Longitudinal Fluctuation of Antibodies to Extractable Nuclear Antigens in Systemic Lupus Erythematosus

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ABSTRACT. ***Objective.*** To examine the appearance, persistence, and disappearance of anti-extractable nuclear antigen (ENA, Sm, U1-RNP, Ro/SSA, and La/SSB) and anti-native DNA (dsDNA) antibodies during systemic lupus erythematosus (SLE) followup.

Methods. One hundred and thirty patients who fulfilled American College of Rheumatology classification criteria for SLE with at least 5 yearly anti-ENA and dsDNA tests between 1987-2002 were retrospectively selected. Four longitudinal antibody data patterns were considered for each antibody: always absent, always present, absent at diagnosis with positive seroconversion, and present at diagnosis with negative seroconversion.

Results. Antibodies to Ro/SSA were present in 47%, U1-RNP in 36%, DNA in 32%, Sm in 23%, and La/SSB in 7% of patients. Among patients ever positive for a given autoantibody, the frequency of the "always present" pattern was 52% for anti-Ro/SSA, 38% for U1-RNP, 17% for Sm, 11% for La/SSB, and 9% for DNA antibodies; the frequency of positive seroconversion was 56% for anti-La/SSB, 33% for DNA, 26% for Sm, 21% for U1-RNP, and 15% for Ro/SSA. Time to positive seroconversion varied from 1 to 8 years after diagnosis. Among patients with a positive test at diagnosis the frequency of those remaining positive between the 2nd and 4th year of followup decreased to 39-78%, depending upon autoantibody specificity; between the 5th and 10th years this rate was 20-75%. Antibody data pattern frequency differed significantly among autoantibody specificities except between anti-U1-RNP and Ro/SSA ($p = 0.15$) and between anti-DNA and Sm antibodies ($p = 0.29$).

Conclusion. The high frequency of longitudinal fluctuation in anti-ENA antibodies suggests that a periodic reappraisal may be appropriate in seronegative patients with a suspect diagnosis of SLE. The clinical significance of such fluctuation deserves future study. (J Rheumatol 2005;32:1267–72)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS — AUTOANTIBODY
ANTINUCLEAR ANTIBODY — EXTRACTABLE NUCLEAR ANTIGENS

The autoimmune nature of systemic rheumatic diseases is strongly suggested, among other facts, by the presence of a multiplicity of antibodies directed to intra and extracellular antigens. Besides their contribution to understanding the pathophysiology of the disease, some aspects of autoantibodies provide useful clinical information. Some autoantibodies are closely associated with specific pathologic traits, such as anti-dsDNA and anti-tRNA synthetases, which are associated with nephritis in systemic lupus erythematosus (SLE) and lung interstitial disease in polymyositis, respectively. Conversely the presence of an autoantibody may be associated with the absence of a given clinical manifestation such as the low frequency of nephritis among SLE patients with anti-La/SSB antibodies[1,2]. Some autoantibodies are markers of more aggressive disease like rheumatoid factor and anti-fibrillarin antibodies in rheumatoid arthritis[3-5] and systemic sclerosis[6,7], respectively. The clinical association of certain autoantibodies can be so specific that they become part of the diagnostic criteria for some diseases, such as anti-Sm and anti-tRNA synthetase antibodies in SLE[8,9] and polymyositis (PM)[10], respectively.

SLE is probably the disease with the greatest variety and frequency of autoantibodies. The most clinically relevant autoantibodies in SLE are against native DNA (anti-dsDNA)[11,12], cardiolipin (aCL)[9,13], extractable nuclear antigens (ENA)[11,12], ribosomal P protein[14], and nucleosome[15]. It is well established that anti-dsDNA antibodies may be present at SLE diagnosis or may appear later in the course of the disease. Moreover, the serum concentration can fluctuate over time and may have a positive correlation with lupus nephritis activity[16-18]. Accordingly, rheumatologists frequently measure anti-dsDNA antibody levels at different stages of disease. In contrast, anti-ENA antibodies (anti-U1-RNP, Sm, Ro/SSA, and La/SSB) are not routinely measured at different stages of disease followup, despite their numerous significant clinical associations. This policy is generally

