) and/or the 2012 SLICC criteria (6). All patients took part in the prospective follow-up program KLURING (Swedish acronym for Clinical Lupus Register In Northeastern Gothia) at the rheumatology clinic, Linköping University hospital, as previously described (32). 45 (83%) were women and 9 (17%) men (mean age 44 years, range 18–84), and the majority (94%) were Caucasians. Clinical follow-up data and serum samples (stored at -70° C) were collected from SLE diagnosis (month 0) and onwards, in most cases yearly. SLE disease activity was assessed by the modified SLE disease activity 2000 (mSLEDAI-2K) score (which excludes items for low complement levels and positive anti-dsDNA) (33). Organ damage, required to have been persistent for at least 6 months, was recorded annually by the SLICC/ACR damage index (SDI), encompassing damage in 12 defined organ systems (34). A detailed description of the study population is given in Table 1. Based on previous observations (27-31), we decided to group patients according to the following characteristics and features; gender, smoking habits, presence of hypocomplementaemia, serositis, lupus nephritis, haematological disorder, and organ damage in order to analyse them in relation to each ANA fine specificity (accumulated analysis) as well as at all available time points (0, 6, 12, 24, 36, 48, 60, 72, 84 and 96 months).

Indirect IF microscopy
IF-ANA was analysed according to the routine at the accredited laboratory of Clinical immunology, University hospital, Linköping, Sweden, using Olympus microscope BX43, lens 20X/0.75 Plan Super Apochromat, illumination with CoolLed pE-100, multi-spot slides with fixed HEp-2 cells (ImmunoConcepts, Sacramento, CA, USA) as antigen substrate, and fluorescein-isothiocyanate (FITC) conjugated γ-chain-specific anti-human IgG as detection antibody (DAKO A/S, Glostrup, Denmark). The cut-off level for a positive IF-ANA test was set at titre 800, corresponding to the 95th percentile (“abnormal titre”) among 752 healthy blood donors (50% women, 50% men) (19). Positive samples were titrated in 2-fold dilution steps up to 1:12 800. Positive IF-ANA tests were categorized regarding staining patterns
according to the International Consensus of ANA Patterns (ICAP) nomenclature as follows: homogenous (H, AC-1), speckled (S), fine (AC-4) or coarse (AC-5), combined homogenous and speckled (HS, AC-1/4), nucleolar (N, AC-8), combined homogenous and nucleolar (HN, AC-1/8-10), combined speckled and nucleolar (SN, AC-4/8-10), and multiple nuclear dots (MND, AC-6) (1). To qualify as H-ANA pattern, chromatin staining was required in metaphase/anaphase cells. Likewise, absence of chromatin staining was required to qualify as a pure S-ANA pattern. To avoid inter-assay variation regarding IF-ANA, all the samples were analysed on the same occasion and was confirmed independently by two experienced evaluators, reaching consensus regarding staining patterns. The stability of fluorescence intensity was routinely checked with an internal control (positive homogeneous/speckled ANA) which was titrated in four 2-fold steps around its endpoint at every occasion. A representative portion of positive (n=22; titre range 800–12 800) and negative (n=8) sera were re-analysed as routine samples at the Clinical immunology and transfusion medicine, Uppsala University hospital, Sweden, using 1:200 as screening dilution and were interpreted by professor Rönnelid. A concordance of 96.7% between laboratories was observed with identical results in 29 of 30 samples regarding both ANA status and staining patterns.

**ANA fine specificities**

All available samples were analysed for IgG-ANA fine specificities, including anti-dsDNA and 8 other autoantibody specificities, by FIDIS™ Connective Profile, Solinium software v 1.7.1.0 (Theradiag, Croissy-Beaubourg, France) at the Clinical immunology laboratory, Linköping University hospital (35). This addressable laser bead assay (ALBIA) simultaneously measures autoantibodies to Ro52/SSA, Ro60/SSA, La/SSB, Sm, Sm/RNP, U1RNP, dsDNA, ribosomal P protein and histone. The manufacturer’s recommended cut-off ≥40 Units per mL (U/mL) was used for all fine specificities except for anti-dsDNA. For anti-dsDNA, a cut-off of 80 was employed as the 2012 SLICC criteria demand ‘2-fold reference range if tested by ELISA’ (6, 35). Results below the cut-off for each antibody analysis were given half the cut-off value. To minimise inter-assay variation, all samples were analysed at the same occasion.

