ORIGINAL PAPER

Antibodies to extractable nuclear antigens (ENAS) in systemic lupus erythematosus patients: correlations with clinical manifestations and disease activity

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SUMMARY

The aim was to explore possible correlations of antibodies to extractable nuclear antigens (ENA) with clinical manifestations and disease activity indices in systemic lupus erythematosus (SLE) patients. A total of 70 consecutive SLE patients (64 females) were included. Disease activity was assessed by SLE activity index (SLEDAI), and British Isles Lupus Assessment Group (BILAG).

Anti-Ro/SSA correlated positively with, headache (r=0.24, p=0.04), blurring of vision (r=0.25, p=0.03) and SLEDAI (r=0.25, p=0.04) and negatively with C3 (r=–0.35, p=0.003). Anti-Ro/SSA correlated with anti La/SSB antibodies (r=0.69, p<0.001), but not with anti-DNA, anti-RNP and anti-Sm antibodies. Anti-La/SSB antibodies correlated with headache (r=0.26, p=0.03), SLEDAI (r=0.25, p=0.03) and negatively with C3 (r=–0.34, p=0.004). Anti-La/SSB did not correlate with anti-RNP or anti-Sm antibodies. Anti-Sm antibodies correlated with disease duration (r=0.34, p=0.003), 24 hours urinary proteins (r=0.31, p=0.008), SLEDAI (r=0.31, p=0.009), BILAG renal score (r=0.29, p=0.02) and negatively with age at onset (r=–0.27, p=0.02), WBCs (r=–0.29, p=0.014) and C4 (r=–0.25, p=0.049). In multivariate analyses, anti-Ro/SSA antibodies remained associated with headache, blurring of vision and C3 and anti-La/SSB antibodies remained associated with C3 and with headache. Anti-Sm antibodies were independently associated with disease duration and total SLEDAI scores, while anti-RNP antibodies remained significantly associated with BILAG mucocutaneous scores only.

Antibodies to ENAs are associated with clinical aspects of SLE and may play a role in the assessment of disease activity. Insight into these ENAs may lead to new approaches to diagnostic testing, accurate evaluation of disease activity and lead to target approach for SLE.

Key words: *Extractable nuclear antigens (ENA); Systemic lupus erythematosus (SLE); Disease activity; SLEDAI; BILAG.*

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INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease that is virtually always accompanied by the production of autoantibodies. In fact, it has been demonstrated that autoantibodies contribute directly to the pathologic changes of SLE. Since autoantibodies are central to the pathogenesis of the disorder, their development must coincide with or precede clinical disease (1). Antibodies to double-stranded DNA (dsDNA) are most closely associated with the clinical manifestations of the condition and appear to have a direct role in pathogenesis. On the contrary, the relationship

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between disease activity in SLE and anti-extractable nuclear antigen (ENA) antibodies has not been well demonstrated (2). 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 (3).

A high number of antinuclear antibody (ANA) specificities can be detected in SLE. Some of these are related to a distinct clinical subset of disease, independently of their frequency. Autoantibodies against ENA are typically present many years before the diagnosis of SLE (4). Furthermore, the appearance of autoantibodies in patients with SLE tends to follow a predictable course, with a progressive accumulation of specific autoantibodies before the onset of SLE, while patients are still asymptomatic (4).

Autoantibodies targeting ENAs are hallmarks in the diagnosis of systemic autoimmune rheumatic diseases such as SLE (5). The primary antigenic targets of anti-ENA antibodies include U1-ribonucleoproteins (RNP), Sm (Smith antigen), topoisomerase I, Jo-1, Ro (SS-A), and La (SS-B) (6). SLE rarely presents with a negative ANA. Antibodies to ENA are sometimes ordered despite a negative ANA and may contribute to the diagnosis of SLE or other forms of connective tissue disease (CTD) (7).

This study was designed to look for possible correlations of antibodies to ENA with clinical manifestations and disease activity indices in a cohort of SLE patients.

PATIENTS AND METHODS

A total of 70 SLE patients agreed to participate. All patients fulfilled the 2012 Systemic Lupus International Collaborating Clinics (SLICC) classification criteria for SLE (8). Clinical data obtained included full medical history, general examination, and cardiovascular, chest, abdominal, neurological and locomotor system examination.

