

Original Article

High prevalence of anti-C1q antibodies in biopsy-proven active lupus nephritis

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Abstract

Background. Anti-C1q antibodies (anti-C1q) have been shown to correlate positively with systemic lupus erythematosus (SLE) nephritis. Several clinical studies indicated a high negative predictive value, suggesting that active lupus nephritis is rarely seen in patients with no anti-C1q. However, the true prevalence of anti-C1q at the time of active lupus nephritis has not been well established. The aim of this study was to determine prospectively the prevalence of anti-C1q in proven active lupus nephritis at the time of the renal biopsy.

Methods. In this prospective multi-centre study, we investigated adult SLE patients undergoing renal biopsy for suspected active lupus nephritis. Serum samples were taken at the time of the biopsy and analysed for the presence of anti-C1q in a standardized way. The activity of lupus nephritis was classified according to the renal histology. Biopsies were also analysed for the presence of glomerular IgG, C1q and C3 deposition.

Results. A total of 38 patients fulfilling at least 4/11 American College of Rheumatology (ACR) criteria for the diagnosis of SLE were included. Out of this, 36 patients had proliferative (class II, III or IV) and two had class V lupus nephritis. All but one patient with proliferative lupus nephritis were positive for anti-C1q (97.2%) compared with the 35% of control SLE patients with inactive lupus nephritis and 25% of SLE patients without lupus nephritis ever. All patients

were positive for glomerular C1q (36/36) and 37/38 patients had glomerular IgG deposits. Anti-C1q strongly decreased during successful treatment.

Conclusions. Anti-C1q have a very high prevalence in biopsy-proven active lupus nephritis, thus a negative test result almost excludes active nephritis. The data support the hypothesis of a pathogenic role of anti-C1q in lupus nephritis.

Keywords: autoantibodies; complement; SLE

Introduction

An important hypothesis about the pathogenesis of systemic lupus erythematosus (SLE) assumes that the disease is driven by a defective clearance of dead and dying cells (apoptosis) [1]. In the context of an altered clearance, apoptotic cells could become antigenic and initiate an autoimmune response. The complement system has been shown to play an important role in the clearance of apoptotic cells, and the deficiency of one of the early components of the classical pathway of complement, i.e. C1q or C4, is strongly associated with the development of SLE [2]. However, the majority of SLE patients has no primary complement deficiency. In contrast, hypocomplementaemia in SLE patients is a secondary event and is most often associated with autoantibodies against C1q (anti-C1q), the first component of the classical pathway of complement [3]. Anti-C1q are best described in patients with SLE (reviewed in [4]). In these patients they strongly correlate with hypocomplementaemia and renal

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flares suggesting that anti-C1q might play a pathogenic role [5]. It would be plausible that anti-C1q alter the normal function of the complement system and consequently impair the course of the disease. However, the true prevalence of anti-C1q in patients with active lupus nephritis remains controversial [6]. Whereas most of the clinical studies have shown a high negative predictive value of anti-C1q for the occurrence of a severe lupus nephritis ranging up to 100% [7], a more recent study found anti-C1q in only 11/18 patients with proliferative lupus nephritis [8]. The variation between the studies can be explained in several ways. First and most importantly, some studies did not precisely indicate the timing of the anti-C1q test in relation to the renal flare. Furthermore, the definition of active lupus nephritis strongly varies between the studies. Third, the assays used have not been standardized, i.e. every group used its own self-made ELISA system with different reagents and standards. Last, the definition of a positive test result varied between the publications. A high cut-off for a positive result reduced the sensitivity of the test and consecutively increased the likelihood of a false negative result. Therefore, in order to determine the prevalence and negative predictive value of anti-C1q for the occurrence of proliferative lupus nephritis, we performed a prospective, multi-centre study measuring anti-C1q in SLE patients undergoing renal biopsy for suspected active lupus nephritis and using a uniform test system [9,10].

Patients and methods

Patients

In this prospective multi-centre study, adult SLE patients undergoing renal biopsy for suspected proliferative lupus nephritis were included. The patients were recruited at the university hospitals in Basel, Geneva, Lausanne, Madrid (University Hospital La Paz) and Prague (Charles University). Patients were excluded from the study when they were <18 years old, did not give written consent or did not fulfil at least 4 out of the 11 American College of Rheumatology (ACR) criteria for the diagnosis of SLE [11]. Serum samples had to be taken at the time of the renal biopsy ± 7 days and were stored at -80°C until further use. Renal histologies obtained were classified according to the International Society of Nephrology/Renal Pathology Society [12]. According to the abbreviated version of the classification, combined classes III/V or IV/V were considered as class III or IV, respectively. In addition to the histological classification, biopsy specimens were also stained for C1q, C3 and IgG. If possible, follow-up serum samples were taken at months +6 and +12 after biopsy.

