Repeated Renal Biopsy in Proliferative Lupus Nephritis — Predictive Role of Serum C1q and Albuminuria

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ABSTRACT. ***Objective.*** Proliferative (WHO III/IV) nephritis in systemic lupus erythematosus (SLE) is a severe disease manifestation for which treatment with cyclophosphamide and high dose corticosteroids is generally recommended. We investigated the effect of this standard treatment on renal histopathology and clinical and serological findings to determine if the therapeutic response could be predicted by these variables.

Methods. We studied 18 patients with SLE and proliferative nephritis in whom repeated renal biopsy was performed after termination of induction therapy with cyclophosphamide and corticosteroids. At the time of renal biopsy, renal function and albuminuria were determined and analyses of anti-dsDNA, anti-C1q, and the complement factors C1q, C3 and C4 were performed.

Results. At repeated biopsy, 6/18 patients still had renal biopsy findings of WHO III/IV, 3 had transformed to WHO V, while 9 exhibited histopathological remission (WHO I/II). In the 9 patients with WHO III–V at the repeat biopsy, all but one patient had low C1q levels at the time of first biopsy and 5/9 at the repeat biopsy. In the 9 patients with WHO I/II at repeated biopsy, 4/9 had low C1q at first biopsy and none at the repeated biopsy ($p = 0.0054$ and $p = 0.017$ vs WHO III–V at repeat and first biopsy, respectively). Albuminuria ≥ 0.5 g/day combined with low C1q levels at repeat biopsy predicted persistent histopathological activity (WHO III–V).

Conclusion. Despite aggressive immunosuppressive therapy, 9/18 patients still had active proliferative or membranous nephritis at a second renal biopsy. Serum C1q levels at both first and repeated renal biopsies were found to be a predictive marker of the histopathological outcome. (J Rheumatol 2002;29:693–9)

Key Indexing Terms:

NEPHRITIS	SYSTEMIC LUPUS ERYTHEMATOSUS		TRANSITION
ANTI-dsDNA	ANTI-C1q	C1q	ALBUMINURIA

Renal survival in lupus nephritis has steadily improved during the past decades, although debate continues about the optimal therapeutic regimen. At most centers, the World Health Organization classification of lupus nephritis[1] is used as a guide for therapy. In patients with focal proliferative lesions (WHO III) and patients with diffuse proliferative disease (WHO IV), immunotherapy with cyclophosphamide in combination with corticosteroids is generally used[2].

How intensive and longstanding such immunotherapy should be has not been clearly established. Many centers follow the protocols recommended by the US National Institutes of Health (NIH)[2], while others have recommended more individualized regimens[3]. After treatment with the NIH protocol some patients still develop persistent renal damage, whereas others have a favorable renal outcome and may not require prolonged treatment. Identification of factors that could predict the renal outcome is thus an issue of major importance.

Low grade proteinuria, normal serum creatinine, and a nearly normal hematocrit after 6 months of therapy have been found to be favorable prognostic indicators of the longterm renal outcome, supporting the use of short courses of pulse cyclophosphamide for proliferative nephritis in selected cases[4]. However, no repeated biopsy was performed in this study, which could have identified patients with unfavorable renal prognosis. Interestingly, in a recent publication, the strongest predictor of an unfavorable renal outcome was determined as persistent renal inflammation at repeated biopsy, performed after termination of induction therapy according to the NIH protocol[5].

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Both anti-dsDNA and anti-C1q antibodies have been reported to precede the onset of nephritis and to decrease after initiation of therapy, thus indicating pathogenic functions[6,7]. Decreases in the classical complement components may be utilized as markers of lupus activity, and both low C3 and C1q levels have independently been reported to predict renal flares[8,9]. Longterm prognosis has also been shown to be better in patients with lupus nephritis in whom serum complement (CH50) was continuously normalized following treatment compared to patients with persistently low complement levels[10]. For specific complement markers there is a lack of data on the predictive value in the therapeutic situation regarding both short term and longterm outcomes.

With this in mind, our objective was to histologically define the therapeutic response of immunosuppressive treatment according to the NIH protocol in patients with proliferative nephritis, here performed by a repeated renal biopsy after 6 months of induction treatment with cyclophosphamide and corticosteriods. We also wanted to identify clinical and serological factors, recorded at both initial and repeated biopsies, that might predict the histological outcome. Better patient monitoring may be achieved by identification of such noninvasive prognostic markers, and serve as an aid in future therapeutic decision making.

