

Relationship of Circulating Anti-C3b and Anti-C1q IgG to Lupus Nephritis and Its Flare

Daniel J. Birmingham,* Joshua E. Bitter,* Ezinne G. Ndukwe,* Sarah Dials,* Terese R. Gullo,* Sara Conroy,[†] Haikady N. Nagaraja,[†] Brad H. Rovin,* and Lee A. Hebert*

Abstract

Background and objectives Autoantibodies to complement C1q (anti-C1q) are associated with the diagnosis of lupus nephritis. In this study, we compare anti-C1q IgG with another complement autoantibody, anti-C3b IgG, as a biomarker of lupus nephritis and lupus nephritis flare.

Design, setting, participants, & measurements Our investigation involved the Ohio SLE Study, a prospective observational cohort of patients with recurrently active lupus who were followed bimonthly. Serum anti-C1q and anti-C3b IgG levels were assessed cross-sectionally by ELISA in 40 normal controls and 114 patients in the Ohio SLE Study (41 nonrenal and 73 lupus nephritis) at study entry, and longitudinally in a subset of patients in the Ohio SLE Study with anti-C1q–positive lupus nephritis in samples collected every 2 months for 8 months leading up to lupus nephritis flare ($n=16$ patients).

Results In the cross-sectional analysis, compared with anti-C1q IgG, anti-C3b IgG was less sensitive (36% versus 63%) but more specific (98% versus 71%) for lupus nephritis. Only anti-C3b IgG was associated with patients with lupus nephritis who experienced at least one lupus nephritis flare during the Ohio SLE Study period ($P<0.01$). In the longitudinal analysis, circulating levels of anti-C1q IgG increased at the time of lupus nephritis flare only in patients who were anti-C3b positive ($P=0.02$), with significant increases occurring from 6 (38% increase) and 4 months (41% increase) before flare. Anti-C3b IgG levels also trended up at lupus nephritis flare, although the change did not reach statistical significance ($P=0.07$). Neither autoantibody increased 2 months before flare.

Conclusions Although not as prevalent as anti-C1q IgG, anti-C3b IgG showed nearly complete specificity for lupus nephritis. The presence of anti-C3b IgG identified patients with lupus nephritis who were prone to flare and in whom serial measurements of markers associated with complement, such as anti-C1q IgG, may be useful to monitor lupus nephritis activity.

Clin J Am Soc Nephrol 11: 47–53, 2016. doi: 10.2215/CJN.03990415

Introduction

Autoantibodies to complement C1q (anti-C1q) have long been viewed as an important biomarker of patients with SLE with major renal manifestations (lupus nephritis [LN]) (1). A recent meta-analysis of >20 of these studies found pooled sensitivity and specificity of 0.58 and 0.75, respectively, for patients with LN compared with patients with SLE without LN and a weighted sensitivity and specificity of 0.74 and 0.77, respectively, for active LN compared with inactive LN (2). Few studies have attempted to address anti-C1q as a biomarker of LN flare through serial measurements leading up to flare.

The role of anti-C1q antibody as a biomarker of LN and its potential as a biomarker of LN flare have led us to investigate other possible autoantibodies to complement proteins in LN. In this study, we screened a small set of patients with LN for IgG autoantibodies reactive to classic complement activation proteins and regulators of early complement activation, and then compared one of these autoantibodies, anti-C3b, to

anti-C1q as a biomarker of LN and LN flare in a larger SLE cohort.

Materials and Methods

Study Population

This study involved 114 patients with SLE from central Ohio who met the American College of Rheumatology (ACR) criteria for SLE diagnosis and were enrolled between 2001 and 2005 in the Ohio SLE Study (OSS), a prospective observational cohort of patients with recurrently active lupus who were methodically tested every 2 months while receiving standard of care. The patient demographic and baseline clinical data (first collected after OSS entry) are shown in Table 1. Forty healthy individuals were also recruited from central Ohio as normal controls (Table 1). All were recruited after institutional review board–approved informed consent and in accordance with the Declaration of Helsinki.

*Department of Medicine and Davis Heart and Lung Research Institute, Ohio State University Medical Center, Columbus, Ohio; and [†]Division of Biostatistics, Ohio State University College of Public Health, Columbus, Ohio

Correspondence:

Dr. Daniel J. Birmingham, The Ohio State University, Department of Medicine, Division of Nephrology, The Davis Heart and Lung Research Institute, 395 West 12th Avenue, Ground Floor, Columbus, OH 43210. Email: dan.birmingham@osumc.edu.

Table 1. Baseline demographics and clinical measurements of the cross-sectional cohorts.

