Original article

Significance of Anti-C1q Antibodies in Lupus Nephritis

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Abstract

Background. The aim of this study is to assess the anti-C1q antibodies (anti-C1q Ab) in the serum of patients with lupus nephritis and to investigate the association of these autoantibodies with other clinical and laboratory parameters, renal histological characteristics and disease activity.

Methods. Sera from 54 patients (49 female, 5 male) with lupus nephritis were collected from peripheral blood at the time of renal biopsy and after mean 64,2 ± 4,97 months (from 56 to 77). The disease activity was assessed by SLEDAI. Renal histopathology was classified according to the ISN/RPS revised criteria for nephritis in systemic lupus erythematosus (SLE).

Results. 43 (79,63 %) patients had positive baseline anti-C1q Ab with mean antibody level of 99,84 ± 35,55 IU/ml. There is a positive correlation between the presence of anti-C1q antibodies and SLEDAI (r=0,71; P<0,001), activity index (r=0,53, P<0,001), proteinuria (r=0,49, P<0,01), glomerular leucocyte infiltration (r=0,38, P<0,001), fibrinoid necrosis (r=0,59, P<0,001), endocapillary hypercellularity (r=0,41, P<0,001). The prevalence of anti-C1q antibody in patients with diffuse proliferative renal lesions (class IV) is significantly higher than in patients with non-diffuse proliferative renal lesions (class II +III) and with membranous lesions (class V).

Conclusions. The anti-C1qAb could be used as a marker for disease course monitoring and to determine the treatment strategy and the prognosis.

Keywords: anti-C1q antibodies, renal biopsy, lupus nephritis

Introduction

Systemic lupus erythematosus (SLE) is a prototype of systemic autoimmune disease characterized by overproduction of various auto-antibodies. It was reported that over 150 auto-antibodies had been identified in sera from patients with SLE; however, only a few of them were associated with the development of lupus nephritis [1]. Autoantibodies (Ab) against C1q (anti-C1q), the first component of the classical pathway of complement, have received much interest in the recent years [2]. The prevalence of anti-C1q in the general population varies between 2% and 8% [3-5]. The positivity for anti-C1q antibodies in healthy individuals does not appear to bear any pathological or prognostic significance [6]. Anti-C1q antibodies in patients with systemic lupus erythematosus were described for the first time in 1984 by Uwatoko *et al*. [7]. Anti-C1q autoantibodies have been found in a number of autoimmune diseases (rheumatoid vasculitis, hypocomplementemic urticarial vasculitis syndrome, mixed connective tissue disease and others) [2,5,8]. A series of studies reported that serum anti-C1q antibodies were common in sera from patients with systemic lupus erythematosus (SLE) and were associated with renal involvement [4,5,9-11]. The prevalence of anti-C1q antibodies in SLE ranges from 30% to 60% [3-5,11]. The marked variance between the results may be due to the differences between individually prepared assays and commercial ELISA kits used to determine the levels of anti-C1q antibodies. Anti-C1q antibodies appear to play a pathogenic role in the development of lupus nephritis [2]. Horvath *et al*. have shown that high levels of anti-C1q antibodies associate with the activity of lupus nephritis but not with other organ manifestation of SLE [12].

The aim of this study was to assess the anti-C1q antibodies in the serum from patients with lupus nephritis with active and nonactive disease. This study also aims to look for correlations with other clinical and laboratory parameters, renal histological characteristics and disease activity.

Patients and methods

All patients included in the study fulfilled four of 11 American College of Rheumatology revised criteria for SLE [13-15]. The disease activity was assessed by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [16-18]. Renal histopathology was classified according to the ISN/RPS (International Society of Nephrology / Renal Pathology Society) revised criteria for nephritis in SLE [19]. In addition to the histological

classification, lupus nephritis activity was scored according to activity (endocapillary hypercellularity, hyaline deposits, fibrinoid necrosis, interstitial inflammation, leucocyte infiltration, cellular crescents) and chronicity (glomerular sclerosis, fibrous crescents, interstitial fibrosis, and tubular atrophy) indices of the National Institutes of Health (NIH) reports [5]. The Systemic Lupus International Collaborating Clinics (SLICC) was used for evaluation the disease damage [17].

During every visit, signs and symptoms of patients and changes in drug treatment were recorded. Laboratory features including urinanalysis, 24-hour urinary protein excretion, serum creatinine, serum albumin, lupus serology (C3, C4, IgG, IgA, IgM and anti-dsDNA antibodies, etc) were obtained. The therapy for individual patients was determined by disease activity and renal histopathology. The medications mainly included corticosteroids (intravenous pulse methylprednisolone for 3 consecutive days), immunomodulating agent (intravenous cyclophosphamide at a dosage 0,5-1 g/m^2 of body surface area monthly) and anticoagulants.