MATERIALS AND METHODS

Patients. From 1996 to 1999, consecutive patients with SLE and biopsy proven proliferative nephritis (WHO class III or IV) were identified for this study. The patients met at least 4 of the American College of Rheumatology criteria for SLE[11]. Renal biopsy was performed as clinical routine if urinary findings had indicated renal involvement (persistent hematuria and/or proteinuria). Duration of nephritis was determined as the time from onset of continuous hematuria and/or proteinuria to renal biopsy.

Treatment. After histological confirmation of proliferative nephritis, a standardized induction treatment with intravenous cyclophosphamide at a dose of 15 mg/kg body weight (or 0.5 g/m^2) monthly for 6 months was initiated, combined with oral prednisolone with a starting dose of 0.8 mg/kg/day as modified from the NIH protocol[2]. The prednisolone treatment was continuously tapered with the aim of reaching a dose of roughly 10 mg/day after 6 months. After treatment termination following 6 months of therapy with cyclophosphamide, a second renal biopsy was performed to study the histopathological tissue response to therapy and to correlate this to clinical and serological measures.

The study was approved by the local ethical committee.

Renal histology and function. Biopsies were performed by percutaneous needle (16 gauge) ultrasonography guided puncture, and the renal tissue obtained was graded according to the WHO classification of SLE nephritis[1]. Biopsies were evaluated by light microscopy, immunofluorescence, and electron microscopy. For estimation of activity and chronicity, the biopsies were graded according to a standardized histological grading protocol[12]. Renal function at the time of renal biopsy was determined by serum creatinine levels (μmol/l) and by glomerular filtration rate (GFR), determined by the clearance of iohexol (ml/min × 1.73 m^2). Grade of proteinuria was measured as grams of urine albumin/24 hours.

Serology and complement. Simultaneously with renal biopsy, analyses of serum IgG anti-dsDNA and IgG anti-C1q antibodies were performed. Anti-dsDNA antibodies were detected by indirect immunofluorescence using *Crithidiae luciliae* as source of antigen according to standard protocols. Anti-C1q antibodies were detected by ELISA as described[13,14]. Serum from an SLE patient with high anti-C1q levels was used for the construction of a standard curve, arbitrarily defined as 1000 U/ml. The mean value of 40 healthy controls + 2 SD was defined as the normal value. Analysis of complement component C1q was performed by rocket electrophoresis[15] using rabbit anti-C1q (Dako) as the antibody. Levels of C1q were expressed as the percentage of the levels of normal blood donors (normal 76–136%). C3 and C4 were determined by nephelometry using a Beckman Array instrument and Beckman reagents. The normal level of C3 was 0.5–1.2 g/l and of C4 0.1–0.4 g/l.

Statistics. For statistical evaluation, Mann-Whitney U test, Spearman rank correlation, Student t test, Wilcoxon signed rank, and Fisher's exact test were employed for paired and unpaired observations. A p value < 0.05 was considered statistically significant.

RESULTS

Outcome at first renal biopsy

Patients. The patients, 15 women and 3 men, had a mean age of 31 years (median 24.5, range 18–58) at the first renal biopsy. The age, sex, duration of nephritis, and treatment at the time of the first renal biopsy are presented in Table 1.

Treatment. After confirmation of nephritis, the intention was to treat all patients according to the protocol described above. However, in one patient (Patient 9), only 2 pulses with cyclophosphamide were administered and then withdrawn due to side effects (severe leukopenia and cystitis). Azathioprine was given instead. In Patient 11, cyclophosphamide was withdrawn after 2 pulses and switched to azathioprine due to an allergic reaction. One patient (Patient 10) received one pulse of cyclophosphamide, which was then altered to oral treatment for 8 weeks due to the patient's fear of parenteral infusions. The other patients adhered to the treatment protocol.

Table 1. Demographic data at first renal biopsy.

Patient	Age, yrs	Sex	Duration of Nephritis, mo	Treatment
1	53	M	ND	None
2	20	F	0.5	None
3	32	F	2	Pred
4	50	F	5	Pred
5	58	F	11	Pred
6	22	F	1	Pred
7	23	F	0.5	Pred, AZA
8	20	F	0.5	Pred
9	22	F	0.5	None
10	19	M	0	None
11	19	F	2	Pred, MTX
12	25	F	6	Pred, MTX
13	24	F	1	Pred, AZA
14	24	F	11	None
15	54	M	4	Pred
16	41	F	2	None
17	35	F	7	Pred
18	18	F	10	Antimalarials

ND: not determined, Pred: prednisolone, AZA: azathioprine, MTX: methotrexate.