Demographic/Clinical Measurement	Controls	Patients in the Ohio SLE Study		
		Nonrenal	Lupus Nephritis	P Value
N	40	41	73	
Age, yr ^a	34 (27–43)	43 (26–44)	31 (25–40)	0.02 ^b
Race, black/white/other	20/20/0	15/26/0	28/40/5	0.69 ^c
Sex, women/men	29/11	38/3	66/7	>0.99 ^c
Urine protein-to-creatinine ratio ^a		0.12 (0.09–0.18)	1.03 (0.28–3.03)	<0.001 ^b
Serum creatinine, mg/dl ^a		0.78 (0.65–0.82)	0.90 (0.72–1.25)	0.004 ^b
C3, mg/dl ^a		126 (103–143)	86 (70–100)	<0.001 ^b
C4, mg/dl ^a		20 (15–28)	15 (8.2–20)	0.003 ^b
Anti-dsDNA, positive/negative ^d		5/31	25/45	0.02 ^c

dsDNA, double stranded DNA.

^aMedian (interquartile range).

^bMann–Whitney test for differences between nonrenal and lupus nephritis.

^cFisher exact test for differences between nonrenal and lupus nephritis in race (black versus white), sex, or anti-dsDNA positivity.

^dAnti-dsDNA Ohio SLE Study data unavailable for five nonrenal patients and three patients with lupus nephritis.

LN Flare Adjudication

LN flares were documented on the basis of criteria for proteinuria, serum creatinine, and urine sediment (Table 2) that closely parallel the current ACR criteria for LN flare, which we have previously detailed (3–6). Proteinuria was quantified as protein-to-creatinine ratios from intended 24-hour urine collections. Individuals adjudicating flares were blinded to biomarker results.

Anticomplement Assays

As an initial screen, serum samples from eight patients with LN and five healthy normal individuals were tested for IgG against the complement proteins C1s, C4b, C2, C3b, C1 inhibitor, Factor H, C4 binding protein, and Factor I using an ELISA format. Complement proteins (Complement Technology, Inc., Tyler, TX), and BSA as a control, were coated separately in 96-well plates at 10 µg/ml in PBS (pH 7.4) overnight at 4°C. The plates were blocked with 5% BSA in PBS (diluent) and washed, and 2% prespun serum samples were added separately to each well and incubated at room temperature for 1 hour. The wells were washed, and anticomplement IgG was detected with mouse anti-human IgG conjugated with horseradish peroxidase (Invitrogen Corporation, Camarillo, CA) followed after washing by the addition of 3,3',5,5'-tetramethylbenzidine. Color development

was measured at OD₄₅₀ after stopping the reactions with 1 N HCl.

Anti-C1q and anti-C3b IgG levels were determined in the OSS samples by this ELISA using wells coated with C1q or C3b. For anti-C1q IgG, the diluent for the samples and the mouse anti-human IgG contained 1.0 M NaCl to prevent binding of coated C1q to IgG through the IgG recognition site. This approach has been used in most of the reports describing circulating anti-C1q autoantibody (as discussed in Seelen *et al.* 7 and Sinico *et al.* 8) and shown to yield results that are highly correlated with measurements of anti-C1q binding to the collagen-like region of C1q at physiologic NaCl concentration (9). The collagen-like region is considered to contain the major anti-C1q epitope in patients with active SLE (10–12), and it is the epitope for anti-C1q antibodies isolated from the glomeruli of patients with LN (13).

For each measurement, background ODs, determined from incubating serum samples in wells coated only with BSA, were subtracted from each sample measurement. All readings were normalized to the same positive control sample, one each for anti-C1q IgG measurements and anti-C3b IgG measurements, and results are reported as normalized OD₄₅₀. All serial samples for a given LN flare interval (see below) were run together in the same plate. Samples were run in duplicate, and each assay was repeated a minimum of two times.

Table 2. Criteria used to adjudicate lupus nephritis flares in the Ohio SLE Study

Type of Flare	Criteria
Mild LN	An increase in hematuria from <5 to >15 RBC/hpf, with >2 acanthocytes/hpf and/or a recurrence of >1 RBC cast, WBC cast (no infection)
Moderate LN	An increase in serum creatinine of 0.2–1.0 mg/dl if baseline is <2 mg/dl or 0.4–1.5 mg/dl if baseline is >2 mg/dl or an increase in urine Pr/Cr to ≥1 if baseline is <0.5 or ≥2 if baseline is 0.5–1 or an increase of ≥2-fold with an absolute Pr/Cr <5 if baseline is >1
Severe LN	An increase in serum creatinine of >1 mg/dl if baseline is <2 mg/dl or ≥1.5 mg/dl if baseline is ≥2 mg/dl or absolute increase in urine Pr/Cr >5

LN, lupus nephritis; RBC, red blood cells; hpf, high-power field; WBC, white blood cells; Pr, protein; Cr, creatinine.