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based on the assumption that anti-ENA antibody status does not vary significantly with disease course in a given patient. It is possible that this idea originated because of the relative longitudinal stability of the qualitative Oüchterlony tests for anti-ENA antibodies compared to the exuberant longitudinal variation in quantitative or semi-quantitative tests for anti-dsDNA antibodies. However, some authors have reported on the fluctuation in anti-ENA antibody status in isolated patients or in small series of patients[19-21]. It would be of great interest to know if the current concept of longitudinal stability of anti-ENA antibodies is valid. What would be the longitudinal data pattern of anti-ENA antibodies if they were examined with the same frequency as anti-dsDNA antibodies? To answer this question we studied the frequency of fluctuation of anti-ENA and anti-dsDNA antibodies by analyzing retrospectively the results of serial determinations in a cohort of SLE patients.

MATERIALS AND METHODS

Patient selection. Patients with SLE were retrospectively selected from registries of the Immuno-Rheumatology Laboratory at the Federal University of São Paulo Medical School Hospital. All patients fulfilled American College of Rheumatology (ACR) classification criteria for SLE[8] and had at least 5-yearly determinations of anti-ENA and anti-dsDNA antibodies between 1987 and 2002. One hundred and thirty patients fulfilled the inclusion criteria [121 women (93.1%)]. The mean number of anti-ENA antibody determinations per patient was 17 (range 5 to 76) over a mean of 8 years (5 to 15).

Laboratory evaluation. Anti-dsDNA antibodies were detected by indirect immunofluorescence on *Crithidia luciliae* at a screening dilution of l/5. Anti-ENA antibodies were detected by Oüchterlony double immunodiffusion (DID). In-house purified calf spleen extract was used as source of antigens and each preparation was calibrated with sequentially diluted secondary standard sera reactive to each of the ENA. All tests were performed when blood was drawn according to standard protocol in the laboratory.

Analysis. We looked for fluctuation in autoantibody status during disease followup. An autoantibody test was considered positive in a given patient if positive on at least 2 different occasions. The same was true for a negative result. Four different antibody data patterns were observed for each autoantibody: (1) always absent; (2) always present; (3) absent at diagnosis with subsequent positive tests (positive seroconversion); and (4) present at diagnosis with subsequent negative tests (negative seroconversion). Kappa test was used to analyze the data pattern for each autoantibody in each patient. Chi-square test was used to compare the frequency of the antibody data patterns among autoantibody specificities. The significance level was established at 0.05.

RESULTS

The most frequently found autoantibody was anti-Ro/SSA, present in 61 patients (46.9%). Fifty-two of these (85.3%) had a positive anti-Ro/SSA test at diagnosis: 20 (32.8%) had negative seroconversion during followup and 32 (52.4%) remained positive (Table 1). Nine of the 61 anti-Ro/SSA-positive patients (14.7%) had a negative test at diagnosis. Six of them (66.6%) had positive seroconversion within one year of diagnosis and 3 (33.3%) between the 2nd and 6th year after diagnosis (Table 2).

Anti-U1-RNP was the second most frequently found autoantibody, occurring in 47 patients (36%). Thirty-seven of these patients (78.7%) had a positive anti-U1-RNP test at diagnosis: 19 (40.4%) had negative seroconversion and 18 (38.3%) remained positive throughout followup. Ten of the 47 anti-U1-RNP positive patients (21.3%) had a negative test at diagnosis (Table 1). Half had positive seroconversion within one year and the remainder between the 3rd and 6th year after diagnosis (Table 2).

Anti-Sm antibodies were detected in 30 patients (23%). Twenty-two of these patients (73.3%) had a positive anti-Sm test at diagnosis: 5 (16.6%) sustained positive tests during followup and 17 (56.7%) had subsequent negative seroconversion. Eight of the 30 anti-Sm positive patients (26.7%) had a negative test at diagnosis (Table 1). Half had positive seroconversion within 2 years of diagnosis and the other half between the 3rd and 6th year after diagnosis (Table 2).

Only 9 patients (6.9%) were positive for anti-La/SSB antibodies. Four (44%) had a positive anti-La/SSB test at diagnosis and only one remained positive during followup. Five anti-La/SSB-positive patients (56%) had a negative test at diagnosis (Table 1). Three had positive seroconversion within the first year of followup and 2 between the 3rd and 6th year after diagnosis (Table 2).

