Title Serum levels and renal deposition of C1q complement component and its antibodies reflect disease activity of lupus nephritis

Running Title C1q and its autoantibodies in lupus nephritis

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Abstract

**Background** Lupus nephritis is considered to be a principal cause of morbidity and mortality in SLE. Few studies focus on the association between anti-C1q antibodies in circulation and renal C1q deposition in human lupus nephritis. In this study, we detected the serum levels of C1q, presence of anti-C1q antibodies in circulation, renal C1q deposition and further analyzed their associations with clinical and pathological activity in a large cohort of Chinese lupus nephritis patients.

**Methods** Sera and renal biopsies from 218 consecutive patients with lupus nephritis with long-term follow up data were studied. Sera were tested for levels of C1q and anti-C1q autoantibodies. Associations of levels of C1q, anti-C1q autoantibodies with renal deposition of C1q, clinical and histopathological data and renal outcome were further investigated.

**Results** The levels of serum C1q were significantly lower in lupus nephritis than that in normal controls [33.81±20.36 v.s. 61.97 ± 10.50 µg/ml \((P<0.001)\)]. The prevalence of anti-C1q antibodies, ratios of glomerular and vascular deposition of C1q in patients with lupus nephritis were 42.7% (93/218), 71.6% (156/218) and 86.2% (188/218), respectively. The serum C1q levels and anti-C1q antibodies were associated with SLEDAI scores \((P<0.001, P=0.012, \text{respectively})\), renal total activity indices scores \((P<0.001, P<0.001, \text{respectively})\). Granular positive staining of C1q and IgG by immunofluorescence was co-localized almost completely along the glomerular capillary wall and mesangial areas. Patients with anti-C1q antibodies presented with significantly lower serum C1q levels than those without it \((23.82 [0.60, 69.62] \mu g/ml \text{v.s.} 37.36 [0.64, 82.83] \mu g/ml, P<0.001)\). The presence of anti-C1q antibodies was associated with the presence of glomerular C1q deposition \((P<0.001)\), but not with the presence of renal vascular C1q deposition \((P=0.203)\).

**Conclusion** Anti-C1q autoantibodies were closely associated with serum levels of C1q and glomerular deposition of C1q. Kidney is at least one of the target organs of anti-C1q autoantibodies.

**Keywords** lupus nephritis; anti-C1q autoantibodies; serum levels of C1q, C1q deposition
Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease and is characterized by the production of various autoantibodies. Lupus nephritis is considered to be a principal cause of morbidity and mortality among the involvement of major organs [1].

C1q is the first component of the classical pathway, which is involved in clearance of immune complexes and apoptotic cells. Interestingly, recent studies showed that C1q could also exert other immuno-regulatory properties, including limiting the differentiation of monocytes into dendritic cells [2] and immune complex–induced interferon-α production in plasmacytoid dendritic cells [3], and participating in neutrophil extracellular trap (NET) degradation [4], etc. It is composed of a collagenous portion and globular heads. Hereditary deficiency of C1q is known to be a risk factor for the development of SLE [5]. Patients with active SLE have lower levels of C1q compared with non-active SLE patients. Furthermore, patients with lupus nephritis showed lower levels of C1q compared with patients with SLE without nephritis, and the presence of the C1q component in isolated SLE immune complex correlated with the presence of renal disease [6].

Anti-C1q antibodies were prevalent in patients with active lupus nephritis [7] and were thought to be closely associated with renal involvement and predictive for a flare of nephritis [8]. The pathogenesis of anti-C1q antibodies involved in lupus nephritis remained unclear.

Animal studies indicated that immune deposition of C1q and anti-C1q antibodies in the kidney was dependent on the presence of glomerular IgG [9-11]. A further study indicated that anti-C1q autoantibodies deposited in glomeruli but were only pathogenic in combination with glomerular C1q-containing immune complexes [12]. However, the composition of the glomerular and extraglomerular immune complex deposits seems to differ from each other, which indicate that there might be different mechanism in the glomerular and extraglomerular lesions.

There were few studies which focus on the association between anti-C1q antibodies in circulation and renal C1q deposition in human lupus nephritis. In this work, we detected the serum levels of C1q, presence of anti-C1q antibodies in circulation, renal C1q deposition and further analyzed their associations with clinical and pathological activity in a large cohort of Chinese patients with lupus nephritis.

Methods

Patients

Sera and renal biopsies from 218 consecutive patients with renal biopsy-proven lupus nephritis, diagnosed from 2000 to 2008 in Peking University First Hospital, were collected on the day of renal
biopsy. All the patients fulfilled the 1997 American College of Rheumatology revised criteria for SLE [13].

Clinical evaluation
The clinical data including gender, fever, malar rash, photosensitivity, oral ulcer, alopecia, arthritis, serositis, neurologic disorder, anemia, leukocytopenia, thrombocytopenia, hematuria, and leukocyturia were collected and analyzed. The clinical disease activity was measured by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [14].

The response to therapy, includes complete remission, partial remission, relapse, and treatment failure were the same as in our previous works [15].

All the patients were followed up in our outpatient clinic specified for lupus nephritis. The primary end point was defined as death and secondary end points were defined as end stage renal disease (ESRD) or doubling of levels of serum creatinine.