**Routine laboratory measurements**

Blood cell counts, plasma creatinine, complement proteins (C3, C4) and urinalysis were followed at each visit.
**Statistical analyses**

Comparing autoantibody levels (at specific time-points, as well as over time) with the characteristics and manifestations described above (see *Patients*), were performed using Mann-Whitney *U* test. For comparisons between groups, \( \chi^2 \) or Fisher’s exact test (\( n \leq 5 \)) were used. Spearman’s rank-order correlation tests were used to examine relations between disease activity and each autoantibody specificity.

**Ethics considerations**

Oral and written informed consent was obtained from all participants. The study protocol was approved by the regional ethics board in Linköping (M75-08/2008).
Results

**ANA staining patterns and titres**

Figure 1 illustrates the longitudinal staining patterns (Fig. 1a) and IF-ANA titres (Fig. 1b) for each included SLE case. At enrolment, all patients were judged IF-ANA positive at a titre of $\geq 800$ ($n=54, 100\%$), and the distribution of staining patterns at inclusion were detailed from most common to least common and coded according to the ICAP nomenclature; homogenous (H, AC-1) ($n=25, 46\%$), speckled (S) ($n=17, 31\%, n=15$ AC-4 and $n=2$ AC-5), homogenous/speckled (HS, AC-1/4) ($n=6, 11\%$), homogenous/nucleolar (HN, AC1/8) ($n=3, 6\%$), nucleolar (N, AC-8-10) ($n=2$, $4\%$) and multiple nuclear dots (MND, AC-6) ($n=1, 2\%$), shown in Figure 1a. None of the included patients displayed dense fine speckled pattern (AC-2) at any time-point (confirmed at the laboratories in Linköping and Uppsala). The majority of patients ($n=50, 93\%$) kept their type of staining pattern during follow-up, whereas 4 cases ($7\%$) switched pattern at least once during the follow-up period.

Overall, IF-ANA titres were relatively consistent (Fig. 1b), yet seroconversions occurred. Decreasing IF-ANA titres over time were more common than the opposite. We observed no significant associations between IF-ANA titre changes and conversion of staining patterns. In addition, no significant correlation was found between IF-ANA titres and mSLEDAI ($r=0.017$; not shown). 7 of 54 patients ($13\%$) had lost their IF-ANA positivity at the last follow-up. All these 7 cases were prescribed antimalarials, 3 in combination with methotrexate and 1 in combination with mycophenolate mofetil. At month 48, when data from 45 cases were at hand, 4 patients ($9\%$) were IF-ANA negative. 1 patient seroconverted from positive to negative, and back again. No significant associations were found between gender, smoking, hypocomplementaemia, serositis, lupus nephritis, haematological disorder and IF-ANA seroconversion (Fisher’s exact test).
Seroconversions and antibody occurrence

Table 2 illustrates the prevalence of IF-ANA, ANA fine specificities and the number of patients seroconverting for each autoantibody specificity. 43 patients (80%) were ever positive regarding ≥1 of the analysed SLE-related fine specificity antibodies. Antibodies which were ever positive and most stable over time included Ro52/SSA (n=22, 41%), Ro60/SSA (n=22, 41%), La/SSB (n=11, 20%), and Sm/RNP (n=8, 15%). Almost half of the study population (n=24, 44%) were anti-dsDNA antibody positive at least once during follow-up. Half of these cases seroconverted, the majority from positive to negative. Seroconversion over time was also observed for anti-Sm, anti-U1RNP, anti-ribosomal protein P and anti-histone antibodies, mostly from positive to negative (Table 2). IF-ANA seroconversion was not significantly associated with the use of antimalarials or the glucocorticoid dose at inclusion.

