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PAPER

Anti-C1q antibodies in systemic lupus erythematosus

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Objective: Anti-C1q has been associated with systemic lupus erythematosus (SLE) and lupus nephritis in previous studies. We studied anti-C1q specificity for SLE (vs rheumatic disease controls) and the association with SLE manifestations in an international multicenter study. **Methods:** Information and blood samples were obtained in a cross-sectional study from patients with SLE ($n = 308$) and other rheumatologic diseases ($n = 389$) from 25 clinical sites (84% female, 68% Caucasian, 17% African descent, 8% Asian, 7% other). IgG anti-C1q against the collagen-like region was measured by ELISA. **Results:** Prevalence of anti-C1q was 28% (86/308) in patients with SLE and 13% (49/389) in controls (OR = 2.7, 95% CI: 1.8–4, $p < 0.001$). Anti-C1q was associated with proteinuria (OR = 3.0, 95% CI: 1.7–5.1, $p < 0.001$), red cell casts (OR = 2.6, 95% CI: 1.2–5.4, $p = 0.015$), anti-dsDNA (OR = 3.4, 95% CI: 1.9–6.1, $p < 0.001$) and anti-Smith (OR = 2.8, 95% CI: 1.5–5.0, $p = 0.01$). Anti-C1q was independently associated with renal involvement after adjustment for demographics, ANA, anti-dsDNA and low complement (OR = 2.3, 95% CI: 1.3–4.2, $p < 0.01$). Simultaneously positive anti-C1q, anti-dsDNA and low complement was strongly associated with renal involvement (OR = 14.9, 95% CI: 5.8–38.4, $p < 0.01$). **Conclusions:** Anti-C1q was more common in

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patients with SLE and those of Asian race/ethnicity. We confirmed a significant association of anti-C1q with renal involvement, independent of demographics and other serologies. Anti-C1q in combination with anti-dsDNA and low complement was the strongest serological association with renal involvement. These data support the usefulness of anti-C1q in SLE, especially in lupus nephritis. *Lupus* (2014) **0,** 1–8.

Key words: Anti-dsDNA antibodies; renal lupus; systemic lupus erythematosus

Introduction

Complement plays a major role in the pathogenesis of systemic lupus erythematosus (SLE) and lupus nephritis. Genetic deficiencies in the early complement components are associated with SLE.[1,2] The strongest association is seen in patients with homozygous C1q deficiency, of whom 88% developed SLE and 30% glomerulonephritis, respectively.[3] In vitro, physiologic concentrations of C1q inhibit interferon alpha production by plasmacytoid dendritic cells stimulated with nucleic acid-containing immune complexes,[4] suggesting a regulatory effect of C1q in response to and clearance of immune complexes. In patients with SLE, levels of C1q were reduced in glomerulonephritis flares.[5] In patients with lupus nephritis, the presence of anti-C1q at the time of renal biopsy was associated with worse renal outcome, by the American College of Rheumatology (ACR) renal response criteria,[6] and with renal tubulointerstitial changes.[7] Acquired antibodies against the collagen-like region of C1q (anti-C1qCLR) were present in the glomerular basement membrane of patients with proliferative lupus nephritis at concentrations more than 50-fold higher per unit immunoglobulin (Ig)G than in the patients' serum, suggesting a role in the pathogenesis of lupus nephritis.[8] C1q were aggregated within IgG in renal subendothelial deposits in active proliferative lupus nephritis as seen on immunogold electron microscopy, further supporting a pathogenic role of anti-C1q.[9] Patients with active lupus nephritis had a higher prevalence of anti-C1q than those without lupus nephritis, 74% vs 32% ($p < 0.0001$).[10] Anti-C1q increased within six months prior to renal involvement in 50% of patients with SLE[11] and was associated with the proliferative form of glomerulonephritis.[12–14] In another study, an increase in anti-C1q level preceded renal flare by 2.3 months and was more specific for renal flare than increases in anti-double-stranded DNA (anti-dsDNA) level.[15] Anti-C1q concentration correlated with activity on the modified Safety of Estrogen in Lupus: National Assessment-Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI) and the Systemic Lupus International Collaborating Clinics (SLICC) Renal Activity Score.[16] With immunosuppressive treatment for membranoproliferative lupus nephritis with either cyclophosphamide or azathioprine, anti-C1q disappeared by week 12 and remained undetectable throughout one year of follow-up.[17] As detailed above, evidence suggests that anti-C1q is associated not only with lupus nephritis, but also with lupus nephritis flares and response to treatment. Therefore anti-C1q might be a candidate for predicting lupus nephritis and monitoring treatment in clinical practice. The purpose of this study was to characterize, in a multinational patient population, the prevalence and clinical associations of anti-C1q in patients with SLE and other rheumatic diseases and to define the association of anti-C1q with renal involvement in patients with SLE.