The SLE disease activity index (SLEDAI) (9) and British Isles Lupus Assessment Group (BILAG) index (10) were used to assess disease activity among the patients. The SLEDAI index consists of 24 variables covering nine organ systems (including some immunological tests) scored according to weights derived using multiple regression techniques. The BILAG index includes 86 items and assesses eight organ-based systems (general, mucocutaneous, neurological, musculoskeletal, cardiorespiratory, vascular, renal and hematological). Each system is given a score ranging from A to E. The disease activity assessed by both indices was classified as mild (score 0-10), moderate (score 11-20), severe (21-45) and very severe (>45) (9, 10).

Laboratory investigations obtained at time of inclusion were: complete blood picture, liver and kidney function tests, urine analysis, 24-hour urinary proteins, serum complement levels (C3 and C4) and ANA. Anti-dsDNA (ELISA) was performed using standard methods.

Anti-extractable nuclear antigens (ENA) assay

Autoantibodies to Smith (anti-Sm), ribonucleoproteins (anti-RNP), SSA/Ro (anti-Ro/SSA), and SSB/La (anti-La/SSB) were assessed by using Alegria® assay which features barcoded 8-well-microstrips, called Alegria® Test Strips. The Alegria® Test Strip holds a complete set of reagents including enzyme conjugate, enzyme substrate, sample buffer and a test specific control. The determination is based on an indirect enzyme-linked immune reaction with the following steps: antibodies present in positive samples bind to the antigen coated on the surface of the two reaction wells forming an antibody antigen complex. After incubation, a first washing step removes unbound and unspecific bound molecules. Subsequently added enzyme conjugate binds to the immobilized antibody-antigen complex. After incubation, a second washing step removes unbound enzyme conjugate. Addition of enzyme substrate solution results in hydrolyzation and color development during incubation. The intensity of the blue color correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 650 nm. The

calculation range of this assay is 0-200 U/mL (normal <15 U/mL, border line 15-25 U/mL, elevated >25 U/mL).

Statistical analysis

Analysis of data was performed with the statistical package for the social sciences (SPSS) version 15. As disease duration and most continuous laboratory and disease activity scores were positively skewed, these values were log-transformed (after adding 1 to eliminate zero values) to normalize their distribution before statistical analysis. Univariate Pearson correlations were computed between ENAs and clinical variables and disease activity indices. Variables significantly ($p<0.05$) associated with the respective ENA antibody were entered as independent variables into four separate multivariate logistic regression analyses with backward deletion ($p<0.05$) to identify independent associations.

Ethics

The design of the study was approved by the ethics committee of the Faculty of Medicine, Cairo University, Cairo, Egypt. All patients gave informed written consent to be enrolled into the study according to the Declaration of Helsinki.

RESULTS

The study included 70 SLE patients with a mean age of 35.4±10.2 years and disease duration of 48.7±40.1 months. The F:M ratio was 10.7:1. Demographic features, clinical manifestations, laboratory investigations and disease activity scores are presented in Table I.

Correlations between antibodies against ENA (Ro/SSA, La/SSB, U1RNP and Sm) and various demographic features, clinical manifestations, laboratory investigations and disease activity scores are presented in Table II. Anti-Ro/SSA correlated positively with blurring of vision ($r=0.26$, $p=0.03$) and total SLEDAI score ($r=0.25$, $p=0.04$) and negatively with C3 ($r=–0.35$, $p=0.003$). Additionally, anti-Ro/SSA significantly correlated with anti-La/SSB antibodies ($r=0.69$, $p<0.001$), but not with the anti-RNP and

Table I - Demographic features, clinical manifestations, laboratory investigations and disease activity scores in the SLE patients.