Renal histology and function. All patients had nephritis confirmed by renal biopsy at the start of this study. Seven patients had WHO grade III and 11 patients WHO grade IV. The renal tissue was examined for activity and chronicity findings as described above. The mean activity index at the first biopsy was 8 (median 8.5, range 4–13) and the chronicity 1 (median 1, range 0–4). The mean serum creatinine value was 91 (± 30) μmol/l and the median albuminuria 1.6 (range 0–19.8; mean 2.9 ± 3.5) g/24 h. The mean GFR was 74 ml/min. Two patients had a serum creatinine level above the normal limit and 10 had GFR below –2 SD.

The histopathology and treatments are shown in Table 2.

Serology and complement. At the time of the first biopsy, anti-dsDNA antibodies were detected in all patients in titers between 1/25 and 1/3200 (cutoff < 1/10). Anti-C1q antibodies above the normal limit (67 U/ml) were detected in 11/18 patients. Serum C1q values below the reference range were observed in 12 patients, low C3 in 7, and low C4 in 9 patients.

Outcome at second renal biopsy

Renal histology and renal function. The median time between the biopsies was 7 months. At the second renal biopsy, WHO I/II was recorded in 9 patients, WHO III in 2, WHO IV in 4, and WHO V in 3 patients. The mean activity index was 4 (median 2, range 0–13) and the chronicity was 2 (median 1.5, range 0–4). A significant decrease in activity index from first to second biopsy was recorded ($p < 0.0001$, Student t test). The mean serum creatinine was 81 μmol/l (± 14) and a value over the normal range (> 120) was observed in only one individual (WHO III). There was no significant reduction of serum creatinine between the 2 biopsies ($p = 0.19$). The median albuminuria at the second biopsy was 0.5 (range 0–3.1) g/24 h (mean 0.7 ± 0.9). Albuminuria < 0.5 g/day was seen in 7/9 patients with WHO I/II, in 2/6 patients with WHO III/IV, and in 0/2 patients with WHO V. No statistical difference in albuminuria was noted between the biopsies ($p = 0.07$). The mean GFR was 83 ml/min (investigated in 15/18 patients); no statistical significance was seen between the 2 examinations ($p = 0.12$). A GFR below –2 SD was observed in 3/9 patients with WHO I/II, 4/6 with WHO III/IV, and 0/2 patients with WHO V.

Serology and complement. At repeated biopsy, anti-dsDNA antibodies were still noted in 14/18 patients (nonsignificant compared to the first examination, Fisher's exact test); however, declining titers were observed in most patients, and 5 had detectable anti-C1q antibodies (NS). Five patients had C1q values below the reference range ($p = 0.10$ compared to first examination), one had low C3 (NS), and another low C4 (NS). A significant increase in C1q levels was noted from first to second biopsy ($p = 0.0013$, Wilcoxon signed rank test). This finding was also observed in the subgroup with WHO III–V at repeated biopsy ($p = 0.012$); however it was not significant in patients with WHO grade I/II ($p = 0.1$).

Factors predicting or indicating the histopathological outcome at repeated biopsy

Treatment. Neither the total dose of cyclophosphamide given nor the time elapsed from the onset of nephritis until the initiation of therapy was found to influence the

Table 2. Histological findings, time elapsed, and treatment given at repeat renal biopsy.

Patient	WHO 1st Biopsy	AI/Cl 1st Biopsy	WHO 2nd Biopsy	AI/CI 2nd Biopsy	Total Dose Cy, g	Prednisolone at 2nd Biopsy, mg/day
1	IVb	6/1	IIa	1/2	6.0	5
2	IVb	9/0	IIb	2/1	5.25	7.5
3	IVb	13/1	IVc	13/4	4.5	30*
4	IVb	8/0	Ib	0/0	6.0	7.5
5	IVc	5/3	IIb	2/1	2.25	7.5
6	IVc	4/2	Vb	2/3	4.6	15
7	IIIa	11/0	IIa	1/4	4.2	5
8	IIIa	11/1	IIIa	10/1	4.8	15
9	IVb	10/1	Vb	2/1	1.15**	5
10	IVb	12/1	IVc	4/3	8.1***	15
11	IIIa	10/0	IVb	8/1	1.4**	15
12	IIIa	10/2	IIa	2/2	5.4	15
13	IIIb	7/1	IIb	1/1	4.5	10
14	IIIa	5/0	IIb	2/0	6.0	10
15	IIIb	7/2	IIIb	7/4	5.9	15
16	IVc	6/4	IVc	7/4	6.7	12.5
17	IVc	9/0	IIb	2/0	5.8	15
18	IVb	8/3	Vb	3/3	5.4	15

AI: activity index, CI: chronicity index.* High prednisolone dose due to pericarditis. ** Azathioprine given after withdrawal of cyclophosphamide. *** Oral cyclophosphamide.

histopathological outcome at repeated biopsy (data not shown).