Sera from patients with lupus nephritis were collected from peripheral blood at the time of renal biopsy and after mean 64,2 ± 4,97 months (from 56 to 77 months). All the sera were stored at -70 ^{0}C until use. All samples were tested by enzyme linked immunosorbent assays (ELISA) using a modification of the method of J. J Wisnieski and S. M. Jones [11]. Whole C1q was purified from human plasma by the method of A. J. Tener *et al.* [22].

All data were analyzed using SPSS 15.0.1. *P*<0.05 was considered to indicate statistical significance. Bivariate correlations between variables in cross-sectional analyses were determined using Spearman's correlation coefficient. Means and standard deviations between two groups were calculated and compared using student's unpaired t-test. Comparisons between serum anti-C1q Ab levels in each group of ISN/ RPS classification of lupus nephritis were determined using analysis of variance (ANOVA).

Results

We studied 54 patients (49 female and 5 male, mean age: 29,48 ± 8,08 years old, range: 19 – 54, mean SLEDAI: 28,89 ± 7,95, mean SLICC: 0,72 ± 0,9) with lupus nephritis. All patients included in the study had proteinuria > 0,5 g/24 h (mean 3,32 ± 1,89 g/24 h), 28 (51,85 %) patients had haematuria (RBC > 8 /HP), 12 (22,22 %) patients had impaired renal function with elevated serum creatinine (up to 242,3 µmol/l). Mean duration of SLE before renal biopsy was 45,48 ± 28,41 months. According to the results of renal biopsy 4 patients had class I ISN/RPS lesions, 8 class II ISN/RPS lesions, 14 class III ISN/RPS lesions, 21 class IV ISN/RPS lesions, 7 class V ISN/RPS lesions.

43 (79,63 %) patients (38 female, 5 male; mean age - 29,12 ± 7,67 years; ISN/RPS class II: 4 patients, ISN/RPS class III: 13, ISN/RPS class IV: 20, ISN/RPS class V: 6 patients; mean proteinuria: 3,55 ± 2,03 g/24

h; SLEDAI: 29,02 ± 7,89) had positive baseline serum anti-C1q Ab (mean antibody levels: 99,84 ± 35,55 IU/ml).

Table 1. Mean values of anti-C1q antibodies in patients with different classes of lupus nephritis

ISN/RPS class	Number of patients	Anti-C1q antibodies (IU/ml)
Class II	4	41,73 ± 9,19
Class III	11	91,09 ± 23,28
Class IV	22	119,5 ± 31,73
Class V	6	82,43 ± 19,80

The prevalence of anti-C1q antibody levels in patients with class IV ISN/RPS lesions were significantly higher than in patients with class II (P<0.001), class III (P<0.01) and class V (P<0.01) lupus nephritis (Table 1). There was a positive correlation between the presence of anti-C1q antibody and SLEDAI (r=0,71; P<0,001) (Fig. 1), proteinuria (r=0,49, P<0,01) (Fig. 2), activity index (r=0,53, P<0,001), glomerular leucocyte infiltration (r=0,38, P<0,001), fibrinoid necrosis (r=0,59, P<0,001), endocapillary hypercellularity (r=0,41, P<0,001). The correlation with SLICC damage index (r=0,46) showed only a bordering significance (p=0,059), which indicates a poor and marginal prognostic value of those tests for the prediction of a severe progressive disease. Levels of the C1q antibodies correlate inversely with levels of C3 (r=-0,68; P < 0,001) and C4 (r=-0,54; P < 0,001) components of complement. No significant correlation was found with serum creatinine and serum albumin. Serum anti-C1q Abs were higher in patients with urinary protein excretion ≥ 3,5 g/24 h than in patients with urinary protein < 3,5 g/24 h (P< 0,001).

After treatment for mean 64,2 ± 4,97 months (range: 56-77 months), marked decrease of anti-C1q Ab levels (the extent of reduction exceeding 50%) was found in 34 patients (anti-C1q Ab became undetectable in 17). In 6 patients (4 with class IV lupus nephritis, 1 with class III and 1 with class V) anti-C1q Ab levels decreased, but less than 50%, and in 3 patients with class IV lupus nephritis anti-C1q Ab levels increased. The median time of disappearance of proteinuria was longer in patients with persistent high level of serum anti-C1q Ab (P< 0,01, χ^2=8,94). These patients were with class IV A/C renal lesions and with higher chronicity index.

Discussion

Although anti-C1q antibodies have received much interest in the recent years, their biological functions remain unclear. Anti-C1q antibodies per se do not seem to activate complement; however, their binding to C1q may amplify complement activation by increasing the amount of the bound IgG in a vicious circle manner. Furthermore, they might attenuate the physiological functions of C1q, including the capacity to activate the classical pathway of complement and to clear immune complexes and apoptotic bodies.