Co-occurrence of some ANA fine specificities was observed. 22 patients had Ro52/SSA and/or Ro60/SSA antibodies, overlapping in 19 cases. Each anti-La/SSB positive patient was also positive regarding Ro52/SSA and/or Ro60/SSA. A similar concordance was observed for antibodies against Sm, Sm/RNP and U1RNP, whereas isolated appearance was only found for anti-U1RNP (n=6). Presence of anti-histone antibodies (n=16) was mainly overlapping with anti-dsDNA, only two cases were not anti-dsDNA positive. No significant associations were seen between specific autoantibodies among patients with consistent HS (AC-1/4) pattern (n=6) compared to those who changed to an HS pattern over time (n=3). 3 of the 6 cases (50%) with consistent HS pattern were dependent on a positive ANA test to classify as SLE (according the 2012 SLICC criteria) compared to 14 of 46 cases (30%) with other patterns (not significant). Furthermore, when comparing cases with HS pattern at any time (n=9) with those displaying other patterns (n=45), we found no significant differences in any of the ANA fine specificities tested or regarding organ involvement.
**Gender and smoking habits**

Anti-Ro52/SSA antibody levels were significantly higher in men compared to women (p=0.0001 median 75 versus (vs.) 49 U/mL). No significant differences in antibody levels were found between smoking habits and any of the analysed fine specificities.

**Hypocomplementaemia and lupus nephritis**

Low complement levels (subnormal C3 and/or C4) were significantly more common at inclusion (p=0.006, median 109 vs. 40 U/mL) and month 48 (p<0.02, median 105 vs. 40 U/mL), and it almost reached significance (p=0.1, median 162 vs. 95 U/mL) overall among cases with anti-dsDNA antibodies (Fig. 2a). As expected, cases with lupus nephritis (meeting the 7th ACR-82 criterion) had significantly higher anti-dsDNA (Fig. 2b) levels overall (p=0.008, median 547 vs. 89 U/mL), as well as at specific time-points; inclusion (p=0.007, median 315 vs. 40 U/mL), month 48 (p=0.02, median 169 vs. 40 U/mL), month 60 (p=0.04, median 164 vs. 40 U/mL) and month 72 (p=0.04, median 590 vs. 40 U/mL).

**Serositis and haematological disorder**

In cases with serositis, the levels of anti-Ro52/SSA (p<0.01, median 64 vs. 49 U/mL), anti-Ro60/SSA (p<0.0001, median 68 vs. 58 U/mL), and anti-dsDNA (p=0.007, median 205 vs. 89 U/mL) antibodies were significantly higher. Patients meeting the ACR criterion for haematological disorder had significantly higher levels of antibodies targeting Ro52/SSA (p=0.007, median 61 vs. 45 U/mL), Ro60/SSA (p=0.003, median 65 vs. 57 U/mL), and U1RNP (Fig. 2c; p<0.0001, median 50 vs. 21 U/mL).

**Organ damage and disease activity**

Among the ANA fine specificities, only anti-Sm/RNP levels correlated significantly with disease activity scores (mSLEDAI) (rho=0.12, p=0.02), whereas anti-Sm (rho=0.09, p<0.09) and anti-histone (rho=−0.10, p<0.08) levels were close to reach statistical significance. We
observed no significance between organ damage at last follow-up (binary variable) and ANA seroconversion (Fisher’s exact test). Serositis was the only manifestation which significantly associated with organ damage, being more common among cases who developed damage (p=0.02, Fisher’s exact test). Compared to cases without damage accrual at last follow-up, patients with any damage (SDI≥1) displayed lower levels of anti-dsDNA (Fig. 2d; p<0.007, median 91 vs. 130 U/mL) but higher levels of antibodies against Ro52/SSA (p<0.02, median 47 vs. 57 U/mL).
Discussion

The main finding in this longitudinal prospective cohort study of Swedish recent-onset SLE patients was that 13% converted from positive to negative IF-ANA during the follow-up period. No apparent associations of IF-ANA seroconversion with pharmacotherapy or development of organ damage were found, as none of the 7 patients who seroconverted received cyclophosphamide or biologics. In addition, we observed that higher IF-ANA titres were more common at SLE onset than later during the disease course. Only 2 of the 7 cases who lost IF-ANA positivity had detectable anti-Ro/SSA antibodies, suggesting that IF-ANA negative cases are not restricted to Ro/SSA positive patients (36). We found no significant correlation between IF-ANA titres and disease activity (mSLEDAI-2K). However, based on our data, such association cannot be excluded as the majority of the patients had low to moderate disease activity.

