TESTING ANTINUCLEAR ANTIBODIES IN RELATIVES OF PATIENTS WITH SYSTEMIC LUPUS ERITHEMATOSUS

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Abstract
Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by the production of autoantibodies, which deposit within tissues and fix complement leading to systemic inflammation (1). It is a heterogeneous disease with a continuum of disease activity. Some patients can have predominant skin and joint involvement, whereas others can present with organ-threatening diseases such as nephritis, cardiac involvement or even neurologic manifestations. Relatives of patients with SLE appear to be at higher risk of SLE and other autoimmune diseases, but estimates of individual familial risks are largely unavailable or unreliable (2,3). The purpose of ANA (antinuclear antibody) determination is generally to screen patients suspected from generalized autoimmune diseases, that is, systemic lupus erythematosus (SLE), rheumatoid arthritis, Sjögren’s syndrome, scleroderma, polymyositis, or mixed connective tissue disease. Clinical and paraclinical studies are needed to reach a definitive diagnosis.

Keywords: systemic lupus erythematous, familial aggregation, autoantibody, autoimmunity

INTRODUCTION
Systemic lupus erythematosus is an autoimmune disease in which a person’s immune system attacks various organs or cells of the body, causing damage and dysfunction. SLE is called a multisystem disease because it can affect many different tissues and organs in the body. Some patients with SLE have very mild disease, which can be treated with simple medications, whereas others can have serious, life-threatening complications. Lupus is more common in women than men, and for reasons that are not precisely understood, its peak incidence is after puberty (4).

The best evidence that SLE is genetically determined is from studies of familial aggregation (an increased frequency of persons with SLE in the same family) (5). For example, an identical twin of a patient with SLE has a 25% to 50% chance of developing the disease, but the risk is 10 times less if the affected twin was nonidentical (risk 2% to 5%). Still, the latter risk is much greater than that in the general population. First degree relatives with a family history of SLE have a 6-fold higher risk of developing SLE and a 4-fold higher risk of developing a non-SLE autoimmune disease (20% to 25%) or have a positive ANA (30%) (6).

For many decades indirect immunofluorescent antinuclear antibody test (IF-ANA) has been the “gold standard” in the diagnosis of these disorders. This test was designed by George Friou in 1957 (7). Since then it has been the most widely used test for diagnosis of connective tissue diseases (CTD). It is inexpensive and easy to perform, with high sensitivity and specificity (8). The test detects the presence of ANA in the blood of the patient which adhere to reagent test cells (substrate), forming distinct fluorescence patterns that are associated with certain autoimmune diseases.

The correct interpretation of the IF-ANA results is important and must always be correlated with the patient’s symptoms and signs. While reporting IF-ANA three parameters are evaluated; these include the pattern of fluorescence, substrate used and the titer of a positive test. A negative IF-ANA result essentially excludes possibility of active CTD.
OBJECTIVE

The aim of the present work was to determine the incidence of ANA in healthy relatives of patients with SLE. Measurements of ANA was carried out in first and second degree relatives of these patients and also in a control group (relatives of patients with non-SLE).

METHOD

18 SLE patients and their healthy relatives were included. We realized a cross-sectional study by enrolling those patients with SLE admitted in our hospital between January 2018 and September 2018. We interviewed 18 SLE patients to ascertain whether they had relatives with SLE and/or other autoimmune diseases. Those patients who had family history of systemic lupus erythematosus or specific autoimmune diseases were excluded for all analyses.

Also, serum samples from 18 individuals (relatives of non-SLE patients) were also tested during the same period.

We studied consecutive lupus probands satisfying the 2012 SLICC Classification Criteria in a hospital-based, probing for 2 generation pedigree charting, clinical and investigational parameters.

Data were analyzed for associations of high ANA levels in healthy relatives with the manifestations in LES patients.

We analysed the data applying Student’s t-test, Chi-Square Test, ANOVA, and Pearson’s correlation.

RESULTS

Multiple variables per patient were collected. Variables were divided into several groups:

1. Demographic data: age, gender and geographic region.
2. The major manifestations of the disease in SLE patients.
3. Coexistence of antiphospholipid syndrome, as defined by the Sydney classification criteria in SLE patients.
4. SLE status, using the activity index SLE-DAI-2K. Laboratory findings, imaging or pathological studies in SLE patients
5. The determination of ANA in healthy relatives of patients with SLE.
6. The determination of ANA in healthy relatives of patients with non-SLE.
7. Any link between positive ANA results in relatives and SLE patients.

The study included 18 SLE patients of whom 17 (94.4%) were women and 1 (5.5%) was a men with a mean age 42.1 years and the mean disease duration was 4.7 years (Fig. 1).

![FIGURE 1. Gender distribution of SLE patients](image)

Of the 17 women who participated in this study, 7 (41.1%) had primary education and 10 (58.8%) had mid-level education or higher (Fig. 2). As for marital status, 67.4% lived with a partner and 32.6% lived alone or with children and families.

![FIGURE 2. Patient education (n=17)](image)

The distribution of rural versus urban patients by geographic region was described. The number of patients from urban areas was higher than the number of patients from rural areas, respectively 61.1% versus 38.8% (Fig. 3).