The study was approved by the Ethical committee of the University Hospital Basel, Switzerland (EKBB) and conformed to standards applied in the involved countries.

Anti-C1q antibodies

Anti-C1q were tested at each participating centre using the same commercially available ELISA kit according to

the assay procedure (kindly provided by Bühlmann Laboratories, Schönenbuch, Switzerland). In this assay, undigested purified human C1q served as the antigen, and sera were diluted and incubated in a high-salt buffer (1 M NaCl). The optical densities were measured at 450 nm converted into units (U/ml) by being plotted against the autoantibody titre of the standards given by the manufacturer. The upper limit of detection of the assay was at 400 U/ml. The technical cut-off for a positive result was as determined by the manufacturer (15 U/ml) was obtained by testing samples from 220 normal blood donors. This cut-off resulted in 14.5% positive blood donors (32/220). In order to achieve comparability to previous reports describing that only about 6% of normal blood donors were anti-C1q positive [3,7,13–16], the cut-off for the definition of a positive test result was increased to 40 U/ml. This new cut-off was verified in cohorts of other normal blood donors ($n=48$), patients with thyroid disease (Graves' disease, chronic autoimmune thyroiditis or multinodular goitre, $n=60$) and patients with ANCA-associated systemic vasculitis as defined by the presence of high-titre PR3-ANCA or MPO-ANCA ($n=23$).

Results in the study population were compared with the anti-C1q concentrations measured in retrospectively analysed cohorts of SLE patients without lupus nephritis at any time ($n=36$) and SLE patients with biopsy-proven proliferative lupus nephritis (classes II, III or IV) without clinical activity at the time of the serum sampling ($n=26$). The SLE control patients all fulfilled at least 4/11 ACR criteria for the diagnosis of SLE. The absence of lupus nephritis was defined as normal urinalysis results and creatinine levels. Inactivity of pre-existing lupus nephritis was defined as either normal or continuously decreasing or stable values for proteinuria, erythrocyturia (<20 erythrocytes/field) and creatinine during the 6 months preceding the analysis for anti-C1q.

Determination of C1q antigen

C1q antigen determinations were all performed at the Department of Immunobiology, Hôpital Européen Georges Pompidou, Paris, France.

The concentration of C1q antigen in serum was measured by means of a double-ligand ELISA. Briefly, Nunc MaxiSorp ELISA plates (Nunc, Roskilde, Denmark) were coated with goat IgG anti-human C1q (Calbiochem, Meudon, France). Free reactive sites were blocked with phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA). The sera to be tested were added at a dilution of 1:2000 for 1 h. After washing, the plates were incubated with biotinylated IgG goat anti-human C1q prior to the addition of streptavidin-horseradish peroxidase and further incubation for 30 min at 37°C . Enzymatic activity was revealed using the orthophenyldiamine substrate.

Values were expressed in percent of the norm (norm=100%). As a standard for the quantification, pooled plasma from 100 healthy donors was used. Normal values established with the plasma from these donors ranged between 70 and 130% of the reference plasma pool.

Statistics

Non-parametric tests (Mann-Whitney *U*-test, Spearman's rank correlation test) and Fisher's exact test were used

throughout using GraphPad Prism versions 3 and 4 (GraphPad Software, San Diego, CA, USA).

Results

Cut-off determination

By the analysis of anti-C1q results of 220 normal blood donors provided by the manufacturer, the cut-off for a positive test result was set at >40 U/ml resulting in 5.9% positive donors. In order to confirm this cut-off, the prevalence of anti-C1q was measured in independent cohorts of 48 normal blood donors, 60 patients with thyroid disease and 23 patients with ANCA-associated vasculitis. In these cohorts, the percentages of anti-C1q positive individuals were 4.2, 6.7 and 0, respectively. The results obtained in normal blood donors were almost identical to those reported previously [7], and the test specificity similar to that reported by other groups [3,13–16].