Laboratory assessment
Serum antinuclear antibodies (ANA) were detected using indirect immunofluorescence assay (EUROIMMUN, Lübeck, Germany) and anti-double-stranded DNA antibodies were detected using Crithidia luciliae indirect immunofluorescence test (EUROIMMUN, Lübeck, Germany). Anti-extractable nuclear antigen (ENA) antibodies, including anti-Sm, anti-SSA, anti-SSB and anti-RNP antibodies, were detected using immunodotting assay (EUROIMMUN, Lübeck, Germany). Serum C3 was determined using rate nephelometry assay (Beckman-Coulter, IMMAGE, USA, normal range>0.85g/L).

Renal histopathology
All the renal biopsy specimens were examined by light microscopy, direct immunofluorescence and electron microscopy techniques. Lupus nephritis was re-classified according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 classification system [16].

Light microscopy examination
Renal biopsy specimens were fixed in 4.5% buffered formaldehyde. Consecutive serial of 3 μm sections were used for histological staining. Stains employed included haematoxylin and eosin (H&E), periodic acid-Schiff, silver methenamine (Meth) and Masson’s trichrome. Pathological parameters including activity indices (AI) and chronicity indices (CI) were assessed by renal pathologists using a previously reported system involving semi-quantitative scoring of specific biopsy features with mild modification [17]. The AI consists of endocapillary hypercellularity, cellular crescents, karyorrhexis/fibrinoid necrosis, subendothelial hyaline deposits, interstitial inflammation, and leukocyte infiltration, and CI includes glomerular sclerosis, fibrous crescents, tubular atrophy, and interstitial fibrosis.
Direct immunofluorescence examination

Fresh frozen renal specimens were stained immediately after the renal biopsy with fluorescein isothiocyanate-labelled rabbit anti-human immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM), C3c, C1q and fibrin antibodies (DAKO A/S, Copenhagen, Denmark). Results were graded from 0 to 4 according to the intensity of fluorescence.

To study immune complex deposited in renal vessels, immunofluorescence staining was performed using rabbit anti-human IgG, IgA, IgM, C3c, C1q, fibrin (DAKO) and C4d (Abcam) on formalin-fixed paraffin-embedded tissue (4-um thick). In brief, optimal antibody dilutions were pre-determined, respectively. Sections were deparaffinized and rehydrated through a series of xylene and graded alcohols. The sections were then treated with 0.4% pepsin (Zhongshan Golden Bridge Biotechnology, Beijing, China) for 40 min. Digestion was terminated by repeated washings in 0.01 mol/L phosphate buffered saline (PBS), pH 7.4. Sections were immersed into freshly prepared 3% hydrogen peroxide in methanol solution for 10 min at room temperature to quench endogenous peroxidase activity. To block non-specific staining, sections were incubated with 3% BSA in PBS at room temperature for 30 min. The primary antibodies against IgG (dilution 1:5000 in PBS), IgA (dilution 1:1000), IgM (dilution 1:400), C3c (dilution 1:300), C4d (dilution 1:400), C1q (dilution 1:50) and fibrin (dilution 1:1000) were added on each section directly. Antibodies were incubated overnight at 4°C. FITC-labeled goat anti-rabbit IgG (Zhongshan Golden Bridge Biotechnology, Beijing, China, and dilution 1:60) were used as secondary antibodies at 37°C for 30 min respectively. Sections were washed with PBS (pH 7.4) between each step (5 min, three times). Finally, Sections were stored shortly at 4°C before being examined using an immunofluorescence microscopy (Nikon Eclipse 80i, Japan). Sections of renal tissue from known patients with lupus nephritis were used as positive controls. Negative controls were performed by omitting or replacing the primary antibodies.

Detection of Co-localization of C1q and IgG on Glomeruli by Immunofluorescence

Sections were cut into a 5 µm slides from frozen renal biopsy tissues, then were air-dried for 25 min at room temperature and fixed in -20°C pre-cooled acetone for 10 min at 4°C. After washed three times with phosphate buffered saline (PBS), sections were incubated in 3% BSA for 30 min at room temperature. Tetramethylrhodamine isothiocyanate (TRITC) -labeled rabbit anti-human IgG (diluted 1:40, Dako, Copenhagen, Denmark) were incubated 30 min at 37°C. After washed with PBS 3 times for 3 min, mouse anti-human C1q (diluted 1:400, Abcam, Cambridge, UK) was added as primary antibody for 30 min at 37°C. After another washing with PBS 3 times for 3 min, FITC-labeled goat anti-mouse IgG (diluted 1:30, Zhongshan Golden Bridge Biotechnology, Beijing, China) was used as secondary antibody for 30 min at 37°C. After washed with PBS 3 times for 3 min, the sections were air-dried at dark and mounted with citifluor. Negative controls were performed by
omitting or replacing the primary antibodies. Sections were stored shortly at 4°C before being scored using a confocal microscope (Olympus viewer 1000, Japan).

**Serum samples**

Sera from patients with lupus nephritis were obtained from peripheral blood at the time of renal biopsy. Sera from 22 healthy controls with matched gender and age were collected as normal controls. All of the sera were stored at -70°C until use.

Written informed consent was obtained for blood sampling and renal biopsy from each patient. The research was in compliance with the Declaration of Helsinki and approved by the ethics committee of Peking University First Hospital.