We also analysed all the manifestations in LES patients. Joint involvement was the most frequent clinical manifestation in patients (94.4%), followed by mucocutaneous manifestation which occurs in 88.8% of patients.
Regarding immunological manifestations, anti-nuclear antibodies (ANA) were found in all patients, anti-dsDNA antibodies, which are incredibly specific for SLE, were positives in 13 patients, anti-Sm in 11 patients, anti-Ro (SSA) in 6 patients and anti-La (SSB) in 2 patients. Also, hypocomplementemia was observed in 14 (77.7%) patients with SLE (Fig. 4).

The antiphospholipid syndrome (APS) is one of the most encountered autoimmunity in SLE patients and these two pathogenesis seem to intricate. The APS diagnosis was sustained according to the 2006 Sydney APS’s criteria. We observed 4 SLE patients with secondary APS, mostly with both IgM and IgG of anticardiolipin antibodies (aCL) positive. Only 3 patients were using oral anticoagulants for the APS.

A particular challenge in patients with lupus has been distinguishing mild, moderate, and severe flares and distinguishing them from ongoing, persistent disease. So, the evaluation of disease activity was performed by SLEDAI-2K. In the present study, the disease was very active (SLEDAI > 11) in 2 (11.1%) of participants, mild to moderate activity (SLEDAI = 1-10) in 10 (55.5%), and inactivity (SLEDAI = 0) in 6 (33.3%) (Table 1).

Using the defined cut-off serum dilution of ≥1/80, we identified 7 (38.8%) first- and second degree relatives with positive ANA. Two of them had high titres of ANA (1:320), 3 individuals had moderate titre of ANA (1:160) and two children had low titre of ANA (1:80) (Fig. 6). All of them don’t have clinical manifestation of SLE or other CTD. The based sample showed a 38.8% prevalence of ANA positivity. The two individuals with high ANA levels had their relatives with SLE with severe manifestations, including neurological, renal and serositis manifestations. The patterns of immunofluorescence staining were as follows: homogeneous, speckled and nucleolar.

In the control group with the same number of patients with non-SLE - who were negative for current
or past autoimmune disease, a screening ANA test was performed, too. Here we identified one relative with ANA positive serum sample at low level (1:80). So, the prevalence of positive ANA in the control group is 5.5%. These data from this analysis are useful in estimating the risk ratio $RR = \frac{38.8\%}{5.5\%} = 7.05$, meaning that relatives of SLE patients have 7.05 the risk of developing in the future SLE or other autoimmune disease compared with control group (a 100% increase in risk).

![FIGURE 6. ANA results in relatives of SLE patients](image)

There are several limitations to the present study. First, it was restricted to Romania, and different findings may occur in other populations and environments. Therefore, additional studies in other countries are required to determine the generalizability of our findings. Second, we do not have detailed information on laboratory testing and examinations for the relatives of SLE patients.

**DISCUSSION**

18 SLE patients were studied of whom 17 (94.4%) were women and 1 (5.5%) was a man with a mean age 42.1 years.

The clinical and immunological characteristics of our SLE patients are largely comparable to most major studies.

In the present study, the disease was very active (SLEDAI $\geq 11$) in 6 (33.3%) of participants, mild to moderate activity (SLEDAI = 1-10) in 10 (55.5%), and inactivity (SLEDAI = 0) in 2 (11.1%).

Biological samples from 18 healthy relatives of SLE patients were obtained. It was found that antinuclear antibodies were present in 7 (38.8%) of the serum of healthy children and mothers despite a lack of clinical symptoms at a dilution of 1:80 or higher. ANA were positive in 11.1% at a serum dilution of 1:320, in 16.6% at 1:160 and in 11.1% at 1:80.

The high levels of ANA are correlated with SLE patients who have severe organ involvement (neurological, renal and serositis manifestations).

In the healthy control group who were negative for current or past autoimmune disease we identified one relative with ANA positive serum sample at low level (1:80).

ANA prevalence was higher in the group with relatives of SLE patients compared with control group. Up to 38% of individuals were ANA positive with majority of moderate titre of antibodies.

These data from this analysis are useful in estimating the risk ratio $RR = \frac{38.8\%}{5.5\%} = 7.05$, meaning that relatives of SLE patients have 7.05 the risk of developing in the future SLE or other autoimmune disease compared with control group (a 100% increase in risk).

**CONCLUSIONS**

The pathogenesis of SLE is multifactorial, including genetic and environmental factors. Genetic predisposition plays a crucial role in susceptibility.

Although a negative IF-ANA test makes a diagnosis of SLE or CTD extremely unlikely (13), a positive test even at moderately high titres of 1:160 or higher is found so frequently in unaffected first degree relatives without a rheumatic disease that a positive result has little or no diagnostic value. The data from this analysis are useful in estimating the probabilities of detecting specific ANA, but further investigation are needed.

Given the low specificity of ANA for systemic autoimmune disease, it is important to counsel the patient about the limitations of a positive test result, particularly if it is requested with low pre-test probability. A positive ANA is not diagnostic of lupus (or any other autoimmune disease) and most individuals with positive ANA will not develop an autoimmune disease in the subsequent three years if they don’t have any suggestive symptoms at the time of initial testing (14).

This data suggested that ANA+ individuals may indicate an increased risk for SLE. Monitoring the auto antibodies in ANA+ population may be beneficial for early diagnosis of lupus or other autoimmune disease.
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