Anti-dsDNA was the third most common autoantibody, being detected in 42 patients (32.2%). Twenty-eight of these patients (66.7%) had a positive anti-dsDNA test at diagnosis but only 4 (9.5%) remained positive during followup. Fourteen of the 42 anti-dsDNA antibody-positive patients (33.3%) had a negative test at diagnosis (Table 1): 6 (42.9%) had positive seroconversion within 2 years of followup, 3 (21.4%) within 3 years, and 5 (35.7%) between the 5th and 8th year after diagnosis (Table 2).

Among patients positive for each autoantibody at diagnosis, there was a progressive decrease in the frequency of patients with positive tests along the years (Figure 1). This was especially notable for anti-Sm, anti-dsDNA, and anti-La/SSB antibodies. The decrease rate was more prominent in the 2 to 4-year interval in comparison to the 5 to 10-year interval of followup. Conversely, among patients negative for each autoantibody at diagnosis, there was a modest but progressive decrease in the frequency of negative results over followup period (Figure 2). This was most prominent for anti-dsDNA, anti-Ro/SSA, and anti-U1-RNP antibodies. This phenomenon was most evident in the 2 to 4-year interval in comparison to the 5 to 10-year interval.

The frequency of the 4 longitudinal antibody data patterns did not differ between anti-U1-RNP and anti-Ro/SSA antibodies or between anti-DNA and anti-Sm antibodies. All other comparisons showed statistically significant differences (Table 1). The "always present" pattern occurred more frequently for anti-Ro/SSA (52.5%) and anti-U1-RNP antibodies (38.3%). Among patients with a specific autoantibody, seroconversion was more frequent for anti-dsDNA (90.5%), anti-La/SSB (88.9%), and anti-Sm antibodies

Table 1. Distribution of 130 patients with SLE according to longitudinal antibody data pattern. Data are expressed by numbers (%).

Autoantibodies	Anytime Positive	Longitudinal antibody data pattern: Initial Visit Positive	Absent at Diagnosis with Positive Seroconversion	Present at Diagnosis With Negative Seroconversion	Always Present
Anti-dsDNA	42	28	14	24	4
	(100)	(66.7)	(33.3)	(57.1)	(9.5)
Anti-U1-RNP	47	37	10	19	18
	(100)	(78.7)	(21.3)	(40.4)	(38.3)
Anti-Sm	30	22	8	17	5
	(100)	(73.3)	(26.7)	(56.7)	(16.6)
Anti-Ro/SSA	61	52	9	20	32
	(100)	(85.3)	(14.7)	(32.8)	(52.4)
Anti-La/SSB	9	4	5	3	1
	(100)	(44)	(56)	(33)	(11)

Chi-square analysis for all autoantibodies: 92,567; $p < 0.001$. Chi-square partition showed no statistical significance for anti-DNA vs anti-Sm antibodies ($p = 0.295$) and anti-U1-RNP vs anti-Ro/SSA ($p = 0.152$). All other pair analyses were statistically significant at 0.05 level.

Table 2. Time to positive seroconversion among patients initially negative to each anti-ENA and anti-DNA antibody.

	Patients with Negative Tests at Diagnosis and Positive Seroconversion	Time to Positive Seroconversion (yrs): Mean	Median	Minimum	Maximum
Anti-dsDNA	14	3.6	3	1	8
Anti-U1-RNP	10	2.8	2	1	6
Anti-Sm	8	3	2	1	6
Anti-Ro/SSA	9	2	1	1	6
Anti-La/SSB	5	2	1	1	4

(83.4%): (1) positive seroconversion had frequencies of 33.3%, 55.6%, and 26.7% in anti-dsDNA, anti-La/SSB, and anti-Sm-positive patients, respectively; (2) negative seroconversion had frequencies of 57.2%, 33.3%, and 56.7% in anti-dsDNA, anti-La/SSB, and anti-Sm-positive patients, respectively.