**Detection of serum C1q levels using enzyme-linked immunosorbent assay (ELISA)**

The method was modified as previously described [18]. Anti-human C1q polyclonal antibody from rabbit (Dako, Denmark) was diluted to 1:5000 (1.26 µg/ml) in 0.05 M bicarbonate buffer (pH 9.6) and coated onto the wells of a polystyrene microtitre plate (Nunc, Denmark). All the plates were coated with 50 µl of this solution and incubated overnight at 4°C, then washed three times with 0.01 M PBST and were blocked with 200µl PBST containing 1% BSA, 37°C for 1 h. The following incubations were carried out at 37°C for 1 h, volume for each well 50µl, and all the plates were washed three times with PBST. Standards and diluted serum sample (1/2,000 in PBST) were added to their respective wells. All the samples were tested in duplicate wells. After that, horseradish peroxidase (HRP)-conjugated goat anti-human IgG monoclonal antibody (Abcam) was diluted in diluent to 1:500. The reaction was developed with 3,3’,5,5’-Tetramethylbenzidine (TMB) liquid substrate system and was stopped with 1 M H₂SO₄. The results were recorded as the net optical absorbance at 450 nm and 570nm in an ELISA reader (Bio-Rad 550, Japan). The cutoff value of low levels of C1q was set as the mean - 2SD of healthy blood donors.

**Detection of anti-C1q IgG autoantibodies with ELISA**

Anti-C1q IgG autoantibodies were detected by ELISA established in our previous study [7]. In brief, purified normal human C1q (Sigma, USA) was diluted at 5 µg/ml in 0.05 M bicarbonate buffer (pH 9.6) and coated onto the wells of one-half of a polystyrene microtitre plate (Costar, USA). The wells in the other half were coated with the same bicarbonate buffer alone as antigen-free wells to exclude non-specific binding. The volumes filled each well for this step and for subsequent steps were 100 µl. All the incubations were carried out at 37°C for 1 h and all the plates were washed three times with 0.01 M PBS containing 0.1% Tween 20 (PBST). Then the wells were blocked with PBST containing 0.01% gelatin. The sera diluted to 1:200 with PBST containing 1% BSA and 0.5 M NaCl, and were added in duplicate to both antigen-coated and antigen-free wells. There were a blank
control, negative control and a known positive control in each plate. After incubation and washing, the wells were then incubated with 1:5000 diluted horseradish peroxidase (HRP)-conjugated goat anti-human IgG (Zhongshan Biotech, China). The reaction was developed with a 0.1 M citrate phosphate buffer (pH 5.0) containing 0.04% O-phenylenediamine (OPD) and 0.1% H$_2$O$_2$, then the reaction was stopped with 1 M H$_2$SO$_4$. The results were recorded as the net optical absorbance (average value of antigen wells minus average value of antigen-free wells) at 490 nm in an ELISA reader (Bio-Rad 550, Japan) and expressed as percentage of the known positive sample. The cutoff value was set as the mean + 2SD of healthy blood donors.

Statistical analysis

Statistical software SPSS 13.0 (SPSS, Chicago, IL, USA) was employed for statistical analysis. Quantitative data were expressed as mean ± SD., median with range (minimum, maximum), or number (%). For comparison of clinical and pathological features of patients, Mann-Whitney Test, X$^2$ test and Spearman’s correlation were used. Survival analysis was performed using the log-rank test. Statistical significance was considered as $P$ <0.05.

Results

General data of patients with lupus nephritis

Clinical and renal histopathological data of 218 lupus nephritis patients at renal biopsy were listed in Table 1.

Levels of serum C1q in patients with lupus nephritis and their associations with clinical and pathological features

The purified human C1q was used to establish standard curve. C1q could be detected by the sandwich ELISA at a range of 0 µg/ml to 125 µg/ml. The average levels of serum C1q were 33.81±20.36 (0.60, 82.83) µg/ml in lupus nephritis group, which were significantly lower than that in normal controls (61.97 ± 10.50 [46.05-86.34] µg/ml, $P$<0.001). The cutoff value for low levels of C1q was 40.97 µg/ml. (Figure 1)

Levels of serum C1q were correlated with age (r=0.161, $P$=0.018). There was no significant difference of serum C1q levels between female and male in lupus nephritis ($P$=0.291).

As to clinical data, patients with lower serum C1q levels (<40.97µg/ml) showed significantly higher levels of SLEDAI ($P$<0.001), significantly lower levels of C3 ($P$<0.001), significantly high incidence of anti-ds-DNA antibody ($P$=0.003), hematuria ($P$=0.021), anemia ($P$=0.004) (Table 2)

As to clinical data, the serum C1q levels were significantly lower in patients presenting with the following clinical manifestations, including hematuria ($P$=0.033), neurologic disorder ($P$=0.028), leukocytopenia ($P$<0.001), anemia ($P$<0.001), thrombocytopenia ($P$=0.017), hypocomplementemia
Serum C1q levels were significantly lower in patients presenting with the following positive parameters, including anti ds-DNA antibody ($P<0.001$), anti SSA antibody ($P=0.040$), compared with those without these parameters in lupus nephritis.

There was a significant inverse association between SLEDAI scores and serum C1q levels in patients with lupus nephritis ($r=-0.242$, $P<0.001$). There was significant association between serum C1q levels and serum C3 levels ($r=0.546$, $P<0.001$) in patients with lupus nephritis.

The levels of serum C1q in different pathological classes of lupus nephritis were 42.39 (9.61, 78.26) µg/ml in class II, 36.00 (0.92, 82.83) µg/ml in class III, 27.21 (0.60, 77.07) µg/ml in class IV, 51.81(1.19, 81.98) µg/ml in class V, 37.92µg/ml in class VI, respectively. There were significant differences of serum C1q levels among various pathological classes ($P<0.001$). The level in class IV was the lowest among the four classes (IV v.s. II, $P=0.046$; IV v.s. III, $P=0.005$; IV v.s. V, $P<0.001$; respectively).