Patients and methods

Patients

We studied anti-C1q specificity for SLE (vs rheumatic disease controls) and its association with SLE manifestations in an international, multicenter, cross-sectional sample of patients with SLE and other rheumatic diseases, assembled to derive the SLICC classification criteria for SLE.[18]

Laboratory methods

Anti-C1q determination was performed at the laboratory of Lennart Truedsson, MD, PhD (Department of Laboratory Medicine, Section of Microbiology, Immunology and Glycobiology, Lund University, Lund, Sweden). An enzyme-linked immunosorbent assay (ELISA) with purified collagenous C1q fragments in the solid phase was used for detection of anti-C1q IgG in all serum samples obtained at the beginning of the study. The assay was previously described,[19] and it is well documented that autoantibodies against C1q in SLE target the collagenous portion of the molecule.[20,21] Use of purified C1q collagenous fragments as antigen in the ELISA prevented nonspecific interactions. The reference interval was defined as <16 AU/l based on analysis of anti-C1q IgG in 96 healthy blood donors.[22]

Laboratory determinations were performed at the Rheumatology Diagnostic Laboratory (Los Angeles, CA) for anti-dsDNA by ELISA, *Crithidia* assay and Farr assay, and for anti-Smith antibody and complement C3 and C4 levels. Another set of blood samples was tested for antiphospholipid antibodies (lupus anticoagulant, and ELISA for IgG, IgM and IgA isotypes of anticardiolipin antibodies and anti-β2-glycoprotein I antibodies) at the laboratory of Joan Merrill, MD (Oklahoma Medical Research Foundation, USA).

Statistical methods

Statistical analyses were carried out using SAS® (SAS® 9.2, SAS Institute Inc, Cary, NC, USA) and Stata statistical software (Stata 12, StataCorp LP, College Station, TX, USA). Patients with SLE and controls with rheumatic disease were compared with respect to demographic characteristics, clinical manifestations, and serologic results using Chi-square tests; p values for Chi-square tests were adjusted for age and ethnicity as specified in the tables. A p value ≤ 0.05 was considered statistically significant. In patients with SLE, we calculated odds ratios of renal involvement by the SLICC classification criteria (urine protein to creatinine ratio or 24-hour urine protein representing 500 mg/24 hours or red blood cell casts),[23] using multiple logistic regression on demographic and serologic characteristics. The first model adjusted for demographics (age, ethnicity, gender) and individual antibodies (antinuclear antibodies (ANA), anti-dsDNA, low complement C3 and/or C4 and anti-C1q). The second model adjusted for demographics and serologic patterns for anti-C1q, anti-dsDNA and low complement C3 and/or C4.

The study was approved by institutional review boards at all institutions involved, and all participants provided written informed consent.

Results

Clinical information and blood samples were obtained from 308 patients with SLE (mean age (SD) 34 (13) years, 89% female, 63% Caucasian, 22% African descent, 12% Asian, 3% other) and 389 patients with other rheumatologic diseases (mean age (SD) 43 (15) years, 80% female, 73% Caucasian, 13% African descent, 5% Asian, 9% other) from 25 clinical sites. SLICC renal involvement was present in 33% of patients with SLE and 4% of controls. Of 308 patients with SLE, 72 (23%) had biopsy-confirmed lupus nephritis (and none of the controls).