Variable mean±SD or n (%)		SLE patients (n=70)
Age (years)		35.37±10.24
Age at onset (years)		31.43±10.52
Disease duration (months)		48.74±40.15
Sex M:F n (%)		6:64 (8.6:91.4)
Headache		37 (52.9)
Nephritis		8 (11.4)
Arthritis		34 (48.6)
Myalgia		58 (82.9)
Laboratory investigations		
ESR (mm/1st h)		49.41±28.61
CRP (mg/dL)		1.97±2.29
Hemoglobin (g/dL)		11.51±1.43
WBCs ($x10^3/mm^3$)		5.67±2.67
Platelets ($x10^3/mm^3$)		278.1±80.67
C3 (µg/mL)		0.59±0.46
C4 (µg/mL)		0.22±0.15
Serum creatinine (mg/dL)		0.69±0.19
Proteinuria (mg/24 hr)		207.59±271.74
Anti-DNA titer		118.37±146.4
Hemolytic anemia		17 (24.3)
ACL antibodies		13 (18.6)
ENA positivity		
Anti-Ro (SS-A)		21(30)
Anti-La (SS-B)		14 (20)
Anti-SM		19 (27.1)
Anti-RNP		7 (10)
Disease activity score		
SLEDAI		14.23±9.38
BILAG score	Musculoskeletal	2.34±0.61
	Renal	0.77±1.16
	Mucocutaneous	1.80±0.65
	CVS/respiratory	0.19±0.46
	Vasculitis	0.47±0.79
	Haematological	1.71±0.98
	CNS	0.49±1.07

SLE, systemic lupus erythematosus; Anti-DNA, anti-deoxyribonucleic acid; ACL, anti-cardiolipin; Anti-Sm, anti-Smith; anti-RNP, antiribonuclear protein; SLEDAI, systemic lupus erythematosus disease activity index; BILAG, British Isles Lupus Assessment Group; CVS, cardiovascular system; CNS, central nervous system.

anti-Sm antibodies. The anti-La/SSB antibodies correlated positively with headache (r=0.26, p=0.03), SLEDAI scores (r=0.26, p=0.04), vasculitis BILAG score (r=0.25, p=0.04) and negatively with C3 (r=–0.34, p=0.004). Moreover, anti-La/SSB showed no significant correlations with anti-RNP or anti-Sm antibodies. Regarding anti-Sm antibodies, a significant correlation was found with age at onset (r=–0.27, p=0.02), disease duration (r=0.36, p=0.003), 24-hour urinary proteins (r=0.31, p=0.008), and SLEDAI score (r=0.31, p=0.009) and renal BILAG (P=0.29, P=0.02). A negative

Table II - Correlation between extractable nuclear antigens and clinical features, laboratory findings and disease activity indices in SLE patients.

Variable r (p)		Anti-ENAs in SLE patients (n=70)							
		Anti-Ro		Anti-La		Anti-Sm		Anti-RNP	
Age		–0.11	(0.37)	–0.02	(0.88)	–0.16	(0.19)	–0.09	(0.45)
Age at onset		–0.17	(0.15)	–0.07	(0.57)	–0.27	(0.02)*	–0.13	(0.28)
Disease duration		0.19	(0.12)	0.15	(0.23)	0.36	(0.003)*	0.14	(0.25)
Headache		0.24	(0.04)*	0.26	(0.03)*	–0.003	(0.98)	–0.07	(0.58)
Blurring of vision		0.26	(0.03)*	0.16	(0.19)	0.08	(0.49)	–0.12	(0.32)
Nephritis		0.16	(0.2)	0.16	(0.19)	0.29	(0.02)*	0.18	(0.14)
Arthritis		0.05	(0.68)	0.09	(0.48)	–0.14	(0.24)	–0.04	(0.75)
Myalgia		0.13	(0.28)	0.13	(0.27)	0.02	(0.86)	0.03	(0.84)
ESR		–0.04	(0.70)	–0.05	(0.7)	0.14	(0.25)	0.17	(0.15)
CRP		0.09	(0.48)	0.12	(0.33)	0.1	(0.43)	0.14	(0.26)
Hemoglobin		–0.07	(0.57)	–0.02	(0.87)	0.13	(0.3)	0.04	(0.73)
WBCs		–0.03	(0.79)	–0.11	(0.35)	–0.29	(0.014)*	–0.25	(0.04)*
Platelets		–0.11	(0.37)	–0.16	(0.19)	–0.16	(0.18)	0.02	(0.87)
C3		–0.35	(0.003)**	–0.34	(0.004)**	–0.11	(0.39)	–0.17	(0.16)
C4		–0.19	(0.12)	–0.2	(0.09)	–0.24	(0.049)*	–0.25	(0.04)*
Serum creatinine		–0.1	(0.42)	–0.02	(0.89)	0.01	(0.93)	0.02	(0.85)
24 h proteinuria		0.12	(0.31)	0.17	(0.15)	0.31	(0.008)*	0.15	(0.22)
Hemolytic anemia		0.07	(0.59)	0.05	(0.68)	0.18	(0.14)	–0.08	(0.52)
Anti-DNA titer		0.01	(0.91)	0.07	(0.54)	0.27	(0.02)	0.31	(0.009)*
ACL syndrome		0.008	(0.95)	0.13	(0.29)	0.12	(0.32)	–0.04	(0.76)
Disease activity score									
SLEDAI		0.25	(0.04)*	0.26	(0.03)*	0.31	(0.009)*	0.09	(0.44)
BILAG	Musculoskeletal	0.14	(0.24)	0.19	(0.12)	–0.13	(0.27)	–0.19	(0.12)
	Renal	0.19	(0.13)	0.22	(0.06)	0.29	(0.02)*	–0.02	(0.89)
	Mucocutaneous	–0.09	(0.48)	0.1	(0.41)	–0.01	(0.94)	0.25	(0.04)*
	CVS/respiratory	–0.06	(0.61)	0.11	(0.37)	–0.11	(0.38)	–0.14	(0.26)
	Vasculitis	0.096	(0.42)	0.25	(0.04)*	0.21	(0.09)	–0.02	(0.88)
	Hematological	0.1	(0.43)	0.037	(0.76)	0.21	(0.08)	0.2	(0.1)
	CNS	0.14	(0.25)	0.07	(0.54)	0.05	(0.66)	–0.15	(0.21)