The most frequently associated autoantibody pair was anti-Sm/anti-U1-RNP, found in 29 patients (22.3% of all patients). These 2 autoantibodies had the same data pattern in 16 (55%) of these patients (kappa test, $p < 0.001$) and in 12 of the latter, anti-Sm and anti-U1-RNP fluctuation occurred at the same time points so that the test status for anti-Sm and anti-U1-RNP antibodies was identical during followup. The always present pattern for anti-U1-RNP antibodies was observed in 13 of 17 patients with isolated anti-U1-RNP antibodies (72%) and in only 5 of 30 patients with both anti-Sm/U1-RNP antibodies (16%) ($p < 0.001$). Other autoantibody associations had frequencies varying from 1.5% (anti-U1-RNP/anti-La/SSB) to 17.7% (anti-U1-RNP/Ro/SSA). None of these less frequent associations showed significant concordance rates for the longitudinal data pattern.

DISCUSSION

In this study we found that anti-ENA antibodies fluctuate over time in patients with SLE. Among patients ever positive for a given autoantibody, negative and positive seroconversion was quite frequent (30 to 57% and 14.7 to 56% of patients, respectively, depending upon autoantibody specificity). The mean interval to positive seroconversion varied from 2 to 3.6 years for the various autoantibodies. It should be emphasized that the observed rates of longitudinal fluctuation of anti-ENA antibodies may represent a rather conservative estimate since at least 2 divergent test results were required for seroconversion definition. Moreover, some patients had only a 5-year followup period precluding detection of possible later seroconversion. Our observation points to the relevance of sequential determination of anti-ENA and anti-dsDNA antibodies in the followup of anti-DNA or anti-ENA-negative patients with suspect but not established SLE diagnosis.

Anti-dsDNA and anti-Sm antibodies had the highest seroconversion rates among all antibodies. The negative seroconversion rate was rather frequent for anti-dsDNA, anti-Sm, and anti-La/SSB antibodies. Anti-Ro/SSA and anti-

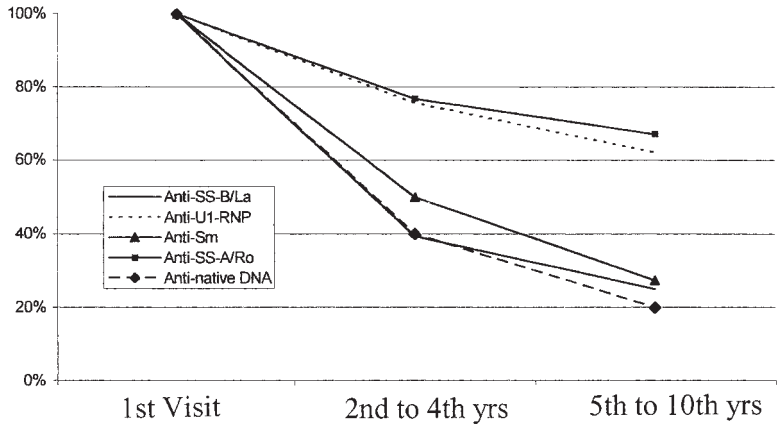

Figure 1. Decrease in the percentage of patients remaining positive for each autoantibody over time (only patients with a positive test at first visit were analyzed). Anti-DNA, anti-La/SSB, and anti-Sm antibodies had a more pronounced decrease in frequency compared to anti-Ro/SSA and anti-U1-RNP antibodies. For all autoantibodies the decrease rate was more marked between the first 2 to 4 years of followup.

