Prevalence and Clinico-Serological Correlations of Anti-α-Enolase, Anti-C1q, and Anti-dsDNA Antibodies in Patients with Systemic Lupus Erythematosus

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ABSTRACT. ***Objective.*** To evaluate the prevalence and clinico-serological correlations of anti-enolase, anti-C1q, and anti-dsDNA antibodies in patients with systemic lupus erythematosus (SLE).

Methods. Sixty-eight sera randomly obtained from SLE patients were examined. Anti-α-enolase antibodies were detected by immunoblot on recombinant protein; anti-C1q and anti-dsDNA antibodies were detected using an ELISA.

Results. Anti-α-enolase, anti-C1q, and anti-dsDNA antibodies were positive in 21%, 62%, and 63% of patients, respectively. A correlation was found between anti-dsDNA and anti-C1q antibodies, while anti-enolase antibodies did not correlate with the other 2 specificities. Anti-α-enolase antibodies were not correlated with any of the clinical and serological variables examined. Anti-C1q antibodies were correlated with ECLAM score, leukopenia, complement levels, and active renal involvement. Anti-dsDNA antibodies correlated with arthritis, leukopenia, complement levels, and the presence of renal involvement, independent of activity. In patients with active renal disease anti-dsDNA antibodies were correlated with a poor renal outcome, occurring after a mean period of 24 months.

Conclusion. These data suggest the association of anti-C1q antibodies with disease flares and active renal disease in SLE. The observed association of anti-dsDNA antibodies and renal disease was expected. Further analysis is required to fully assess the clinical significance of anti-α-enolase antibodies. (J Rheumatol 2006;33:695–7)

Key Indexing Terms:
ANTI-α-ENOLASE ANTIBODIES ANTI-C1Q ANTIBODIES ANTI-dsDNA ANTIBODIES
SYSTEMIC LUPUS ERYTHEMATOSUS DISEASE ACTIVITY

Systemic lupus erythematosus (SLE) is a very complex disease: to properly follow up and treat patients, identification of variables predictive of future organ involvement and outcome is of great importance. The autoantibody profile may represent an important prognostic indicator for the treating physician; therefore, as different antibody specificities are detected, the question arises as to their prevalence, their association with the clinical manifestations, and their predictive significance for the evolution of the disease[1].

We analyzed the prevalence, clinico-serological correlations, and prognostic value for future organ involvement of anti-α-enolase, anti-C1q, and anti-dsDNA antibodies in a cohort of patients with SLE.

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MATERIALS AND METHODS

Study design. Serum samples, randomly obtained from 68 patients with SLE, were examined. Clinical and laboratory data required to assess disease activity and renal activity at the time of blood sampling were recorded. Disease activity was measured using the ECLAM index[2]; an ECLAM score > 2 was considered to be indicative of active disease.

Renal activity was categorized as (1) nephritic: increased plasma creatinine of at least 30% over the normal value, nephritic urinary sediment, and proteinuria; or (2) proteinuric: proteinuria > 500 mg/day, without any modification in the plasma creatinine level. A poor renal outcome was defined as a doubling of serum creatinine values for at least 6 months, with plasma creatinine ≥ 2 mg/dl[3].

To assess a predictive role of these autoantibodies for disease flares, we recorded manifestations occurring within 3 months of evaluation in patients with inactive disease at the time of antibody detection.

Sera from healthy subjects, age and sex matched with the patients, served as controls.

Antibody detection. Anti-α-enolase antibodies were detected by immunoblot on recombinant α-enolase, obtained from *E. coli* transfected with a cDNA encoding amino acids 10–434. The immunoactive bands were measured by densitometry; results were expressed as percentage of a positive control (upper limit of normal was 21%)[4].

Anti-C1q antibodies were detected in the sera by an ELISA[5]. Results were expressed as percentage of a positive control; the upper normal limit was 18%.