SLE, systemic lupus erythematosus; Anti-DNA, anti-deoxyribonucleic acid; ACL, anticardiolipin; SLEDAI, systemic lupus erythematosus disease activity index; BILAG, British Isles Lupus Assessment Group; Anti-Sm, anti-Smith; anti-RNP, antiribonuclear protein; CVS, cardiovascular system; CNS, central nervous system. *Significant at p<0.05.

association was found with the white blood cells (WBCs) count (r=–0.29, p=0.01), and C4 (r=–0.24, p=0.04). Finally, the anti-RNP antibodies correlated positively with the BILAG mucocutaneous score (r=0.25, p=0.04) and negatively with the WBCs (r=–0.25, p=0.04) and C4 (r=–0.25, p=0.04). The C3 was significantly reduced in patients with a positive anti-Ro and anti-La. The SLEDAI score was significantly higher in SLE patients with positive anti-La and anti-Sm.

Lupus nephritis (LN) significantly correlated with anti-dsDNA titer (r=0.37, p=0.002), positive anti-Sm antibodies (r=0.29, p=0.017), while no significant correlations were observed with anti-Ro (r=0.16, p=0.2), anti-La (r=0.16, p=0.19) and anti-RNP (r=0.18, p=0.14). Moreover, the SLEDAI score significantly correlated with anti-ds-DNA titer (r=0.28, p=0.01), anticardiolipin (aCL) antibodies (r=0.47, p<0.001), ESR (r=0.46, p<0.001), CRP (r=0.5, p<0.001), 24-hour urinary proteins (r=0.56, p<0.001), nephritis (r=0.57, p<0.001) and Coomb's test (r=0.28, p<0.01), and negatively correlated with C4 (r=–0.39, p=0.001) and platelet count (r=–0.39, p=0.001).

Furthermore, Anti-dsDNA titer showed positive correlations with the BILAG renal score (r=0.320, p=0.007), SLEDAI score (r=0.280, p=0.019), 24-hour urinary proteins (r=0.285, p=0.017), and ESR (r=0.268, p=0.025). No other significant correlations were observed between anti-dsDNA and other BILAG scores for other major systems.

In multivariate analyses Anti-Ro/SSA antibodies remained associated with headache, blurring of vision and C3 and Anti-La/SSB antibodies remained associated with C3 and with headache. Anti-Sm antibodies were independently associated with disease duration and total SLEDAI scores, while anti-RNP antibodies remained significantly associated with BILAG mucocutaneous scores only.