Renal function. No correlation was noted between serum creatinine levels at repeated biopsy and WHO grade (p = 0.13). The level of serum creatinine at first biopsy did not predict the histological outcome at repeated biopsy (p = 0.07) (Tables 3 and 4). Low grade albuminuria (defined as < 0.5 g/day) at the time of second renal biopsy correlated with transformation into WHO class I/II as compared with patients with WHO III–V (p = 0.0013, Mann-Whitney U test). The positive predictive value of significant albuminuria (> 0.5 g/day) at first biopsy and an unfavorable histopathological outcome (WHO III–V at repeated biopsy) was 50%, with sensitivity 89% and specificity 33%. At repeat biopsy, the predictive value of significant albuminuria and an unfavorable histopathological outcome was 78%, the sensitivity 88%, and the specificity 78%.

Despite histologically active disease with persistence of WHO III/IV or transformation to WHO V at repeat biopsy, an apparent improvement in laboratory measures was noted in the majority of the patients. However, the clinical findings could not discriminate patients with transformation to WHO V from patients with persistent WHO grade III/IV. However, as the number of patients we studied was limited, no firm conclusion can be drawn.

Complement components. The patients with persistent WHO grade III/IV and transformation to WHO V at repeated biopsy had significantly lower C1q levels compared to patients with WHO I/II at repeated biopsy (p = 0.0054, Mann-Whitney) and also at the time of the first renal biopsy

Table 3. Clinical and immunological measures at the first renal biopsy in relation to histopathological outcome at second biopsy.

	WHO I/II, n = 9	WHO III-V, n = 9	p, Mann-Whitney U
S-creatinine, normal < 120 μmol/l	78 (±13)	103 (±38)	NS (0.07)
Albuminuria, g/24 h	3.9 (±6.5)	1.8 (±1.3)	NS
Median	1.4 (0–19.8)	1.9 (0.2–4.7)	
C3, normal 0.5–1.2 g/l	0.54 (±0.22)	0.58 (±0.28)	NS
C4, normal 0.1–0.4 g/l	0.1 (±0.07)	0.09 (±0.03)	NS
Clq, normal 76–136%	82 (±29)	46 (±27)	0.017
Anti-dsDNA, no. patients positive	9/9	9/9	NS*
Anti-Clq, no. patients positive	6/9	5/9	NS*

Data are calculated as mean values (± SD). * Fisher's exact test.

Table 4. Clinical and immunological measures at second biopsy in relation to histopathological outcome.

	WHO I-II n = 9	WHO III-V n = 9	p, Mann-Whitney U
S-creatinine, normal < 120/μmol/l	76 (±10)	86 (± 16)	NS
Albuminuria, g/24 h	0.1 (±0.2)	1.3 (±0.9)	0.0013
Median	0 (0–0.5)	1.5 (0.3–3.1)	
C3, normal 0.5–1.2 g/l	1.0 (±0.32)	0.87 (± 0.35)	NS
C4, normal 0.1–0.4 g/l	0.18 (± 0.08)	0.12 (± 0.04)	NS
Clq, normal 76–136%	100 (± 16)	69 (± 25)	0.0054
Anti-dsDNA, no. patients positive	7/9	7/9	NS*
Anti-Clq, no. patients positive	2/9	3/9	NS*

Data calculated as mean values (± SD). *Fisher's exact test.