In a second step, 26 SLE patients with a history of lupus nephritis in the past but without clinical signs of the activity at the time of serum sampling and 36 SLE patients without a history of lupus nephritis ever were analysed for the presence of anti-C1q. In these patients anti-C1q were found in 35 and 25%, respectively. SLE patients were significantly more often positive and had higher titres of anti-C1q than the pooled patients of non-SLE cohorts. In addition, SLE patients with inactive lupus nephritis had higher titres of anti-C1q than those without a history of lupus nephritis ever (Figure 1).

Anti-C1q in patients with biopsy-proven active lupus nephritis

A total of 40 patients undergoing renal biopsy for suspected severe lupus nephritis were included in the study. Two of these 40 patients had to be excluded for not fulfilling at least 4 of the 11 ACR criteria for the diagnosis of SLE. Both the patients were anti-C1q negative although the renal histology would have been compatible with class II and IV lupus nephritis, respectively. These two patients were, moreover, diagnosed as having C1q nephropathy according to the criteria described by Jennette and Hipp [17] and Markowitz *et al.* [18].

Of the remaining 38 patients, 32 were females and six males. The age median was 32.5 years (range 19–68). The histological characteristics are summarized in Table 1.

The majority of patients had class III or IV lupus nephritis. All patients had a positive staining for glomerular C1q and C3, and all but one patient had a positive staining for glomerular IgG. Anti-C1q were found in 36/38 patients (94.7%, Figure 1). Two of the 38 patients had class V (membranous) lupus nephritis corresponding to a class Va nephritis according to the modified 1982 WHO classification [12]. This entity was considered as being distinct from the other classes of

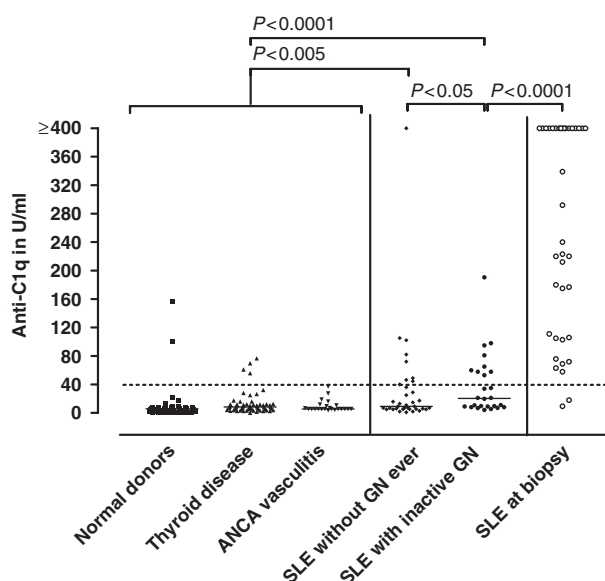


Fig. 1. Anti-C1q in SLE and control patients. Anti-C1q titres in cohorts of normal blood donors, patients with thyroid disease and patients with ANCA-associated vasculitis, compared with SLE patients without lupus nephritis ever, with clinically inactive lupus nephritis and patients with lupus nephritis at the time of the biopsy. SLE patients had significantly higher titres and were more often anti-C1q positive than pooled control cohorts. In SLE patients, increased titres of anti-C1q were most frequently detected, and the titres were highest among patients with active nephritis at the time of the biopsy. Interestingly, SLE patients with inactive lupus nephritis had significantly higher anti-C1q titres than those without lupus nephritis ever.

Table 1. Immunohistological characteristics of the renal biopsy obtained for suspected proliferative lupus nephritis

Histological classification (abbreviated form)	Numbers (n = 38)	Glomerular C1q (n = 36)	Glomerular C3 (n = 38)	Glomerular IgG (n = 38)
I	0	–	–	–
II	3	2/2	3/3	3/3
III	10	10/10	10/10	10/10
IV	23	22/22	23/23	22/23
V	2	2/2	2/2	2/2

lupus nephritis observed in our study population with regard to prognosis and immunohistological characteristics [17,19]. One of the two patients with membranous lupus nephritis was anti-C1q negative, the other was low-level positive (Figure 2). Of the remaining 36 patients with class II, III and IV lupus nephritis, all but one were positive for anti-C1q at the time of the renal biopsy (97.2%). The results are demonstrated in Figure 2. SLE patients with proliferative lupus nephritis were significantly more often positive for anti-C1q, and the titres were significantly higher than in SLE controls ($P < 0.0001$). The only patient being anti-C1q negative had a 10-year history of cutaneous lupus erythematosus before developing lupus nephritis