The serum C1q levels were negatively associated with renal activity indices score ($r=-0.347$, $P<0.001$), endocapillary hypercellularity ($r=-0.343$, $P<0.001$), fibrinoid necrosis ($r=-0.367$, $P<0.001$), subendothelial hyaline deposits ($r=0.407$, $P<0.001$), glomerular leukocyte infiltration ($r=0.215$, $P=0.001$), positively associated with glomerular sclerosis ($r=0.140$, $P=0.039$). The lower levels of serum C1q (<40.97µg/ml) were negatively associated with renal activity indices score ($r=-0.327$, $P<0.001$), endocapillary hypercellularity ($r=-0.337$, $P<0.001$), cellular crescents ($r=-0.182$, $P=0.007$), fibrinoid necrosis ($r=-0.310$, $P<0.001$), subendothelial hyaline deposits ($r=-0.312$, $P<0.001$), glomerular leukocyte infiltration ($r=-0.246$, $P<0.001$), positively associated with glomerular sclerosis ($r=0.146$, $P=0.031$). No association was found in other indices. (Table 3)

Prevalence of serum anti-C1q IgG auto-antibodies in patients with lupus nephritis and their associations with clinical and pathological characteristics

The cutoff value for detection of anti-C1q IgG auto-antibodies was 29%. The prevalence of anti-C1q antibodies in patients with lupus nephritis was 42.7% (93/218), which was significantly higher than that in normal control (0/22). Patients with positive anti-C1q antibodies showed significantly higher SLEDAI (18.72 ± 5.70 v.s. 16.74±5.69, $P=0.012$), lower levels of C3 (0.39 ± 0.19 g/l v.s. 0.50±0.25 g/l, $P<0.001$) and hemoglobin (96.30 ± 25.79 g/l v.s. 104.36±26.36 g/l, $P=0.025$), compared with those without it. (Table 2)

Anti-C1q antibodies were also associated with arthralgia (64.5% [60/93] v.s. 44.0% [55/125], $P=0.003$), anemia (78.5% [73/93] v.s. 59.2% [74/125], $P=0.003$) and hematuria (84.9% [79/93] v.s.71.2% [89/125], $P=0.017$). (Table 2)

The prevalence of anti-C1q antibodies in patients with type II, III, IV, V, VI were 25.0% (2/8),
33.3% (13/39), 55.1% (70/127), 16.3% (7/43), 100% (1/1), respectively. The prevalence of anti-C1q antibodies in patients with diffuse proliferative renal lesions (class IV) was significantly higher than that in patients with non-diffuse proliferative renal lesions (class II, III, V) (55.1% [70/127] v.s. 36.7% [33/90]) (P<0.001).

Anti-C1q antibodies were associated with most of histopathological parameters of activity indices including activity indices score (9.02±4.34 v.s. 6.67±4.52; P<0.001), endocapillary hypercellularity (P<0.001), cellular crescents (P=0.013), karyorrhexis/fibrinoid necrosis (P=0.009), subendothelial hyaline deposits (P=0.012) and leukocyte infiltration (P=0.019). (Table 3)

Co-localization of C1q and IgG deposited in glomeruli by Laser Scanning Confocal Microscopy

The prevalence of glomerular and vascular deposition of C1q in patients with lupus nephritis were 71.6% (156/218) and 86.2% (188/218), respectively. Renal biopsies from four patients with lupus nephritis with positive anti-C1q autoantibodies were stained for C1q and IgG. Granular positive staining of C1q and IgG by immunofluorescence was shown along the glomerular capillary wall and mesangial area and was co-localized almost completely in the same areas. (Figure 21)

Correlations among levels of serum C1q, autoantibodies against C1q, glomerular and vascular depositions of C1q in lupus nephritis

Patients with anti-C1q antibodies positive presented with significantly lower serum C1q levels than those without it (23.82 [0.60, 69.62] µg/ml v.s. 37.36 [0.64, 82.83] µg/ml., P<0.001) (Figure 2). However, the serum C1q levels were not associated with the intensity of glomerular or vascular C1q depositions in kidneys (P=0.469, P=0.198, respectively).

The presence of anti-C1q antibodies were positively associated with the intensity presence of glomerular deposition (87.1% patients with anti-C1q antibodies positive [81 patients with C1q deposition/93 patients with antibodies positive] v.s. 60.0% patients without anti-C1q antibodies [75 patients with C1q deposition /125 patients with antibodies negative], P<0.001), but not with the intensity presence of vascular C1q deposition (82.8% patients with anti-C1q antibodies [77 patients with C1q deposition /93 patients with antibodies positive] v.s. 88.8% patients without anti-C1q antibodies [111 patients with C1q deposition /125 patients with antibodies negative], P=0.203).

The intensity of glomerular deposition was not associated with the the intensity of vascular deposition (r=-0.081, P=0.239).