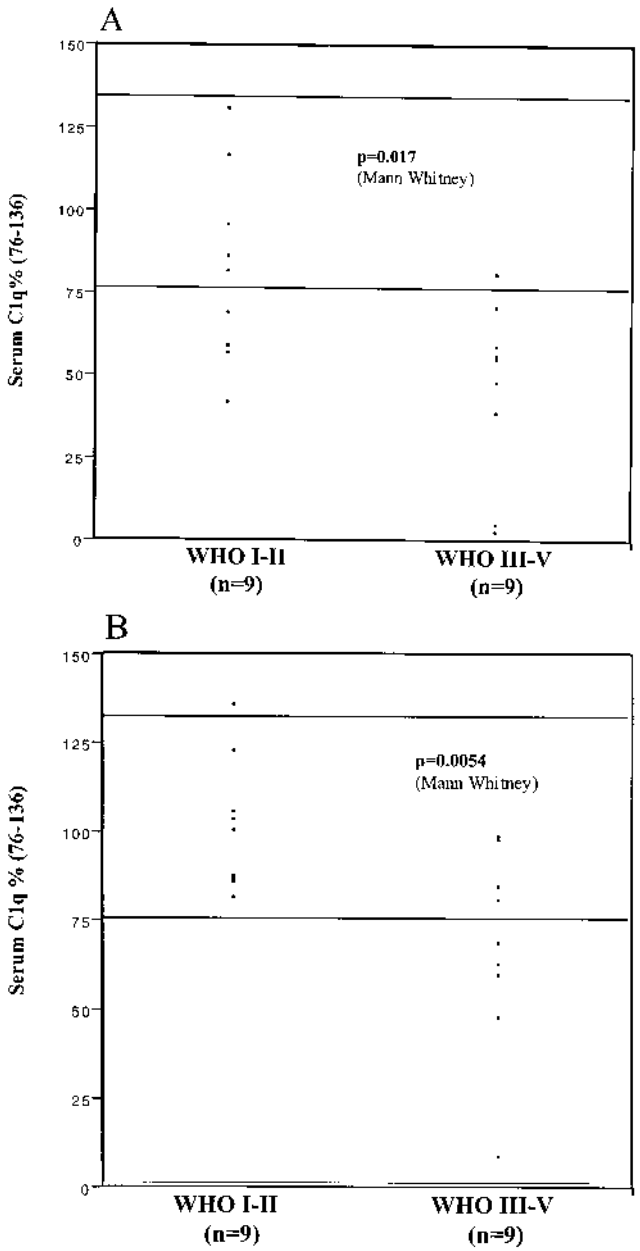

Figure 1. A. Serum C1q at first renal biopsy and histopathological group at repeat biopsy. B. Serum C1q and histopathological group at repeat biopsy.

(p = 0.017, Mann-Whitney). The positive predictive value of a low C1q value and an unfavorable histopathological response (WHO III–V at repeat biopsy) was 67% at first biopsy, the sensitivity 89%, specificity 56%. At second biopsy, the positive predictive value of a normal C1q level and a favorable outcome was 100%, the sensitivity of a low C1q to predict an unfavorable outcome was 56% and specificity was 100% (Tables 3 and 4, Figure 1).

A finding of a low C1q level combined with significant albuminuria at first biopsy and an unfavorable histopathological outcome at repeated biopsy had a sensitivity of 100%, specificity of 40%, and a positive predictive value of 70%. Interestingly, at repeated biopsy, the sensitivity was 100%, specificity was 100%, and positive predictive value was 100%.

No predictive value of the histological outcome was provided by C3 or C4 levels at the first or second examination (Tables 3 and 4).

Analyzing the levels of complement components and activity index at first and repeated biopsy, an inverse correlation between low C1q levels and activity index at repeated biopsy was observed (p = 0.048).

An inverse correlation was evident between C1q levels and anti-C1q antibody titer in all determinations (p = 0.0009, Spearman rank correlation) and at first renal biopsy (p = 0.01), although it did not reach statistical significance at the second biopsy (p = 0.09).

Serology. Anti-dsDNA or anti-C1q antibody titers did not predict the histopathological outcome, at neither the first nor the repeated renal biopsy. However, a correlation between anti-dsDNA antibody levels and the renal activity index was recorded in all biopsies (p < 0.0001, Spearman rank correlation) as well as in the second biopsy (p = 0.032). No significant correlation was noted between anti-dsDNA and renal activity in the first biopsy (p = 0.11). No correlation was found between activity index and anti-C1q antibodies, neither at first nor at repeated biopsy (p = 0.76 and 0.63, respectively) (Tables 3 and 4).

DISCUSSION

Nine out of 18 patients with proliferative lupus nephritis treated with induction therapy with cyclophosphamide and corticosteroids still had histologically active disease of WHO grade III to V at repeated biopsy despite the apparent clinical response. Continuing histological renal activity at repeated biopsy was associated with decreased C1q levels and significant albuminuria at the time of repeated biopsy, and as well was predicted by low C1q levels at first renal biopsy. Indicators of beneficial histological outcome (i.e., transition to WHO I/II) were low grade albuminuria and normal C1q levels at the time of repeated biopsy.