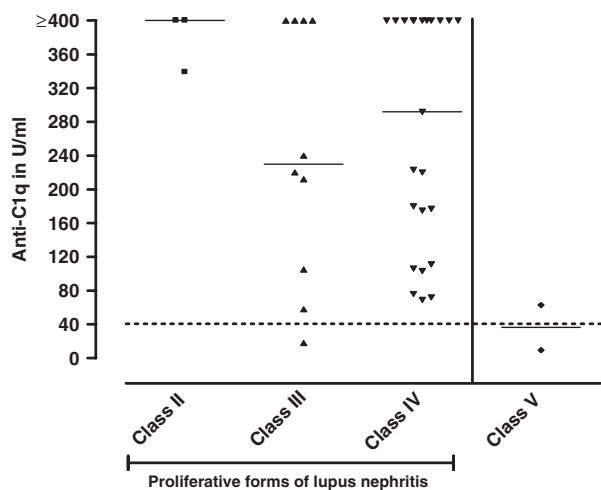


Fig. 2. Anti-C1q concentrations according to the class of lupus nephritis. Anti-C1q titres in the study population of SLE patients at the time of renal biopsy. Patients were grouped according to the histological classification of the lupus nephritis. Lupus nephritis classes II–IV were considered as active (proliferative) forms and separated from the two patients with class V (membranous) lupus nephritis. All but one of the patients with proliferative lupus nephritis (35/36) were anti-C1q positive. Almost half of these patients ($n = 14$) had very high titres, i.e. ≥ 400 U/ml.

accompanied by oral ulcers, arthralgias, anti-nuclear antibodies (ANA) and anti-dsDNA antibodies. The patient's immunohistology was positive for glomerular C1q and IgG.

For the detection of an active glomerulonephritis in SLE patients, the anti-C1q assay showed a particularly high sensitivity (97.2%) and negative predictive value (97.8%). Specificity and positive predictive value were 70.3 and 68.4%, respectively. The corresponding receiver operating characteristic (ROC) curve is demonstrated in Figure 3 [area under the curve = 0.958, 95% confidence interval (CI) 0.92–0.996, $P < 0.0001$].

Anti-C1q at follow-up

Fifteen of the 36 patients with proliferative lupus nephritis at biopsy, i.e. classes II, III and IV, could be followed at 6 months and 11 patients at 12 months after the biopsy. As demonstrated in Figure 4, there was a significant drop of anti-C1q titres between the time of the biopsy and month +6, but no further decrease between months +6 and +12. In only three patients, a mild increase of anti-C1q titres at month +6 could be observed (from 58 to 73, 72 to 91 and 105 to 120 U/ml). Furthermore, the two patients with persistently high titres of anti-C1q (>400 U/ml) at month +6 were the only two of the cohort in whom no sustained response to treatment could be observed as judged by the elevated markers of inflammation and unchanged or reappearing proteinuria and glomerular erythrocyturia. The patient without response to treatment also had a persistently elevated creatinine whereas the patient with renal relapse, i.e. reappearing

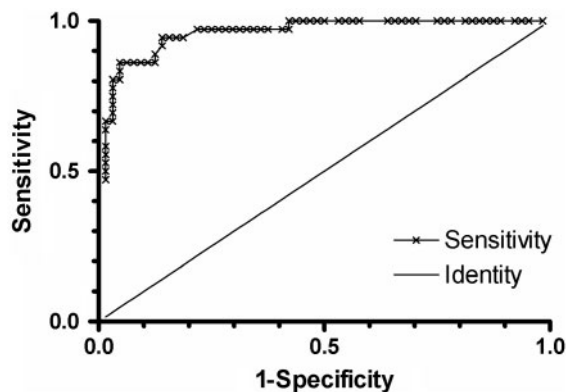


Fig. 3. ROC curve of anti-C1q for the discrimination between active and inactive/no lupus nephritis. The ROC curve of the anti-C1q antibody test used for the discrimination between active and inactive/no nephritis in SLE patients. The curve was generated using anti-C1q results from the study population (Figure 2) and the two SLE control cohorts shown in Figure 1 (area under the curve = 0.958, 95% CI 0.92–0.996, $P < 0.0001$).