Serum Samples Tested

To explore the relationship between circulating levels of anti-C1q IgG or anti-C3b IgG and LN and LN flare, both a cross-sectional analysis and a longitudinal analysis were performed. The cross-sectional analysis involved measuring serum anti-C1q and anti-C3b IgG levels in patients at OSS entry and 40 healthy age- and race-matched individuals.

The longitudinal analysis involved measuring anti-C1q and anti-C3b IgG in serial bimonthly serum samples from 24 LN flare intervals. A flare interval was defined as the 8 months leading up to LN flare (–8, –6, –4, –2, and 0 months [at flare]). All of the flare intervals from anti-C1q-positive patients with LN with serum samples available from at least four of five time points were analyzed. These represented 24 flare intervals from 16 patients with LN. Of these 24 flares, two were mild, and the remaining 22 were on the basis of increases in proteinuria (21 moderate and one severe). Of 16 patients with LN, two experienced three flares each, four experienced two flares each, and ten experienced one flare each. In patients with multiple flares, each flare was considered an independent event because the minimum time between LN flares was 10 months (occurred once), the average time between LN flares was 26 months, and there were no clear trends in flare intervals from the same patient (Supplemental Figure 1).

Statistical Analyses

Differences in anticomplement IgG levels during the initial screen between patients with LN and healthy normal controls (Figure 1) were determined by two-tailed

unpaired *t* test (for normalized data) or the Mann–Whitney test (for non-normalized data).

For the cross-sectional analyses, serum samples were identified as positive for anti-C1q or anti-C3b IgG if their normalized OD values were at least 3 SDs above the mean normalized OD for the normally distributed data of the controls (0.195 for anti-C1q and 0.134 for anti-C3b). Differences in the proportion of anti-C1q or anti-C3b IgG-positive samples between groups (Table 3) were determined by two-tailed Fisher exact tests.

For the longitudinal analyses, repeated measures mixed effects multiple regression models were run (JMP, version 10.0.2; SAS Institute Inc., Cary, NC), with flare month (comparing –8, –6, –4, –2, and 0 months) as the nominal predictor, the flare interval as random effect, and anti-C1q IgG or anti-C3b IgG levels as the response. Other covariates that were tested included age, race, World Health Organization (WHO) classification, and use during the approximately 60-day period before each interval month visit of prednisone (mean daily dose), mycophenolate mofetil (none, >0 but ≤1000 mg/d, >1000 but <2000 mg/d, and ≥2000 mg/d), azathioprine (yes or no), and hydroxychloroquine (yes or no). All 16 patients with LN were women, and therefore, sex was not a testable covariate.

The regression models were run as follows. Each covariate was run in a separate model that included flare month (predictor) and flare interval (random effects). Those covariates with *P* ≤ 0.25 were then combined back into the model, which was then repeated in a stepwise fashion, eliminating covariates with *P* > 0.10 at each step until only covariates