Anti-C1q prevalence by diagnosis

The prevalence of anti-C1q was 28% (86/308) in patients with SLE and 13% (49/389) in controls with other rheumatologic disorders (OR = 2.7, 95% confidence interval (CI) 1.8–4.0, $p < 0.001$). The frequency of anti-C1q in rheumatic disease controls was: 26% in scleroderma, 19% in rheumatoid arthritis, 15% in undifferentiated connective tissue disease, 15% in chronic cutaneous lupus, 14% in Sjögren's syndrome, 8% in fibromyalgia, 7% in antiphospholipid antibody syndrome, 6% in dermatomyositis, and 5% in vasculitis.

Anti-C1q and demographic characteristics in patients with SLE

Anti-C1q was more common in Asians ($n = 37$, 40.5%) than in Caucasians ($n = 192$, 27.6%) or patients of African descent ($n = 69$, 21.7%), but these differences were not statistically significant. Anti-C1q was more common in younger individuals with SLE, using an age cutoff of 30 years (35.5% vs 23%, $p = 0.02$) (Table 1).

Anti-C1q and clinical SLE manifestations

Sensitivity of anti-C1q for a classification of SLE was 28% and specificity was 87%. In an age-adjusted analysis we assessed the clinical features of SLE associated with anti-C1q antibodies. Patients with anti-C1q were significantly more likely to have proteinuria (OR = 3.0, 95% CI 1.7–5.1, $p < 0.001$) and urinary red cell casts (OR = 2.6, 95% CI 1.2–5.4, $p = 0.015$). There was a trend towards an association with psychosis (OR = 9.5, 95% CI 0.9–98.5, $p = 0.06$). No significant associations were seen with arthritis, cutaneous lupus or hematologic manifestations (Table 2).

Anti-C1q and serologic SLE manifestations

In patients with SLE positive for anti-C1q (compared to patients negative for anti-C1q), there were positive associations with anti-dsDNA (OR = 3.4, 95% CI 1.9–6.1, $p < 0.001$) and anti-Smith (OR = 2.8, 95% CI 1.5–5.0, $p = 0.01$) and no association with antiphospholipid antibodies after adjustment for age (Table 2).

Anti-C1q and lupus nephritis

Sensitivity of anti-C1q for SLE renal involvement was 41% and specificity was 85%. Anti-C1q

Table 1 Association between demographic characteristics and anti-C1q in SLE: Percentage of patients with anti-C1q, by demographic variables

Demographics	*Percentage for anti-C1q*	p *value*
Ethnicity		
African descent	21.7	0.15
Caucasian	27.6	
Asian	40.5	
Other	30.0	
Gender		
Female	26.9	0.25
Male	36.4	
Age (years)		
≤30	35.5	0.01
>30	23.0	

SLE: systemic lupus erythematosus.

Table 2 Association between ACR criteria and anti-C1q in SLE: Percentage of patients with various clinical conditions, by anti-C1q status

ACR criteria	*Anti-C1q positive (%)*	*Anti-C1q negative (%)*	p *value*	*Odds ratio (95% CI)*	*Adjusted* p *value for age*
Malar rash	47.7	46.9	0.90	0.9 (0.5, 1.5)	0.69
Discoid rash	19.8	19.4	0.94	1.1 (0.6, 2.1)	0.71
Photosensitivity	53.5	53.2	0.96	1.0 (0.6, 1.7)	1.00
Oral ulcers	38.4	46.4	0.20	0.7 (0.4, 1.1)	0.14
Arthritis	64.0	65.8	0.76	0.9 (0.5, 1.5)	0.70
Serositis	37.2	34.7	0.68	1.1 (0.6, 1.8)	0.84
Pleurisy	31.4	28.4	0.60	1.1 (0.6, 1.9)	0.74
Pericarditis	14.0	12.2	0.67	1.2 (0.6, 2.5)	0.66
Proteinuria	50.0	22.5	<0.01	3.0 (1.7, 5.1)	**<0.01**
Red cell casts	18.6	7.2	<0.01	2.6 (1.2, 5.4)	**0.02**
Seizure	5.8	4.1	0.51	1.2 (0.4, 3.8)	0.72
Psychosis	3.5	0.5	0.04	9.5 (0.9, 98.5)	0.06
Hematologic	64.0	58.1	0.35	1.2 (0.7, 2.0)	0.49
Leukopenia	40.7	35.1	0.36	1.2 (0.7, 2.0)	0.48
Lymphopenia	38.4	36.5	0.76	1.1 (0.7, 1.8)	0.73
Thrombocytopenia	15.1	12.2	0.49	1.1 (0.5, 2.2)	0.86
Anti-dsDNA	77.9	47.8	<0.01	3.4 (1.9, 6.1)	**<0.01**
Anti-Smith	33.7	14.4	<0.01	2.8 (1.5, 5.0)	**0.01**
Antiphospholipid	57.0	54.5	0.70	1.1 (0.7, 1.8)	0.70