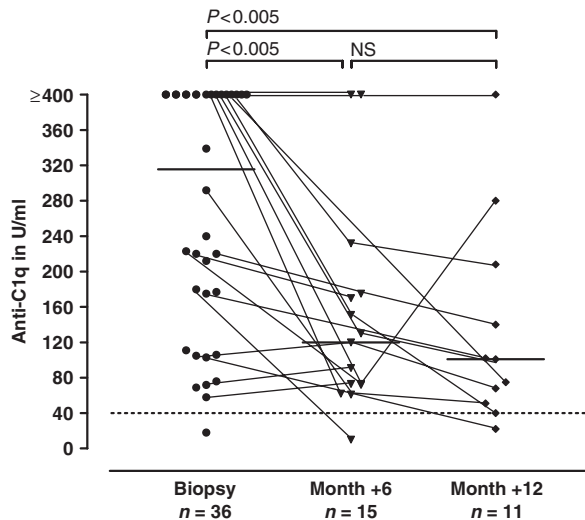


Fig. 4. Follow-up of anti-C1q in patients with proliferative lupus nephritis. Anti-C1q titres during follow-up significantly decreased at 6 months after renal biopsy without further decrease at month +12. Interestingly, the two patients with persisting high titres of anti-C1q (≥ 400 U/ml) at month +6 did not show a clinical response to treatment nor had a relapse after initial response as confirmed by a second biopsy.

proteinuria and erythrocyturia after initial improvement, underwent a second biopsy at month +6 revealing a persistent lupus nephritis class III.

It is interesting to note that the majority of patients included at the time of the biopsy did not become negative for anti-C1q during follow-up despite decreasing titres.

C1q antigen concentrations

Since anti-C1q had been shown to be associated with low levels of C1q antigen in serum, the sera from the

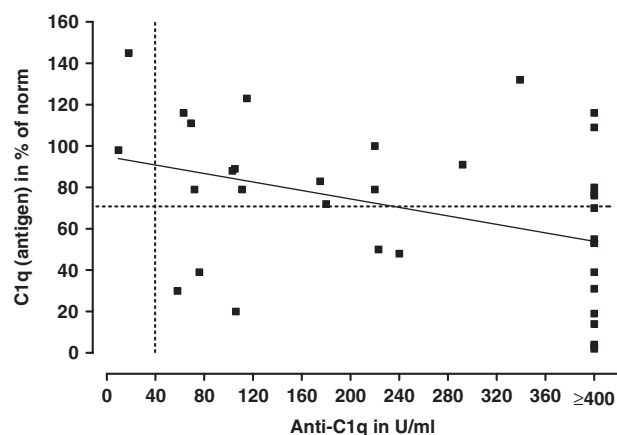


Fig. 5. Correlation between anti-C1q and serum C1q concentrations. C1q (antigen) concentrations compared with anti-C1q antibody titres in the SLE patients at the time of the renal biopsy. Although there was a significant negative correlation between the two parameters, measuring C1q concentrations was less useful for the detection of active lupus nephritis than the determination of anti-C1q since more than half of the patients (58%) had C1q levels within the norm. C1q concentrations were expressed in percent of norm. The dotted lines indicate the cut-off for decreased C1q concentrations and elevated titres of anti-C1q, respectively.

study patients were also analysed for C1q concentrations. Forty-two percent of the sera had C1q concentrations below the norm with a median of 76.5% and a range of 2–145%. As expected, there was a significant negative correlation between anti-C1q titres and C1q concentrations in serum (Spearman's $r=0.426$, $P<0.005$). The data are demonstrated in Figure 5.

Discussion

In the present study, we prospectively investigated the prevalence of autoantibodies against complement C1q in a cohort of SLE patients undergoing renal biopsy for suspected proliferative lupus nephritis. We hypothesized that anti-C1q play an important, though not exclusive, role in the pathogenesis of lupus nephritis. Therefore, the majority of patients were expected to be anti-C1q positive. Indeed, we found a strong positive correlation between anti-C1q and the occurrence of active proliferative lupus nephritis. All but one of the patients with class II, III or IV lupus nephritis were positive for anti-C1q at the time of active nephritis, corresponding to a prevalence of >97%. In comparison, anti-C1q were found in only about one-third of SLE patients having either inactive lupus nephritis or no lupus nephritis at all. Interestingly, the last group had the lowest prevalence of anti-C1q. In addition to the high prevalence of anti-C1q in patients with biopsy-proven active lupus nephritis, these patients had the highest titres observed in this study. Furthermore, anti-C1q titres strongly decreased during successful treatment.