Associations between levels of serum C1q, autoantibodies against C1q, glomerular, vascular deposition of C1q and renal outcomes

Levels of serum C1q, autoantibodies against C1q, glomerular or vascular deposition of C1q was not the risk factors for long-term renal outcomes in lupus nephritis with the log-rank test for univariate survival analysis of renal prognosis (P=0.200, P=0.301, P=0.548, P=0.697, respectively).
Patients were divided into three groups according to C1q level (patients with "normal C1q" (> 40 µg/ml), "low levels of C1q" (20-40 µg/ml) and "very low levels of C1q" (<20 µg/ml)). No significant difference between the three groups was found regarding long-term renal survival and relapse-free renal survival ($P=0.237$, $P=0.185$, respectively) (Figure 3).

**Discussion**

Kidney is one of the most common involved organs in SLE. C1q and its autoantibodies have been considered to be associated closely with SLE and lupus nephritis. Controversies still existed in the organ specification of anti-C1q autoantibodies. Recently, Yasuhiro et al indicated that anti-C1q antibodies were associated with SLE global activity but not specifically with active lupus nephritis [19]. With a large lupus nephritis cohort in our referral center, we firstly demonstrated the associations among levels of C1q, presence of anti-C1q antibodies, renal C1q deposition and their correlations with clinical and pathological disease activity of lupus nephritis, which might shed a light on the roles of C1q and it autoantibodies in lupus nephritis.

Previous studies showed that there was negative correlation between anti-C1q autoantibodies and serum levels of C1q in patients with lupus nephritis. Levels of C1q were significantly lower in patients with lupus nephritis and in active patients [20]. Animal studies indicated that the severity of lupus nephritis seemed to correlate with the amount of C1q present in the glomeruli [12]. And some studies found that anti-C1q antibody was positively associated with glomerular C1q deposition [21].

In our study, we confirmed that the presence of anti-C1q autoantibodies was associated with the intensity of glomerular deposition of C1q, both of which were associated with disease activity of SLE and lupus nephritis. And the intensity of glomerular deposition of C1q was also associated with IgG and was co-localized with IgG in the glomeruli. The co-localization indicated that C1q could deposit on the kidney with immunoglobins, including anti-C1q autoantibodies as Mannik et al found in previous studies [22]. It strengthened the findings by Daha et al that anti-C1q autoantibodies may induce renal disease by deposition in glomeruli only together with C1q and immune complex [12]. Thus, kidneys are at least one of the target organs of anti-C1q autoantibodies.

Though the intensity of vascular deposition of C1q was associated with vascular deposition of IgG and IgM, presence of anti-C1q autoantibodies was not associated with the intensity of vascular deposition of C1q. As recent study indicated that different immune complex might deposit in glomerular and extraglomerular regions, the pathogenesis of vascular lesions still needs further investigation [12].

Serum levels of C1q were not associated with glomerular deposition of C1q or vascular deposition of C1q. It indicates that serum C1q may contribute little to the deposition of C1q in the
kidneys, while local production of C1q by endothelial cells [23, 24], dendritic cells, macrophages in 
the kidneys might be the main source of the local C1q deposition [25].

Animal studies suggested that immune deposition of C1q and anti-C1q autoantibodies in the 
kidney was dependent on the presence of glomerular IgG deposition and the further study indicated 
that anti-C1q autoantibodies deposit in glomeruli but were only pathogenic in combination with 
glomerular C1q-containing immune complexes, and the certain amount deposition of C1q in the 
glomeruli, as may in the form of glomerular C1q-containing immune complexes, will induce overt 
renal disease [12]. Our study supported the theory that auto-antibodies of C1q were co-localized with 
C1q and other immunoglobulins in the glomeruli and might induce the glomerular injury in patients of 
lupus nephritis. The severity was positively associated with the intensity of C1q deposition in the 
glomeruli. However, the constitution of the immune complex was still in discussion. Previous 
studies indicated that anti-dsDNA antibody was the best serological biomarker for lupus nephritis. 
Lately, Krishnan et al reported that those cross-reacting with glomerular basement membrane 
components produced immune deposits, [26] which suggested that anti-dsDNA antibodies might be 
the component of immune complex. Interestingly, recent studies indicated that anti-C-reactive 
protein (CRP) autoantibodies and anti-PTX3 autoantibodies were also correlated with 
histopathological activity and disease activity in lupus nephritis [27, 28]. As CRP and PTX3 could 
bind to C1q as reported [29], and local production of CRP and PTX3 in the kidneys were also 
detected in some studies [30, 31]. Thus, the correlations between C1q, CRP, PTX3 and its 
auto-antibodies in the formation of immune complex in lupus nephritis need further investigation.

Conclusions

Anti-C1q autoantibodies were closely associated with serum levels of C1q and glomerular 
deposition of C1q. Kidney is at least one of the target organs of anti-C1q autoantibodies.

Competing interests

All the authors declare no conflicts of interest.

Authors’ contributions

Dr. Ying Tan carried out the immunoassays, participated in the design of the study, performed 
the statistical analysis and drafted the manuscript. Di Song carried out the immunoassays and drafted 
the manuscript. Dr. Li-hua Wu carried out the renal histology studies. Prof. Feng Yu, Ming-hui Zhao 
participated in the design of the study and revised the manuscript. All authors read and approved the 
final manuscript.
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Table 1. General clinical and renal histopathological profiles of patients with lupus nephritis at renal biopsy