Anti-dsDNA antibodies were assayed as described[4] using calf thymus DNA. Results were expressed as percentage of a positive control; the upper normal limit was 5%.

Statistical analysis. All variables were analyzed independently using the Mann-Whitney U-test and chi-squared test as appropriate. Continuous variables were compared by Spearman's rank correlation coefficient.

RESULTS

Table 1 shows the prevalence of the antibody specificities; levels are shown in Figure 1. A correlation was observed between anti-C1q and anti-dsDNA antibodies ($p < 0.001$). No correlations were observed between anti-α-enolase antibodies and anti-C1q or anti-dsDNA antibodies.

Table 1. Anti-enolase, anti-C1q, and anti-dsDNA antibodies in 68 sera from patients with SLE.

Specificity	Positive, n (%)	Mean (range, median, IQR)
Anti-enolase	14 (21)	63.7 (30.4–100, 58.6, 52.660)
Anti-C1q	43 (62)	51.6 (18.3–131.3; 50.4; 35.4)
Anti-dsDNA	43 (63)	35.2 (4.3–107.3; 30.9; 50.4)
All positive	5 (7)	—
Anti-enolase + anti-dsDNA	4 (6)	—
Anti-enolase + anti-C1q	1 (1)	—
Anti-C1q + anti-dsDNA	26 (38)	—
Anti-enolase alone	4 (6)	—
Anti-C1q alone	10 (15)	—
Anti-dsDNA alone	8 (12)	—
All negative	10 (15)	—

IQR: interquartile range.

No significant correlations were observed between anti-α-enolase antibodies and any of the analyzed variables, particularly with renal involvement.

Anti-C1q antibodies were associated with active renal disease ($p < 0.05$), leukopenia ($p < 0.01$), low C3 levels ($p < 0.01$), and ECLAM index ($p < 0.001$).

Anti-dsDNA antibodies were associated with arthritis ($p < 0.05$), leukopenia ($p < 0.01$), low C3 levels ($p < 0.01$), and the presence of renal involvement ($p < 0.05$). Five patients with active renal involvement reached a poor outcome a mean of 30 months after evaluation (median 24, range 6–60). Anti-dsDNA antibodies were correlated with a poor outcome: 64.2% versus 20.9% in patients with poor and good outcomes, respectively ($p < 0.05$).

The prognostic significance of the autoantibodies was assessed by identifying clinical manifestations presented by 42 patients with inactive disease in the 3 months following antibody determination. Nine patients (22%) presented with a flare, but no correlation was observed between antibody specificity and either occurrence of disease flares or specific clinical manifestations.

DISCUSSION

α-enolase is a glycolytic enzyme that catalyzes dehydration of 2-phosphoglycerate to phosphoenolpyruvate; although virtually expressed in every tissue, kidney and thymus contain the