ACR: American College of Rheumatology; SLE: systemic lupus erythematosus; Anti-dsDNA: anti-double-stranded DNA; CI: confidence interval. Bold values represent $p<0.05$.

prevalence in patients with SLE with, vs without ACR renal disorder (persistent proteinuria >0.5 g/24 hour or proteinuria >3+, or red blood cell casts) was 45.5% compared to 19.3%, respectively (OR = 3.2, 95% CI 1.8–5.6, $p < 0.001$). Additional serologic associations observed for ACR renal disorder were with anti-dsDNA (OR = 4.7, 95% CI 2.5–8.6, $p < 0.001$), low complement (OR = 2.8, 95% CI 1.5–4.9, $p = 0.001$) and anti-Smith (OR = 1.9, 95% CI 1.1–3.6, $p = 0.03$), after adjustment for age and ethnicity (Table 3).

The first logistic regression model was applied to all patients with SLE ($n = 308$) to estimate the independent contribution of demographic characteristics and serologies to odds of SLICC renal involvement ($n = 101$). Odds of SLICC renal involvement were two times lower in patients above age 30 than below age 30 (OR = 0.4, 95% CI 0.3–0.8, $p = 0.005$) and, independently of age, three times lower in Caucasians compared to African Americans (OR = 0.3, 95% CI 0.1–0.6, $p < 0.001$), after adjustment for gender and serologies (Table 4).

Odds of SLICC renal involvement in the presence of anti-dsDNA were four times higher than in the absence of anti-dsDNA, after adjustment for age, ethnicity, gender and serologies (OR = 4.1, 95% CI 2.1–7.9, $p < 0.001$). In the same model, for anti-C1q positive, odds of SLICC renal involvement were independently 2.3 times higher than in the absence of anti-C1q (OR = 2.3, 95% CI 1.3–4.2, $p = 0.007$) (Table 4). Low complement C3 and/or C4, compared to normal, was associated with double the odds of SLICC renal involvement, a finding that was not statistically significant after adjustment for anti-dsDNA and anti-C1q (OR = 1.9, 95% CI 1.0–3.6, $p = 0.06$).

The second logistic regression model estimated odds of SLICC renal involvement using possible combinations of serology results for anti-dsDNA, anti-C1q and low complement and adjusted for age, ethnicity and gender. By patterns of positivity for anti-C1q, anti-dsDNA and low complement, odds of SLICC renal involvement were 15 times higher for patients with all three serologies positive compared to all negative (OR = 14.9, 95% CI 5.8–38.4, $p < 0.001$). In the same model, combinations of simultaneously positive anti-dsDNA and low complement, and simultaneously positive anti-dsDNA and anti-C1q were associated with five times and six times increase in odds of SLICC renal involvement than all three serologies negative, respectively (OR = 5.2 95% CI 2.1–13.1, $p < 0.001$ and OR = 5.7 95% CI 1.2–28.3, $p = 0.03$) (Table 5).

Discussion

Anti-C1q has been associated with SLE and SLE nephritis in previous studies.[10,12,14,24–28] We confirmed this association in the SLICC international

Table 3 Association with renal involvement: Percentage of patients with SLE serologies among those with and without ACR lupus nephritis

Variable	*Renal involvement (%)*	*No renal involvement (%)*	p *value*	*Odds ratio (95% CI)*	*Adjusted* p *value for age and ethnicity*
Anti-C1q	45.5	19.3	<0.01	3.2 (1.8, 5.6)	<**0.01**
Anti-dsDNA	80.2	44.4	<0.01	4.7 (2.5, 8.6)	<**0.01**
Anti-Smith	29.7	15.0	<0.01	1.9 (1.1, 3.6)	**0.03**
Low complement	78.2	50.2	<0.01	2.8 (1.5, 4.9)	<**0.01**

ACR: American College of Rheumatology; SLE: systemic lupus erythematosus; Anti-dsDNA: anti-double-stranded DNA; CI: confidence interval. Bold values represent $p<0.05$.