<table>
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<tr>
<th></th>
<th>Number of patients</th>
<th>C3 (mean ± SD) (g/l)</th>
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<tr>
<td>Number of patients</td>
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<td></td>
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<tr>
<td>Gender (male/female)</td>
<td>33/185</td>
<td>Anti-nuclear antibody (ANA) (+) No.(%)</td>
<td>215 (98.6)</td>
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<td>Age (mean ± SD) (years)</td>
<td>32.5 ± 11.3</td>
<td>Anti-double stranded DNA antibody (ds-DNA) (+) No.(%)</td>
<td>153 (70.6)</td>
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<td>Follow-up time (median, range) (months)</td>
<td>41 (1,360)</td>
<td>Anti-SSA antibody (+) No.(%)</td>
<td>102 (46.8)</td>
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<td>Fever (non-infectious) No.(%)</td>
<td>69 (31.7)</td>
<td>Anti-SSB antibody (+) No.(%)</td>
<td>25 (11.5)</td>
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<td>Malar rash No.(%)</td>
<td>118 (54.1)</td>
<td>Anti-Smith antibody (Sm) (+) No.(%)</td>
<td>53 (24.3)</td>
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<td>Photosensitivity No.(%)</td>
<td>47 (21.6)</td>
<td>Anti-ribonucleoprotein (RNP) antibody (+) No.(%)</td>
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<td>Oral ulcer No.(%)</td>
<td>64 (29.4)</td>
<td>Anti-cardiolipin antibody (+) No.(%)</td>
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<td>Alopecia No.(%)</td>
<td>68 (31.2)</td>
<td>SLEDAI (mean ± s.d.)</td>
<td>17.6 ± 5.8</td>
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<td>Arthralgia No.(%)</td>
<td>115 (52.8)</td>
<td>Activity indices (AI) score (median, range)</td>
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<td>Serositis No.(%)</td>
<td>34 (15.6)</td>
<td>Endocapillary hypercellularity (median, range)</td>
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<td>Neurologic disorder No.(%)</td>
<td>14 (6.4)</td>
<td>Cellular crescents (median, range)</td>
<td>0 (0.6)</td>
</tr>
<tr>
<td>Anemia No.(%)</td>
<td>147 (67.4)</td>
<td>Karyorrhexis/fibrinoid necrosis (median, range)</td>
<td>0 (0.6)</td>
</tr>
<tr>
<td>Leukocytopenia No.(%)</td>
<td>102 (46.8)</td>
<td>Subendothelial hyaline deposits (median, range)</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Thrombocytopenia No.(%)</td>
<td>64 (29.4)</td>
<td>Interstitial inflammatory cell infiltration (median, range)</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Hematuria No.(%)</td>
<td>168 (77.1)</td>
<td>Glomerular leukocyte infiltration (median, range)</td>
<td>1 (0.12)</td>
</tr>
<tr>
<td>Leukocyturia (non-infection) No.(%)</td>
<td>118 (54.1)</td>
<td>Chronicity indices (CI) score (median, range)</td>
<td>2 (0.10)</td>
</tr>
<tr>
<td>Acute renal failure No.(%)</td>
<td>39 (17.9)</td>
<td>Glomerular sclerosis (median, range)</td>
<td>0 (0.3)</td>
</tr>
<tr>
<td>Hemoglobin (mean ± SD) (g/l)</td>
<td>100.92 ± 26.36</td>
<td>Fibrous crescents (median, range)</td>
<td>0 (0.3)</td>
</tr>
<tr>
<td>Urine protein (median, range) (g/24 hours)</td>
<td>4.36 (0-17)</td>
<td>Tubular atrophy (median, range)</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Serum creatinine (median, range) (µmol/l)</td>
<td>82.0 (37,100)</td>
<td>Interstitial fibrosis (median, range)</td>
<td>1 (0.3)</td>
</tr>
</tbody>
</table>

SSA/SSB, Sjögren’s syndrome A/Bantigen; SSB, Sjögren’s syndrome B antigen; SLEDAI, SLE Disease Activity Index.
Table 2. Associations between presence of anti-C1q autoantibodies, levels of C1q and clinical parameters of patients with lupus nephritis