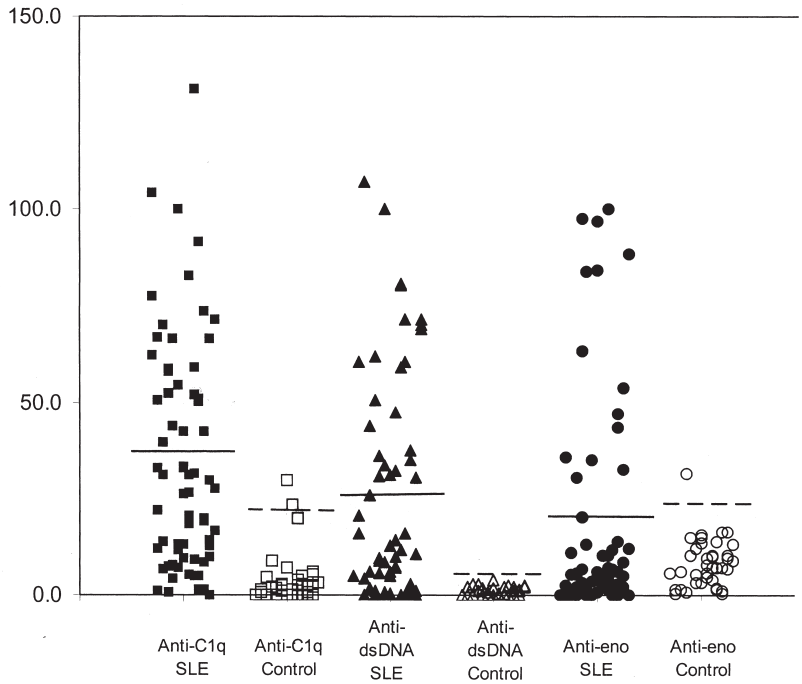

Figure 1. Levels of anti-C1q, anti-dsDNA, and anti-α-enolase (anti-eno) antibodies in patients with SLE and healthy blood donor controls. Results are expressed as percentage of a positive control. Upper limits of normal (mean + 2 standard deviations of controls) are 18%, 5%, and 21% for anti-C1q, anti-dsDNA, and anti-α-enolase antibodies, respectively (broken lines). Mean autoantibody levels in patients are indicated with a solid line (anti-C1q: 34.7; anti-dsDNA: 22.3; anti-α-enolase: 16.6).

highest amount of the enzyme. Anti-α-enolase antibodies have been described in systemic autoimmune disorders with renal involvement and have been proposed as a marker of nephritis in mixed cryoglobulinemia[6]. In a study of patients with membranous nephritis, anti-α-enolase antibodies were detected in 69% of patients with primary and in 58% with secondary disease[7]. Anti-α-enolase antibodies have also been detected in antineutrophil cytoplasmic antibody-positive vasculitis[8] and in SLE[4] with active renal disease.

In our study, the frequency of anti-α-enolase antibodies was lower than reported by us in an earlier study[6], and the hypothesized relationship with active renal disease was not confirmed; further, no correlation with any of the examined clinical and serological variables was observed. These differences may be attributed to the different assay used for autoantibody detection (recombinant vs tissue α-enolase), but further studies are required to confirm this hypothesis.

Anti-C1q antibodies have been associated with different clinical manifestations of SLE[9-12], particularly hypocomplementemia, active nephritis, proteinuric flares, and future development of renal involvement. Our results are partially in agreement with published data, since we found an association with disease activity and with active renal disease, although no differences were observed between proteinuric and nephritic flares. As far as the predictive significance of anti-C1q antibodies for future organ involvement is concerned, we were unable to relate the positivity of anti-C1q antibodies with events occurring within 3 months from detection. We chose to evaluate disease course in this short period of time with the idea that a correlation with events occurring later may not be related to the autoantibodies.

In our study anti-dsDNA antibodies were a good indicator of renal disease; further, the positivity of anti-dsDNA antibodies in patients with active renal disease was correlated with a poor renal outcome.

It is generally held that anti-dsDNA antibodies of high affinity are endowed with a higher pathogenicity[13,14]. The ELISA used in this study aims at detection of higher affinity antibodies, limiting the reactivity of the lower affinity ones by high detergent concentration in washing buffers. Our results suggest that these conditions probably allowed us to measure anti-dsDNA antibodies that are more likely to induce lesions. The fact that anti-dsDNA antibodies but not anti-C1q appear to be correlated with a poor renal outcome may be consistent with the data recently reported by Trouw, *et al*[15] suggesting that the presence of anti-C1q antibodies alone is not sufficient to cause renal damage.

Our data show that anti-α-enolase antibodies were present in a small percentage of patients with SLE and did not confirm previous correlations with clinical manifestations, particularly renal involvement. Anti-C1q antibodies were related to disease activity and renal involvement, but did not give any prognostic information relative to relapses or to the occurrence or outcome of renal involvement. As expected, anti-dsDNA antibody positivity predicted severe renal involvement and a poor renal outcome.

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