The high prevalence of anti-C1q in our patients might have important consequences for the clinical management of SLE patients as well as for the understanding of pathogenic mechanisms in SLE. From a clinical point of view, anti-C1q might be of important help in the diagnosis of suspected proliferative lupus nephritis, particularly in situations when standard parameters such as urinalysis, creatinine, serum complement levels and anti-dsDNA antibodies do not allow a clear-cut decision about treatment modifications and/or the necessity of a renal biopsy. Very high titres of anti-C1q strongly increase the likelihood of the presence of severe lupus nephritis. Vice versa, and maybe more importantly, a negative test result almost excludes the presence of an active glomerulonephritis and therefore might help avoid unnecessary renal biopsies and/or treatment modifications.

For the judgement about the occurrence of renal flares in SLE, anti-C1q have been shown to be more helpful than the determination of anti-dsDNA antibodies [7,20,21]. Furthermore and in contrast to a previous study using a different technique [8], our data demonstrate that measuring anti-C1q in the diagnostic process of active lupus nephritis is also more helpful than the determination of C1q antigen in serum. Although there was a strong negative correlation between anti-C1q and C1q antigen in serum, more than half of our patients had C1q concentrations within the normal range.

Up to now, the prevalence of anti-C1q in active lupus nephritis has not been uniformly established [6,7]. Although many studies demonstrated a high prevalence of anti-C1q in active lupus nephritis, the data presented in other reports suggested lower negative predictive values for anti-C1q [8,13,22]. Differences between our and previous studies might be partially attributed to differences in the assay used and the definition of a positive test result. In our study we used a well-established [9,10] and robust commercially available kit that was designed to well-discriminate low-level positive from negative results. The cut-off used in our study was set in order to allow comparisons with previous studies, and resulted in a low prevalence of anti-C1q in controls. However, the timing of blood sampling in relation to the renal flare appears to be the most important difference with previous studies. It is likely that many of the negative anti-C1q measurements were from serum samples that were not obtained shortly before or during the nephritic episode. Finally, in many studies, the activity of the nephritis at the time point of blood sampling was judged by clinical parameters but not controlled by histology.

Since anti-C1q were detected by an assay using undigested C1q as the antigen, it might be argued that some of the positive results are due to unspecific binding of immune complexes. However, in a previous report it was demonstrated that binding of immune complexes is almost completely abrogated by the use of 1 M NaCl, and the correlation of results obtained by

the use of undigested C1q and collagen-like region of C1q was particularly high ($R=0.983$) [23]. Therefore, this factor is unlikely to have substantially affected our results.

Independent of the clinical relevance of anti-C1q as a diagnostic parameter, our results support the hypothesis of anti-C1q playing an important role in the pathogenesis of lupus nephritis, since all but one patient were positive for anti-C1q. In a more sensitive assay, even this patient might have been positive for anti-C1q [24].

As suggested in a previous study, anti-C1q appear to be necessary but not sufficient for the occurrence of severe lupus nephritis [7]. However, the role of anti-C1q in the pathophysiology of SLE remains unclear. As the binding of anti-C1q to fluid phase C1q is weak, their functional role might be limited to tissues/organs where C1q is deposited, e.g. the kidney [25]. Indeed, anti-C1q could be isolated from glomerular basement fragments of patients with proliferative lupus nephritis and the deposition seemed to occur via binding to deposited C1q [25,26]. Interestingly, anti-C1q could not only be isolated from the glomerular basement membranes of patients with proliferative lupus nephritis but were enriched about 50 times in the glomeruli compared with the total IgG deposition and anti-C1q serum concentrations of the same patients.

In a recent experimental study, the effect of a monoclonal anti-C1q antibody was investigated in mice [26]. It could be demonstrated that the anti-C1q alone resulted in glomerular deposition of the antibody and C1q as well as in mild neutrophil influx but could not cause severe renal damage. However, when glomerular immune complexes were induced by a pre-injection of subnephritogenic doses of a C1q-fixing anti-glomerular basement membrane (anti-GBM) antibody, the following injection of anti-C1q could exacerbate the pre-existing subclinical renal disease. Although not being a final proof, the study strongly supported the view of anti-C1q having a direct pathogenic effect.

Thus, in the context of our clinical findings, the role of autoantibodies and B cells should be re-emphasized in lupus nephritis, with the use of treatment strategies targeting B cells, such as rituximab, as the logical consequence.

In conclusion, we found a high prevalence of anti-C1q in biopsy-proven active lupus nephritis at the time of the biopsy. The absence of anti-C1q argues strongly against the presence of proliferative lupus nephritis and therefore may serve as a tool to avoid unnecessary renal biopsies and/or treatment intensifications.

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Conflict of interest statement. None declared.

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