<table>
<thead>
<tr>
<th></th>
<th>Patients with anti-C1q autoantibodies</th>
<th>Patients without anti-C1q autoantibodies</th>
<th>P value</th>
<th>Patients with levels of C1q above 40.97µg/ml</th>
<th>Patients with levels of C1q under 40.97µg/ml</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients (%)</td>
<td>93/218 (42.7%)</td>
<td>125/218 (57.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>14/79</td>
<td>19/106</td>
<td>NS</td>
<td>12/63</td>
<td>21/122</td>
<td>NS</td>
</tr>
<tr>
<td>Age (mean ± SD) (years)</td>
<td>31.27 ± 9.96</td>
<td>33.47 ± 11.21</td>
<td></td>
<td>34.15±10.92</td>
<td>31.69±11.40</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Clinical and laboratory data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever (non-infectious) No.(%)</td>
<td>31 (33.3%)</td>
<td>38 (30.4%)</td>
<td>NS</td>
<td>19 (25.3%)</td>
<td>50 (35.0%)</td>
<td>NS</td>
</tr>
<tr>
<td>Malar rash No.(%)</td>
<td>51 (54.8%)</td>
<td>67 (53.6%)</td>
<td>NS</td>
<td>42 (56.0%)</td>
<td>76 (53.1%)</td>
<td>NS</td>
</tr>
<tr>
<td>Photosensitivity No.(%)</td>
<td>20 (21.5%)</td>
<td>27 (21.6%)</td>
<td>NS</td>
<td>16 (21.3%)</td>
<td>31 (21.7%)</td>
<td>NS</td>
</tr>
<tr>
<td>Oral ulcer No.(%)</td>
<td>28 (30.1%)</td>
<td>38 (30.4%)</td>
<td>NS</td>
<td>20 (26.7%)</td>
<td>44 (30.8%)</td>
<td>NS</td>
</tr>
<tr>
<td>Alopecia No.(%)</td>
<td>30 (32.3%)</td>
<td>38 (30.4%)</td>
<td>NS</td>
<td>26 (34.7%)</td>
<td>42 (29.4%)</td>
<td>NS</td>
</tr>
<tr>
<td>Arthralgia No.(%)</td>
<td>60 (64.5%)</td>
<td>55 (44.0%)</td>
<td>0.003</td>
<td>36 (45.6%)</td>
<td>79 (55.2%)</td>
<td>NS</td>
</tr>
<tr>
<td>Serositis No.(%)</td>
<td>13 (14.0%)</td>
<td>21 (16.8%)</td>
<td>NS</td>
<td>10 (13.3%)</td>
<td>24 (16.8%)</td>
<td>NS</td>
</tr>
<tr>
<td>Neurologic disorder No.(%)</td>
<td>7 (7.5%)</td>
<td>7 (5.6%)</td>
<td>NS</td>
<td>1 (1.3%)</td>
<td>13 (9.1%)</td>
<td>NS</td>
</tr>
<tr>
<td>Anemia No.(%)</td>
<td>73 (78.5%)</td>
<td>74 (59.2%)</td>
<td>0.003</td>
<td>41 (54.7%)</td>
<td>106 (74.1%)</td>
<td>0.004</td>
</tr>
<tr>
<td>Leukocytopenia No.(%)</td>
<td>49 (52.7%)</td>
<td>53 (42.4%)</td>
<td>NS</td>
<td>23 (30.7%)</td>
<td>79 (55.2%)</td>
<td>NS</td>
</tr>
<tr>
<td>Thrombocytopenia No.(%)</td>
<td>26 (28.0%)</td>
<td>38 (30.4%)</td>
<td>NS</td>
<td>19 (25.3%)</td>
<td>45 (31.5%)</td>
<td>NS</td>
</tr>
<tr>
<td>Hematuria No.(%)</td>
<td>79 (36.2%)</td>
<td>89 (40.8%)</td>
<td>0.017</td>
<td>51 (68.0%)</td>
<td>117 (81.8%)</td>
<td>0.021</td>
</tr>
<tr>
<td>Leukocyturia No.(%)</td>
<td>52 (25.2%)</td>
<td>63 (28.9%)</td>
<td>NS</td>
<td>34 (45.3%)</td>
<td>84 (58.7%)</td>
<td>NS</td>
</tr>
<tr>
<td>Acute kidney injury No.(%)</td>
<td>18 (8.3%)</td>
<td>21 (9.6%)</td>
<td>NS</td>
<td>13 (17.3%)</td>
<td>26 (18.2%)</td>
<td>NS</td>
</tr>
<tr>
<td>Hemoglobin (mean ± SD) (g/l)</td>
<td>96.30±25.78</td>
<td>104.36±26.36</td>
<td>0.025</td>
<td>102.89±31.52</td>
<td>99.89±23.77</td>
<td>NS</td>
</tr>
<tr>
<td>Urine protein (mean ± SD) (g/24 hours)</td>
<td>4.42±3.05</td>
<td>5.40±3.39</td>
<td>NS</td>
<td>5.64±3.70</td>
<td>4.64±3.00</td>
<td>0.045</td>
</tr>
<tr>
<td>C3 (mean ± SD) (g/l)</td>
<td>0.39±0.19</td>
<td>0.50±0.25</td>
<td>&lt;0.001</td>
<td>0.60±0.25</td>
<td>0.37±0.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anti-nuclear antibody (ANA)</td>
<td>92 (98.9%)</td>
<td>123 (98.4%)</td>
<td>NS</td>
<td>73 (97.3%)</td>
<td>142 (99.3%)</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-double stranded DNA antibody (ds-DNA) (+) No.(%)</td>
<td>71 (76.3%)</td>
<td>82 (65.6%)</td>
<td>NS</td>
<td>43 (57.3%)</td>
<td>110 (76.9%)</td>
<td>0.003</td>
</tr>
<tr>
<td>Anti-SSA antibody (+) No.(%)</td>
<td>45 (48.4%)</td>
<td>57 (45.6%)</td>
<td>NS</td>
<td>43 (57.3%)</td>
<td>59 (41.3%)</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-SSB antibody (+) No.(%)</td>
<td>8 (8.6%)</td>
<td>17 (13.6%)</td>
<td>NS</td>
<td>9 (12.0%)</td>
<td>16 (11.2%)</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-Smith antibody(Sm) (+)</td>
<td>26 (28.0%)</td>
<td>27 (21.6%)</td>
<td>NS</td>
<td>21 (28.0%)</td>
<td>32 (22.4%)</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-ribonucleoprotein (RNP) antibody (+) No.(%)</td>
<td>34 (36.7%)</td>
<td>32 (25.6%)</td>
<td>NS</td>
<td>26 (34.7%)</td>
<td>40 (28.0%)</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-cardiolipin antibody (+) No.(%)</td>
<td>10 (10.8%)</td>
<td>6 (4.8%)</td>
<td>NS</td>
<td>2 (2.7%)</td>
<td>14 (9.8%)</td>
<td>NS</td>
</tr>
<tr>
<td>SLEDAI</td>
<td>18.72±5.70</td>
<td>16.74±5.69</td>
<td>0.012</td>
<td>15.47±4.90</td>
<td>18.69±5.89</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