Arbuckle et al. found that IF-ANA as well as ANA fine specificities may be detected several years before SLE onset, and a gradually increasing rate of appearing autoantibodies are seen up to the time of diagnosis (i.e. epitope spreading), thereafter the autoantibody accrual rate was halted (37). Similar results have been presented from Swedish biobanks (38). Our finding of seroconversion of IF-ANA from positive to negative is in line with studies showing that a remaining IF-ANA positivity over time is less frequent than previously assumed (10, 14, 15, 18). In contrast, Ippolito et al. did not report disappearance of IF-ANA over time (25).

A positive IF-ANA test prevails as a hallmark of SLE and plays an important role in all classification criteria sets, but the clear international recommendation for IF-ANA cut-off level is not always complied (19). As equipment and procedures vary between laboratories, and on the subjective evaluation at ocular inspection under the microscope, IF-ANA titres cannot be compared directly in-between them (39). Depending on selected cut-off level, the presence of IF-ANA is not uncommon among healthy individuals, and thus it is of utmost importance that all IF-ANA laboratories calibrate their cut-off levels based upon defined reference material and use a 95th percentile cut-off level of a healthy population (4, 10, 39-41). In the recently published SLE criteria set from EULAR and ACR, a positive IF-ANA test at a titre $\geq 1:80$ is used as an entry criterion. Unsatisfactory though, at many laboratories, IF-ANA at a titre of $\geq 1:80$ does not reflect a prevalence of 5% positive ANA tests among healthy
blood donors but a considerable larger proportion, depending on different microscope performances regarding e.g. illumination and lens equipment (2, 7, 10, 39).

To our knowledge, prospective longitudinal data on ANA staining patterns in SLE are scarce. In the majority of our cases (93%), the staining pattern was stable over time, but in 4 patients the pattern changed at least once. Nevertheless, the distribution herein was largely similar to what was found in a previous Swedish SLE cohort but with a higher observed proportion of H-ANA positive individuals compared to both a large Japanese study with volunteers where S-ANA constituted the dominating staining pattern and in a Mexican report which evaluated relatives of patients with autoimmune disease (10, 42, 43).

In clinical practice, anti-dsDNA antibodies are often measured repeatedly as it is well-known that the concentration can fluctuate longitudinally and may correlate with renal involvement and flares (44). In contrast, other ANA fine specificities are usually not followed despite their multiple clinical associations. Agarwal et al. assessed disease activity in SLE and compared with different non-dsDNA ANA reactivities using enzyme-linked immunoassay, but did not find any clear associations (45). However, evaluation of ANA fine specificities with IF in a Brazilian SLE cohort indicated that fluctuations of antibody levels as well as seroconversion over time was common (26). Anti-dsDNA and anti-Sm were the most frequent autoantibodies to change over time in the present study. Although our results were comparable with the Brazilian study, both methodological differences and different ethnicity of the study population should be emphasized and hinders a direct comparison. Furthermore, we showed that also cases with antibodies to histone and ribosomal P protein seroconverted; whereas anti-Ro52/SSA was the most stable antibody, corroborating the finding by Faria et al (26). The levels of antibodies targeting Ro60/SSA and La/SSB showed only limited fluctuations over time (Table 2), which is in line with earlier observations (46, 47).