Table 4 Odds ratios (OR) of SLICC renal involvement[a] in patients with SLE ($N=308$) by individual antibody status, adjusted for demographic and serologic characteristics

Covariates	*SLICC renal (OR)*	*95% CI*	p *value*
Age (years)			
≤30 (ref.)[b]	1.00	0.25–0.78	<**0.01**
>30	0.44		
Ethnicity			
African American (ref.)	1.00	0.14–0.60	<**0.01**
White	0.28	0.16–1.08	0.07
Asian	0.42	0.09–1.71	0.22
Hispanic/Latino	0.40		
Gender			
Male (ref.)	1.00	0.19–1.14	0.09
Female	0.46		
ANA	0.22	0.05–1.01	0.05
Anti-dsDNA	4.05	2.10–7.90	<**0.01**
Low complement	1.87	0.98–3.59	0.06
Anti-C1q	2.30	1.26–4.19	<**0.01**

SLICC: Systemic Lupus Collaborating Clinics; SLE: systemic lupus erythematosus; Anti-dsDNA: anti-double-stranded DNA; CI: confidence interval.

[a]SLICC renal involvement is defined as urine protein-to-creatinine ratio (or 24-hour urine protein) representing 500 mg/24 hours or red blood cell casts; estimates from multivariable logistic regression model, constant term 3.44, 95% CI 0.51–23.07, p value 0.2).

[b]ref.: the reference group for each category. Bold values represent $p<0.05$.

patient population, of whom we studied 308 patients with SLE and 389 controls with other rheumatologic diseases. We also showed, for the first time, the association of anti-C1q with lupus renal involvement by SLICC classification criteria.

The presence of anti-C1q antibodies in other autoimmune diseases, as we have found, and even in healthy individuals (4% to 6.4%), has been previously reported.[21,29] Patients with scleroderma were anti-C1q positive in a higher proportion in our study, 26%, than observed in other studies, 5.5%.[24] None of these patients had renal involvement. Patients with rheumatoid arthritis were anti-C1q positive in a higher proportion in our study, 19%, than observed in other studies, 5%.[30] However, a review by Seelen et al. reported anti-C1q prevalence of 77% in rheumatoid vasculitis.[31] Patients with vasculitis were less often positive in the population we studied, 5%, vs 12%–35% in other studies.[29] We did not collect information on type of vasculitis and anti-neutrophil cytoplasmic antibody (ANCA) status. Anti-C1q has not been previously described in dermatomyositis in which we found a prevalence of anti-C1q of 6% (based on 55 patients). Anti-C1q was more common in Asians (40.5%) than in Caucasians (27.6%) and patients of African descent (21.7%), but these differences were not statistically significant, consistent with previous studies.[32] We found that younger individuals with SLE were more likely to be anti-C1q positive than older individuals, using an age cutoff of 30 years. Siegert et al., similarly, found a higher prevalence of IgG anti-C1q antibodies in younger individuals with SLE compared to random selected controls (highest titer and highest prevalence below age 30); in patients with SLE anti-C1q prevalence decreased with age while in random controls the opposite was true.[33].Anti-C1q antibody prevalence in patients with SLE with ACR renal involvement was 45.5% in our study. Braun et al. found a prevalence of 61.7% in biopsy-proven lupus nephritis cases[29] and Wener et al., 48%.[21] The strongest clinical association we observed for anti-C1q was with proteinuria, consistent with published data.[12,14,16,27,34] Our study was undertaken in patients with SLE from a multicenter, multiethnic patient population and a similar number of patients with other rheumatic diseases (controls), in which complete clinical, serologic and candidate criteria variables were assessed for the purpose of deriving SLE classification rules. We did not have flare data, treatment data, or repeat anti-C1q antibody levels because of the cross-sectional nature of this study. Therefore, any temporal relationship of anti-C1q antibody levels to flares of lupus nephritis or change in treatment could not be