Spontaneous transformation from one WHO class of lupus nephritis to another is not uncommon[16]. Histological transition after treatment has also been reported in a substantial number of patients with lupus nephritis[17-19]. In previous reports, the transformation rate in proliferative nephritis at repeated biopsy was lower than described here, reported in 34–42% of patients with WHO grade IV seen at the initial biopsy[17,19]. However, in previous reports, no standardization of therapy was performed and the time elapsed between biopsies was longer, making comparisons with the present study difficult. Further, patients with more active renal disease were selected to undergo a second biopsy in previous studies, probably biasing the results.

In our study, 12 patients displayed a transition in WHO class, of which 3 had developed WHO class V with subepithelial depositions of immune complexes in a membranous pattern. Six patients had persistent proliferative histopathological findings, of which 5 also had high activity scores (> 6) despite aggressive immunosuppressive therapy. The transition pattern was not primarily due to the cumulative dose of cyclophosphamide, as the patients who switched to WHO class I/II received equal doses compared to the others, nor did duration of nephritis at the time of the first biopsy influence the histopathological outcome.

Low C1q levels analyzed at the first and second renal biopsy were associated with and even predicted an unfavorable histopathological outcome with persistent WHO III/IV or development of WHO V. Interestingly, low C1q levels combined with significant albuminuria at the time of repeated biopsy were also associated with persistent histopathological activity. However, we could not discriminate between the persistence of WHO III/IV from the transition to WHO V by the C1q levels, neither at the first nor at the second evaluation. By contrast, neither serum creatinine levels nor C3 or C4 had predictive prognostic value. These findings may thus be important in the identification of subpopulations who respond to immunosuppressive induction therapy or not.

Complement components of the classical pathway vary in relation to disease activity and low values of C1q and C3 have been reported in active renal disease[8,9,20]. Low C1q levels were determined to be the strongest predictor of subsequent development of renal flare compared to levels of other complement factors[9].

A well established function of C1q is the binding and subsequent removal of immune complexes from the circulation or tissue depositions. Interestingly, recent reports have also suggested a new role of C1q in the clearance of apoptotic cell material in the absence of antibodies[21]. The findings of multiple apoptotic bodies in the glomeruli of C1q deficient mice, which develop SLE-like disease with a high incidence of nephritis, also indicates apoptosis as a contributing factor in the development of nephritis[22]. Further, in an experimental model C1q was shown to protect against the development of glomerulonephritis independently of C3 activation[23], suggesting that C3 may not be essential for development of nephritis.

Anti-dsDNA antibodies have been reported to increase before the development of nephritis and to be a marker of general disease activity in lupus[6,7]. Depositions of anti-dsDNA in the glomeruli have also been documented in early studies, indicating a pathogenetic importance[24]. The finding of a correlation between renal activity index and the titer of IgG anti-dsDNA, as observed for both IgM and IgG anti-dsDNA[25,26], confirms the role of anti-dsDNA in lupus nephritis.

A strong correlation between anti-C1q and proliferative forms of nephritis has been documented[7,27,28]. Depositions of anti-C1q antibodies in renal tissues of patients with proliferative lupus nephritis have also been described, strengthening a putative pathogenetic role of these antibodies[29]. Although a majority of patients in our study had detectable anti-C1q at the time of first biopsy, occurrence or persistence of anti-C1q antibodies did not predict the histopathological outcome at repeated biopsy. This contrasts with reports describing a higher proportion of declining anti-C1q antibody levels in patients with clinical response to treatment compared to nonresponders[30]. The absence of anti-C1q antibodies in this study cohort in many of the patients with persistent proliferative nephritis at repeated biopsy also contrasts with the report describing a lack of lupus nephritis among anti-C1q negative patients with SLE[31]. However, as our patient population was small, the role of anti-C1q antibodies as a prognostic marker may need to be further evaluated in a larger patient cohort.

The patients in this study represent a consecutive SLE population with proliferative nephritis who underwent renal biopsy, treatment, and repeated biopsy according to a standardized protocol. The objective was to discriminate between individuals with histologically persistent active nephritis in need of prolonged cytotoxic treatment and those in histological remission, in whom treatment may be reduced. As the intention to treat was equal in all patients, all were included and followed despite the fact that not all adhered fully to the intended protocol. Extended studies with larger patient cohorts and longterm followup data are required to further determine the prognostic value of repeated renal biopsies and monitoring of serology and complement components, with special focus on the predictive value of serum complement C1q.

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