Fairly established associations between ANA reactivities and distinct clinical features in autoimmune diseases, such as anti-Ro52/Ro60/SSA antibodies with skin involvement and sicca symptoms, anti-dsDNA with renal involvement and anti-U1RNP with Raynaud’s phenomenon have been reported (29, 48, 49). Several additional associations have been described, but conflicting results were achieved in different studies, possibly due to
differences in ANA methodology, cut-off levels, patient selection and ethnicity of engaged study populations (29, 30, 32, 48). In line with many previous observations, we confirmed that increased anti-dsDNA levels and low complement were associated with renal involvement and this seemed particularly true at SLE onset (29, 44, 50). In line with our findings, Hoffmann et al. reported an association between haematological disorder and presence of anti-U1RNP (30). Also similarly to our observations in this mainly Caucasian study population, a study which included ethnically diverse cases of SLE observed higher levels of autoantibodies to Ro52/SSA, Ro60/SSA, and U1RNP among Caucasians with haematological disorder as compared to other ethnicities (51).

The impact of gender on SLE autoantibodies has been studied previously (52). In contrast to our finding of higher Ro52/SSA levels among men, the opposite was observed in two larger non-European studies (53, 54). The previously shown association between cigarette smoking and anti-dsDNA positive SLE could not be confirmed herein (28). Neither did we find any association between smoking habits and other ANA fine specificities.

Ethnicity is well-known to influence autoantibody reactivity, but since only a minor percentage of the investigated cohort was of non-Caucasian origin, the impact of ethnicity was not systematically evaluated. In previous multi-ethnic studies, the prevalence of ANA fine specificities has been found to be less frequent among Caucasians than in other ethnic groups (27, 55). In the British study, no associations between fluctuation and disease activity were observed, and the proportion of positive ANA fine specificities was nearly the same after 10 years of observation, although individual staining patterns changed over time (27).

An asset of this study is the prospective approach as well as the well-characterized patients with few missing data points. To minimise inter-assay variations IF-ANA, staining patterns and ANA fine specificities were all analysed at the same occasion and judged by the same two experienced persons at the Clinical immunology routine laboratory (Linköping). A major strength of the study is that a number of samples were re-evaluated regarding IF-ANA at another Swedish accredited laboratory with a concordance rate of >96%. A limitation is the relatively small number of included patients, with potential statistical power issues in analysing correlations between rare manifestations and different autoantibody specificities. After 36 months, data from 7% of the study population were not available, but over time
further cases were lost to follow-up mainly due to death or migration (missing data are illustrated in Fig. 1).

Although some ANA fine specificities are associated with clinical manifestations, and all fluctuate in varying degree over time, the clinical relevance of repeated measurement of the autoantibodies, except for anti-dsDNA, seem limited. No clear associations were found between clinical features, smoking status, gender or complement levels, comparing the cases becoming IF-ANA negative to those remaining IF-ANA positive. It is not clarified whether or not seroconversion in general from IF-ANA positive to IF-ANA negative, which we observed in this study, reflects the natural history of disease, consequences of treatment, or the variability in test kits. Therefore, further prospective studies are required.

To conclude, a considerable proportion of Swedish SLE patients lose ANA positivity over time. In patients remaining IF-ANA positive, a consistent staining pattern was most common. The clinical relevance of IF-ANA seroconversion remains uncertain and deserves further evaluation in larger longitudinal studies.

**Acknowledgements**

We thank Marianne Petersson for biobank administration, all the clinicians for their efforts, and the staff at the Clinical Immunology laboratories in Linköping and Uppsala. This work was supported by grants from the Swedish Rheumatism Association, the Region Östergötland (ALF Grants), the Swedish Society of Medicine, the King Gustaf V’s 80-year Anniversary foundation and the King Gustaf V and Queen Victoria’s Freemasons foundation.

**Disclosures**

The authors declare that they have no disclosures related to this manuscript.
Figure legends:

Figure 1. ANA over time of the 54 cases. (a) shows the distribution of ANA titres, whereas (b) demonstrates the frequencies of ANA staining patterns, (c) displays anti-dsDNA and (d) illustrates anti-U1RNP levels longitudinally. Each line represents one single patient, in the same order in all panels.