NS, no significance
Table 3. Associations between presence of anti-C1q autoantibodies, levels of C1q and histopathological parameters of patients with lupus nephritis

<table>
<thead>
<tr>
<th>Patients with anti-C1q autoantibodies Median (range)</th>
<th>Patients without anti-C1q autoantibodies Median (range)</th>
<th>r value (P value)</th>
<th>Patients with levels of C1q above 40.97 µg/ml Median (range)</th>
<th>Patients with levels of C1q under 40.97 µg/ml Median (range)</th>
<th>r value (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Histopathological data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity Indices score</td>
<td>9 (0.19)</td>
<td>7 (0.16)</td>
<td>0.238 (&lt;0.001)</td>
<td>4 (0.19)</td>
<td>9 (0.19)</td>
</tr>
<tr>
<td>Endocapillary hypercellularity</td>
<td>3 (0.3)</td>
<td>2 (0.3)</td>
<td>0.249 (&lt;0.001)</td>
<td>3 (0.3)</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Cellular crescents</td>
<td>2 (0.6)</td>
<td>0 (0.6)</td>
<td>0.167 (0.013)</td>
<td>2 (0.6)</td>
<td>0 (0.6)</td>
</tr>
<tr>
<td>Karyorrhexis/fibrinoid necrosis</td>
<td>2 (0.6)</td>
<td>0 (0.4)</td>
<td>0.175 (0.009)</td>
<td>2 (0.6)</td>
<td>0 (0.2)</td>
</tr>
<tr>
<td>Subendothelial hyaline deposits</td>
<td>1 (0.3)</td>
<td>1 (0.3)</td>
<td>0.171 (0.012)</td>
<td>1 (0.3)</td>
<td>0 (0.3)</td>
</tr>
<tr>
<td>Interstitial inflammation</td>
<td>1 (0.3)</td>
<td>1 (0.3)</td>
<td>NS</td>
<td>1 (0.3)</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Leukocyte infiltration</td>
<td>1 (0.3)</td>
<td>1 (0.3)</td>
<td>0.159 (0.019)</td>
<td>1 (0.3)</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Chronicity Indices score</td>
<td>2 (0.9)</td>
<td>2 (0.10)</td>
<td>NS</td>
<td>2 (0.10)</td>
<td>2 (0.9)</td>
</tr>
<tr>
<td>Glomerular sclerosis</td>
<td>0 (0.3)</td>
<td>0 (0.3)</td>
<td>NS</td>
<td>0 (0.3)</td>
<td>0 (0.3)</td>
</tr>
<tr>
<td>Fibrous crescents</td>
<td>0 (0.3)</td>
<td>0 (0.3)</td>
<td>NS</td>
<td>0 (0.3)</td>
<td>0 (0.2)</td>
</tr>
<tr>
<td>Tubular atrophy</td>
<td>1 (0.3)</td>
<td>1 (0.3)</td>
<td>NS</td>
<td>1 (0.3)</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Interstitial fibrosis</td>
<td>1 (0.3)</td>
<td>1 (0.3)</td>
<td>NS</td>
<td>1 (0.3)</td>
<td>1 (0.3)</td>
</tr>
</tbody>
</table>

NS, no significance
Figure legends

Figure 1. Comparison of serum C1q levels in patients with lupus nephritis and normal controls
The horizontal lines indicated the mean value and standard deviation.

Figure 2. Co-localization of C1q and IgG staining in glomeruli in lupus nephritis.
A&D. Granular positive staining of C1q by immunofluorescence along the glomerular capillary wall and mesangial area in a patient with lupus nephritis.
B&E. Granular positive staining of IgG by immunofluorescence along the glomerular capillary wall and mesangial area in the same section in Figure 1A or D.
C&F. C1q and IgG co-localized completely along the glomerular capillary wall and mesangial area, merged in yellow.
(Original magnification×800)

Figure 1. Co-localization of C1q and IgG staining in glomeruli in lupus nephritis.
A&D. Granular positive staining of C1q by immunofluorescence along the glomerular capillary wall and mesangial area in a patient with lupus nephritis.
B&E. Granular positive staining of IgG by immunofluorescence along the glomerular capillary wall and mesangial area in the same section in Figure 1A or D.
C&F. C1q and IgG co-localized completely along the glomerular capillary wall and mesangial area, merged in yellow.
(Original magnification×800)

Figure 2. Comparison of serum C1q levels in patients with lupus nephritis with positive or negative anti-C1q antibodies and normal controls
The horizontal lines indicated the median value and range.

Figure 3. Comparison of renal outcomes and relapse-free renal survival among patients with different levels of C1q
A. Comparison of renal outcomes among patients with different levels of C1q; B. Comparison of relapse-free renal survival among patients with different levels of C1q
Group 1. patients with "normal C1q" (> 40 µg/ml); Group 2. patients with “low C1q” (20-40 µg/ml); Group 3. patients with "very low C1q" (<20 µg/ml).
Figure 1
Levels of C1q (µg/ml)

- Anti-C1q positive
- Anti-C1q negative
- Normal controls

P<0.001
Figure 3

A. 
P = 0.237, HR 1.663 (95% CI: 0.908-3.048)

B. 
P = 0.185, HR 1.312 (95% CI: 0.915-1.881)