Table 5 Odds ratios (OR) of SLICC renal involvement[a] in patients with SLE ($N = 308$) by antibody patterns, adjusted for demographics (age, ethnicity and gender)

Serologic patterns						
Anti-C1q	*Anti-dsDNA*	*Low complement*	N *(308)*	*SLICC renal (OR*)*	*95% CI*	p *value*
Negative (ref.)[b]	Negative	Negative	75	1.00		
Negative	Negative	Positive	41	1.83	0.62–5.34	0.27
Negative	Positive	Negative	35	2.46	0.79–7.61	0.12
Negative	Positive	Positive	71	5.23	2.10–13.05	**<0.01**
Positive	Negative	Negative	6	4.06	0.60–27.30	0.15
Positive	Negative	Positive	13	0.66	0.07–6.11	0.72
Positive	Positive	Negative	9	5.74	1.16–28.29	**0.03**
Positive	Positive	Positive	58	14.89	5.77–38.44	**<0.01**

SLICC: Systemic Lupus Collaborating Clinics; SLE: systemic lupus erythematosus.
[a]SLICC renal involvement is defined as urine protein-to-creatinine ratio (or 24-hour urine protein) representing 500 mg/24 hours or red blood cell casts; estimates of odds ratios (OR) are from multivariable logistic regression model, constant term 0.95, 95% CI 0.26–3.49, p value 0.94).
[b]ref.: the reference group for each category. Bold values represent $p<0.05$.

assessed. Others noted that anti-C1q antibody levels increased prior to flares of lupus nephritis and disappeared with immunosuppressive treatment.[11,15,26] Moroni et al. showed an association with active lupus nephritis for anti-C1q and low complement.[14] Yang et al. showed concomitant presence of anti-C1q and anti-dsDNA was associated with higher lupus nephritis activity and poor renal outcome compared to only one or none of these antibodies.[28] Anti-C1q in our study had the highest prevalence in patients with SLE with ACR renal involvement and was strongly associated with anti-dsDNA and low complement. It was the second highest antibody associated with a diagnosis of ACR renal involvement, after anti-dsDNA. By the SLICC classification criteria, age above 30 years and being Caucasian were protective from SLICC renal involvement: These characteristics were independently associated with decreased odds of renal involvement in patients with SLE by two and three times, respectively, which is consistent with the literature on the subject. Independently of each other, anti-dsDNA (vs negative) was associated with four times higher odds of SLICC renal involvement and anti-C1q (vs negative) was associated with two times higher odds of SLICC renal involvement, after adjustment for age, ethnicity, gender and low complement. In patients with SLE, odds of SLICC renal involvement were highest in the presence of simultaneously positive anti-dsDNA, anti-C1q and low complement (15 times higher than all negative). Increases in odds of SLICC renal involvement with concomitantly positive anti-dsDNA and low complement were similar to concomitantly positive anti-dsDNA and anti-C1q. As seen in the logistic regression models, the three serologies (anti-C1q, anti-dsDNA, low complement) had a multiplicative relationship in increasing the odds of SLICC renal involvement after adjustment for demographics.

Many studies of anti-C1q antibodies are performed with methods using whole C1q molecules as antigen and a buffer with high ionic strength to prevent nonspecific interaction between the globular heads of C1q and antibodies. In this study the purified collagenous fragment was used as antigen in the ELISA and the nonspecific interactions were thereby avoided.[19] Comparisons between the method used here and the high-salt buffer method in 100 patients with high and low disease activity have given very similar results (Truedsson et al. 2014, personal communication). The reason anti-C1q was eliminated, at the end of the derivation phase of the SLICC classification criteria for SLE, was mainly because of a lack of a high-quality, standardized, less laborious assay. As new laboratory techniques develop and further uses of anti-C1q determinations become important for clinical care and disease prognosis, anti-C1q can be reconsidered for inclusion in classification criteria and in the clinical management of SLE.

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Conflicts of interest statement

The authors have no conflicts of interest to declare.

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