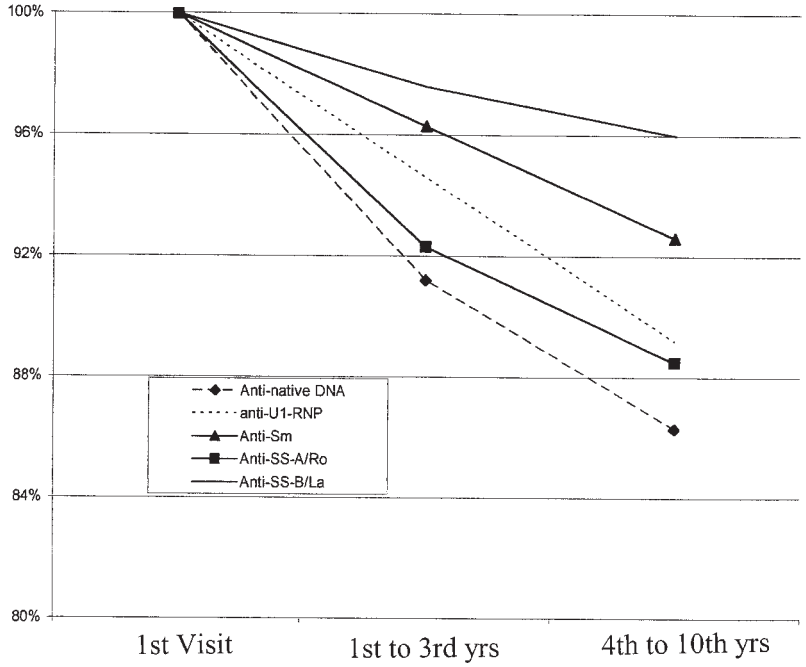

Figure 2. Decrease in the percentage of patients remaining negative for each autoantibody over time (only patients with a negative test at first visit were analyzed). A modest but progressive decrease in patients remaining negative for each autoantibody was observed, more marked during the first 3 years of followup.

U1-RNP antibodies presented a more stable data pattern in comparison with the other anti-ENA antibodies. More than 60% of patients with positive tests at diagnosis for one of these 2 autoantibodies were still positive within 5 to 10 years. A high percentage never had negative tests. Moreover, these 2 autoantibodies had the lowest rates of positive seroconversion along disease followup. The observation that anti-Sm antibodies had a high frequency of positive seroconversion and anti-Ro/SSA antibodies had a low positive seroconversion rate is in accordance with the recent finding that anti-Ro/SSA were the first and anti-Sm were the last autoantibodies to appear in the pre-clinical stage of a cohort of 130 SLE patients[22].

Fluctuation of anti-ENA antibodies has been reported in several studies[20,21,23-25]. Ten of 16 patients with SLE showed fluctuation in anti-Ro/SSA antibody titer when

measured prospectively by counter immunoelectrophoresis at 3-month intervals during a 2-year followup, but the majority did not have negative seroconversion[20]. No association between fluctuation and disease activity was found. Tench and Isenberg[21] found anti-Ro/SSA positive seroconversion in the absence of other anti-ENA antibodies to be the most common antibody pattern to emerge over a 10-year followup in a cohort of 61 patients with SLE. The same study found a considerable rate of anti-Sm negative seroconversion even using a detection method as sensitive as ELISA[21]. Others have also shown anti-Sm titer fluctuation[23-25] and eventually negative seroconversion[23] over time. These results, based on more sensitive techniques than ours, corroborate our findings, suggesting that the fluctuation of anti-ENA autoantibodies in this study was not a consequence of the low sensitivity of the DID assay. From a practical point of view, it is important that this fluctuation has been validated by DID, since this is a widely used assay, and the traditional clinical associations for anti-ENA antibodies were established on the basis of this technique.

We found that the most common association of autoantibodies in the same patient was anti-Sm and anti-U1-RNP, as reported[26]. The opportunity of following anti-ENA antibody status in our cohort showed that in half the patients with both autoantibodies, anti-Sm/U1-RNP status remained the same over longitudinal fluctuation periods, i.e., when anti-Sm antibody became negative, the same occurred to anti-U1-RNP, and *vice versa*. This concordant pattern was not observed for other combinations of anti-ENA antibodies. Additionally, anti-U1-RNP patients without anti-Sm antibodies presented a rather stable data pattern for anti-U1-RNP in contrast to patients with anti-U1-RNP plus anti-Sm antibodies.

This observation raises the interesting possibility of a conjugated control of immune response to Sm and U1-RNP in patients with anti-Sm antibodies and a distinct and more stable immune response in patients with isolated anti-U1-RNP antibodies. Unfortunately, the retrospective nature of our study does not allow exploration of the clinical and immunological significance of this phenomenon.

Our results show clearly that anti-ENA autoantibody status frequently fluctuates in SLE patients over time. No attempt to address possible associations of anti-ENA antibody fluctuation with disease activity and/or treatment was made due to the retrospective nature of the study. However, the considerable frequency of longitudinal autoantibody fluctuation that we found indicates that further studies should be designed to address this particular issue. Apart from possible pathophysiologic and clinical implications, longitudinal anti-ENA antibody fluctuation *per se* may have practical clinical relevance, especially for anti-ENA antibody-negative patients with a suspect diagnosis of SLE, since the demonstration of anti-ENA autoantibodies due to positive seroconversion may contribute to definitive diagnosis.

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