Earlier investigations suggested that anti-C1q antibodies are found in SLE patients, particularly in patients with renal involvement [2,12]. From a clinical point of view,

anti-C1q Ab might be an important biomarker in lupus nephritis. This promoted us to further explore the potential role of anti-C1q Ab in evaluation of renal lesion and its prognostic significance in lupus nephritis.

Our data of prevalence of anti-C1q Ab in 79,63% of patients with active lupus nephritis patients were higher than data in the study of Sinico *et al.* (60%) and Fang *et al.* (56%) [1,23]. High levels of serum anti-C1q Ab correlated with proliferative lupus nephritis. Levels of anti-C1q Ab in Class IV were much higher than that of Class II. Although the difference between Class III and V was not significant, high levels of anti-C1q Ab may be a reliable predictor of proliferative lupus nephritis. Furthermore, serum anti-C1q Ab levels were found to be associated with activity and chronicity indices. These data suggest that in patients with lupus nephritis, anti-C1q Ab measurement could serve as a noninvasive investigation for assessing the renal lesions in lupus nephritis.

Proteinuria is the main mode of expression of lupus nephritis. Moroni *et al.* [24] reported that titers of anti-C1q Ab were significantly greater during flares of renal activity than in quiescent disease, and titers were elevated in all cases of proteinuric flares and in only two thirds of the cases of nephritic flares. We demonstrated in our study that serum anti-C1q Ab level strongly correlated to proteinuria. It suggests that lupus nephritis patients with persistently high levels of anti-C1q Ab should be prescribed intensive treatment.
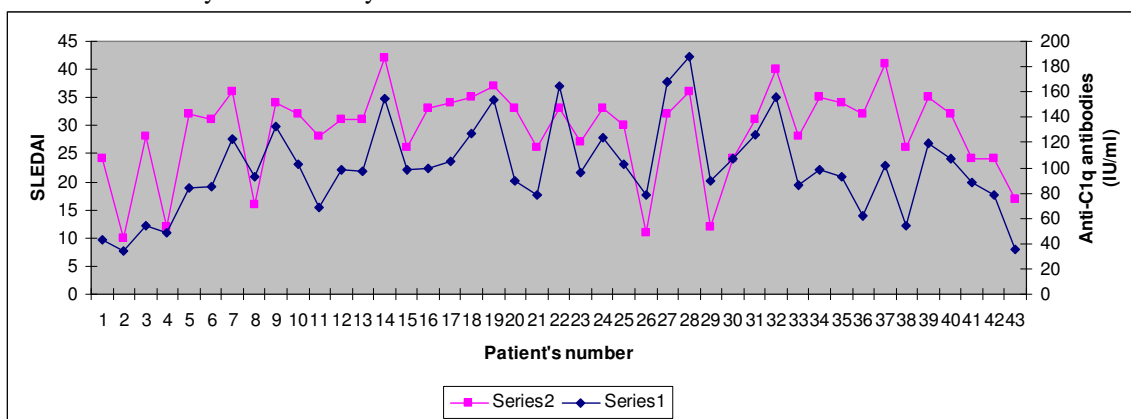
Fig. 1. Correlation between serum levels of anti C1q antibodies (series 1) and SLEDAI (series 2)
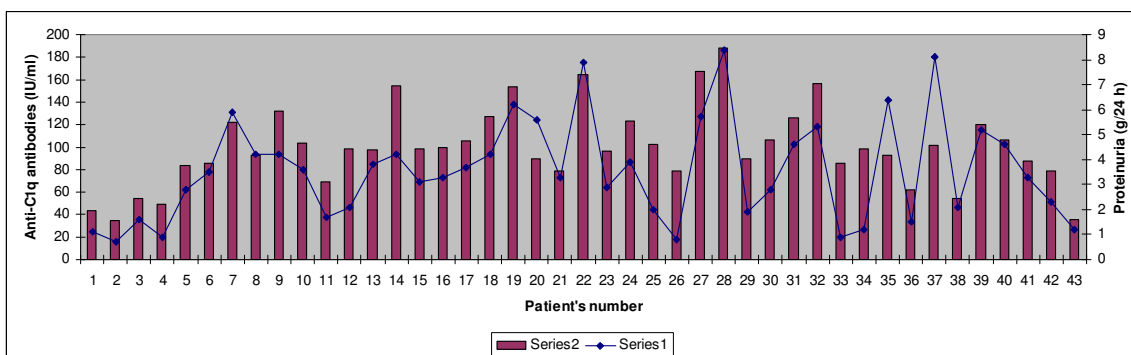
Fig. 2. Correlation between serum levels of anti C1q antibodies (series 1) and proteinuria (series 2)

The presence of anti-C1q Ab is not absolutely specific and necessarily sufficient for lupus nephritis. Serum anti-C1q Ab serial determination is a perspective test, which may help to determine prognosis and further treatment strategy. Serum anti-C1q Ab could be used as a biomarker for disease course monitoring, prognosis evaluation and treatment guidance.

Conflict of interest statement. None declared.

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