H=homogenous; S=speckled; HS=homogenous/speckled; HN=homogenous/nucleolar; N=nucleolar; MND=multiple nuclear dots

Figure 2. ANA fine specificities versus (vs.) variables and disease phenotypes. a–d show significant associations between ANA fine specificity levels and features: (a) anti-dsDNA vs. complement; (b) anti-dsDNA vs. lupus nephritis; (c) anti-U1RNP vs. haematologic disorder; and (d) anti-dsDNA vs. organ damage. Please note axis break in a–b and d.
Table 1. Characteristics of the 54 included patients with systemic lupus erythematosus.

<table>
<thead>
<tr>
<th>Background variables</th>
<th>At inclusion</th>
<th>At last follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females, n (%)</td>
<td>45 (83.3)</td>
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<tr>
<td>Age at inclusion, mean years (range, years)</td>
<td>44 (18-84)</td>
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<tr>
<td>Caucasian ethnicity, n (%)</td>
<td>51 (94.4)</td>
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<tr>
<td>Ever smoker (former or current), n (%)</td>
<td>23 (42.6)</td>
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### Disease variables

<table>
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<tr>
<th>Disease variables</th>
<th>At inclusion</th>
<th>At last follow-up</th>
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<tr>
<td>Meeting ACR-82 criteria, n (%)</td>
<td>44 (82.0)</td>
<td>47 (87.0)</td>
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<tr>
<td>Number of fulfilled ACR-82 criteria, mean (range)</td>
<td>4.4 (3-9)</td>
<td>4.6 (3-9)</td>
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<tr>
<td>Meeting SLICC-12 criteria (%)</td>
<td>52 (96.3)</td>
<td>52 (96.3)</td>
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<tr>
<td>mSLEDAI, mean (range)</td>
<td>2.6 (0-20)</td>
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<td>SLICC/ACR damage index, mean (range)</td>
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<td>0.8 (0-5)</td>
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<td>Low complement (%)</td>
<td>24 (44.4)</td>
<td>15 (27.8)</td>
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<tr>
<td>Any anti-phospholipid antibody</td>
<td>23 (42.6)</td>
<td>23 (42.6)</td>
</tr>
</tbody>
</table>

### Clinical SLE phenotypes (ACR-82 defined), n (%)

1. Malar rash | 17 (31.5) | 17 (31.5) |
2. Discoid lupus | 6 (11.1) | 6 (11.1) |
3. Photosensitivity | 30 (55.6) | 30 (55.6) |
4. Oral ulcers | 8 (14.8) | 9 (16.7) |
5. Arthritis | 41 (75.9) | 41 (75.9) |
6. Serositis | 18 (33.3) | 19 (35.2) |
7. Renal disorder | 6 (11.1) | 8 (14.8) |
8. Neurologic disorder | 1 (1.9) | 2 (3.7) |
9. Hematologic disorder | 25 (46.3) | 30 (55.6) |
10. Immunologic disorder | 27 (50.0) | 28 (51.9) |
11. Antinuclear antibody | 54 (100) | 54 (100) |

### Treatment, n (%)

<table>
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<tr>
<th>Treatment</th>
<th>At inclusion</th>
<th>At last follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antimalarials</td>
<td>41 (74.1)</td>
<td>41 (74.1)</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>4 (7.4)</td>
<td>3 (5.6)</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>1 (1.9)</td>
<td>0</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>7 (13.0)</td>
<td>4 (7.4)</td>
</tr>
<tr>
<td>Mycophenolate mofetil</td>
<td>0</td>
<td>3 (5.6)</td>
</tr>
<tr>
<td>Prednisolone, median dose in mg (range)</td>
<td>5 (0-60)</td>
<td>5 (0-30)</td>
</tr>
<tr>
<td>Rituximab</td>
<td>1 (1.9)</td>
<td>0</td>
</tr>
<tr>
<td>Belimumab</td>
<td>0</td>
<td>1 (1.9)</td>
</tr>
</tbody>
</table>

* Positive by immunofluorescence microscopy (IF-ANA)

ACR = American College of Rheumatology;
Any anti-phospholipid antibody = Positive test for lupus anticoagulant, medium- or high-titer anticardiolipin antibody level (IgA, IgG, or IgM) and/or positive test result for anti-β2-glycoprotein I (IgA, IgG, or IgM)
mSLEDAI = modified SLE disease activity 2000 (SLEDAI-2K) score;
SLICC = Systemic Lupus International Collaborating Clinics
**Table 2.** Antinuclear antibodies (ANA) and ANA fine specificities over time among the 54 systemic lupus erythematosus patients.