DISCUSSION

The current cross-sectional study was conducted to investigate ENA autoantibodies among a cohort of SLE patients and to examine possible associations with different disease manifestations as well as disease activity indices. Specifically in SLE, the appearance of ENA autoantibodies precedes the clinical onset of the disease, a finding that underscores their potential importance in the pathogenesis. ANA, anti-Ro, anti-La, and aCL antibodies appear first, followed by anti-ds DNA antibodies, and then by anti-Sm and anti-RNP antibodies (11-16). Furthermore, the appearance of autoantibodies in patients with SLE tends to follow a predictable course, with a progressive accumulation of specific autoantibodies before the onset of SLE, while patients are still asymptomatic (11).

It is noteworthy that anti-Ro, anti-La, aCL, and ANA are in fact relatively common in normal persons who never have clinical symptoms of an auto-immune rheumatic disease. In contrast, anti-dsDNA, anti-Sm, and anti-RNP antibodies are very rare in normal persons (17, 18).

Hoffman et al. described the presence of 5 clusters of autoantibodies (anti-Sm/RNP, anti-Ro/La, anti-ribosomal P, anti-histone, and anti-dsDNA antibodies) in the setting of SLE (19). Tapanes et al. reported that the presence of anti-Sm/ RNP or anti-Ro/La/Sm/RNP was associated with a more benign form of lupus nephropathy (20). It was found that SLE patients with Sm/RNP antibodies had a lower prevalence of urine cellular casts (19) and represent a subset of lupus patients with less major organ involvement (21).

Antigen-antibody reactions involving the ENAs including Ro, RNP, and Sm may also contribute to the pathogenesis of LN; however a definitive relationship has not been fully established (22). In our study, a significant correlation was observed between LN with anti-dsDNA titer and anti-Sm antibodies. These results can be explained by the fact that ANAs, with the ability to fix complement, are primarily immunoglobulin (Ig) G1 and G3, and these subclasses correlate with the presence of LN and levels of anti-dsDNA antibodies. Of the ENAs, anti-Ro, anti-La and U1 RNP antibodies are primarily IgG1, whereas

anti-Sm antibody contains equal amounts of IgG1 and IgG2 (23). In a recent study, anti-Sm identified at kidney biopsy was suggested to have a predictive value for the early poor outcome of biopsy-proven LN during the follow-up period (24).

An established association has been reported between anti-Sm antibody levels and the SLEDAI as well as between anti-U1-RNP antibodies and the occurrence of LN (25). In an Afro-Caribbean cohort of SLE patients, rash, alopecia, mouth ulcers, serositis, neurological, joint and renal involvement were significantly associated with the presence of anti-Sm and anti-RNP antibodies while joint involvement was associated with the presence of anti-Ro and anti-La antibodies (26). In our study we observed that anti-Ro was positive in 30%, anti-La in 20%, anti-Sm in 27.1% and anti-RNP in 10% of the cases. In a recent study that included a cohort of 552 SLE patients, antinuclear antibodies were detected in 99.8% of patients, followed by anti-ds DNA (81.3%), anti-SSA/Ro (58.7%), anti-RNP (36.8%), anti-Sm (35.7%), and anti-SSB/La (15%) (27).

Among ANAs, anti-Sm and anti-RNP antibodies are of the utmost importance in clinical practice and the study of the mechanisms inducing their production has opened up new perspectives and helped to elucidate the pathogenesis of autoimmune disorders (28). The study of autoantibodies, their production and their role in the immunopathology of SLE is complex. Insight into these issues is not only of theoretical interest but may also lead to new approaches to diagnostic testing, accurate assessment of disease activity and more preventative or specific therapies (29).

The cross sectional design of the study forms a limitation as we cannot say whether the correlations will persist over time. For that reason, we did not study specifically relations with, for example, pregnancy and other detailed clinical manifestations. We encourage more in-depth analysis of the relation of ENAs to the detailed clinical manifestations, damage scores, pregnancy and outcome.

CONCLUSIONS

Antibodies to ENAs are associated with clinical aspects of SLE, seem to contribute to the pathogenesis of LN and play an important role in the assessment of disease activity. Insight into these ENAs may lead to new approaches to diagnostic testing, accurate evaluation of disease activity and lead to target approach for SLE. A larger scale longitudinal study is recommended in the future to confirm our findings and verify the core role of the ENAs in the prognosis and course of SLE disease.

Conflict of interest: none of the authors has any conflict of interest regarding this study.

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