<table>
<thead>
<tr>
<th></th>
<th>Cut-off (titre)</th>
<th>Ever positive, n (%)</th>
<th>Consistently positive, n (%)</th>
<th>Seroconversion: from positive to negative, n (%)</th>
<th>Seroconversion: from negative to positive, n (%)</th>
<th>Consistently negative, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANA (HEp-2)</td>
<td>≥1:800</td>
<td>54 (100)</td>
<td>47 (87.0)</td>
<td>7 (13.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Any positive ANA fine specificity</td>
<td></td>
<td>43 (79.6)</td>
<td>37 (86.0)</td>
<td>4 (9.3)</td>
<td>2 (3.7)</td>
<td>11 (20.4)</td>
</tr>
<tr>
<td>Ro52/SSA</td>
<td>≥40</td>
<td>22 (40.7)</td>
<td>19 (86.4)</td>
<td>3 (13.6)</td>
<td>0 (0.0)</td>
<td>32 (59.3)</td>
</tr>
<tr>
<td>Ro60/SSA</td>
<td>≥40</td>
<td>22 (40.7)</td>
<td>21 (95.5)</td>
<td>0 (0.0)</td>
<td>1 (1.9)</td>
<td>32 (59.3)</td>
</tr>
<tr>
<td>La/SSB</td>
<td>≥40</td>
<td>11 (20.4)</td>
<td>8 (72.7)</td>
<td>3 (27.3)</td>
<td>0 (0.0)</td>
<td>43 (79.6)</td>
</tr>
<tr>
<td>Sm</td>
<td>≥40</td>
<td>6 (11.1)</td>
<td>2 (33.3)</td>
<td>3 (50.0)</td>
<td>1 (1.9)</td>
<td>48 (88.9)</td>
</tr>
<tr>
<td>Sm/RNP</td>
<td>≥40</td>
<td>8 (14.8)</td>
<td>6 (75.0)</td>
<td>1 (12.5)</td>
<td>1 (1.9)</td>
<td>46 (85.2)</td>
</tr>
<tr>
<td>U1RNP</td>
<td>≥40</td>
<td>15 (27.8)</td>
<td>9 (60.0)</td>
<td>4 (26.7)</td>
<td>2 (3.7)</td>
<td>39 (72.2)</td>
</tr>
<tr>
<td>dsDNA</td>
<td>≥80</td>
<td>24 (44.4)</td>
<td>12 (50.0)</td>
<td>9 (37.5)</td>
<td>3 (5.6)</td>
<td>30 (55.6)</td>
</tr>
<tr>
<td>Ribosomal P protein</td>
<td>≥40</td>
<td>6 (11.1)</td>
<td>2 (33.3)</td>
<td>3 (50.0)</td>
<td>1 (1.9)</td>
<td>48 (88.9)</td>
</tr>
<tr>
<td>Histone</td>
<td>≥40</td>
<td>16 (29.6)</td>
<td>2 (12.5)</td>
<td>11 (68.8)</td>
<td>3 (5.6)</td>
<td>38 (70.4)</td>
</tr>
</tbody>
</table>

ANA = Antinuclear antibody; HEp-2 = Human epithelial cells-2
Fig. 2c

Anti-U1RNP (U/mL)

Time in months

- Red circle: Haematologic disorder
- Black square: No haematologic disorder

Fig. 2d

Anti-dsDNA (U/mL)

Time in months

- Black square: SDI = 0
- Red circle: SDI $\geq 1$
References