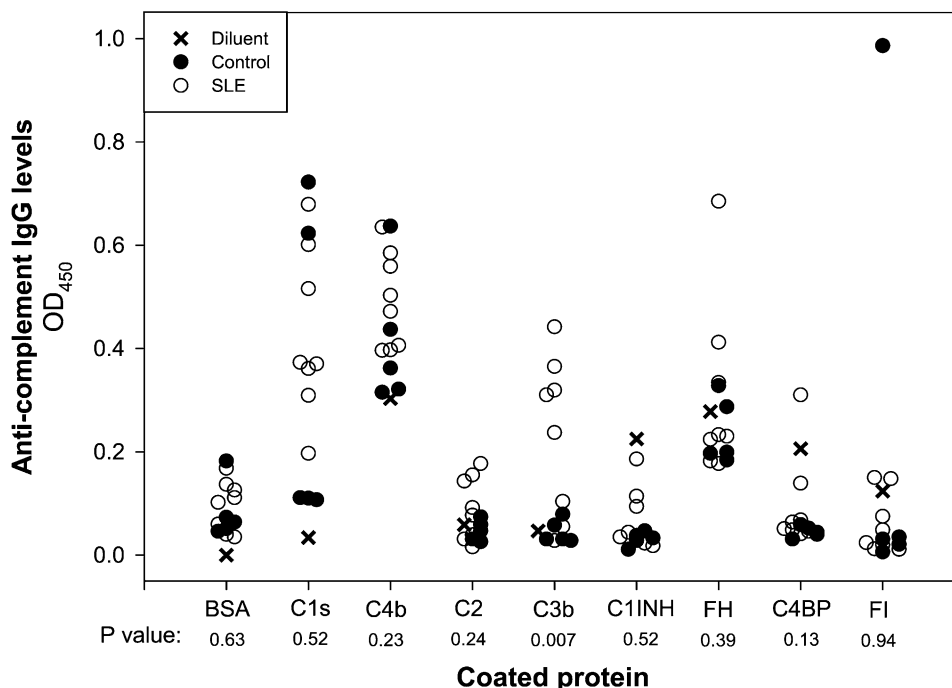


Figure 1. | Anticomplement IgG levels. The degrees of IgG reactivity (measured by ELISA OD₄₅₀) to the coated complement proteins in serum samples from eight patients with lupus nephritis (LN) and five normal controls are shown. Also shown are the baseline values in the absence of serum (diluent). The *P* values refer to the levels of significance for the difference in IgG levels between controls and patients with LN for each coated complement protein (two-tailed unpaired *t* test [for normalized data] or the Mann–Whitney test [for non-normalized data]). C1INH, C1 inhibitor; C4BP, C4 binding protein; FH, Factor H; FI, Factor I.

Table 3. Cross-sectional analysis of the prevalence of anti-C1q or anti-C3b in healthy controls versus patients with SLE, nonrenal patients versus patients with lupus nephritis, and patients with lupus nephritis without versus with at least one lupus nephritis flare during the Ohio SLE Study period

Cohort	N	Anti-C1q			Anti-C3b				
		No. of positive ^a	P Value ^b	Sensitivity ^c (%)	Specificity ^c (%)	No. of positive ^a	P Value ^b	Sensitivity ^c (%)	Specificity ^c (%)
Control	40	2	<0.001	51	95	0	<0.001	24	100
SLE	114	58				27			
Nonrenal	41	12	<0.001	63	71	1	<0.001	36	98
LN	73	46				26			
LN no LNF	36	20	0.23	70	44	7	<0.01	51	81
LN yes LNF	37	26				19			

anti-C1q, autoantibodies to complement C1q; LN, lupus nephritis; LNF, lupus nephritis flare.
^aNumber of samples in each cohort that were positive for anti-C1q or anti-C3b IgG.
^bResults of analysis comparing prevalence of anti-C1q or anti-C3b IgG between each pair of cohorts.
^cSensitivity and specificity of anti-C1q or anti-C3b IgG for SLE (versus control), LN (versus nonrenal), and LN with at least one LNF (LN yes LNF, versus LN without at least one LNF [LN no LNF]).

with $P < 0.05$ remained along with flare month and flare interval. *Post hoc* Tukey tests were performed as needed.

For the anti-C1q IgG analysis, because eight of 24 flare intervals involved patients who were anti-C3b negative (from the cross-sectional analysis), the interaction between anti-C3b positivity (yes or no) and flare month was also included as fixed effects. The final model had an R^2 of 0.87, with $F(34,80)=16.1$ ($P < 0.001$).

For the anti-C3b IgG analysis, we considered only 16 flare intervals with measurable levels. Two of these exhibited high variation, as described below, and were excluded from the model. The final model for the remaining 14 flare intervals had an R^2 of 0.83, with $F(17,48)=13.5$ ($P < 0.001$).

To assess the relationships between circulating C3 levels, C4 levels, anti-C1q IgG levels, anti-C3b IgG levels, and LN flare, two analyses were performed. First, the relationship of C3 or C4 levels (predictor) to the levels of anti-C1q or anti-C3b IgG during the flare intervals (response) was assessed by univariate regression model. Second, the relationship between C3 or C4 and LN flare as influenced by C3b positivity was assessed by the same stepwise multiple regression model described above but with C3 or C4 levels as the response instead of anti-C1q or anti-C3b IgG levels. This was done separately for anti-C3b–positive and anti-C3b–negative patients. In all of the analyses, flare intervals were set as random effects.

Results

Screen for Anticomplement IgG

Serum samples from eight different patients with LN and five healthy normal individuals were screened for IgG reactivity against a panel of complement proteins that included activators (C1s, C4b, C2, and C3b) and regulators (C1 inhibitor, Factor H, C4 binding protein, and Factor I). As can be seen in Figure 1, there were various degrees of overlap between normal individuals and patients with LN in IgG reactivity. Among these reactivities, anti-C3b IgG seemed to exhibit the least overlap between normal and LN serum samples, and it was the only antibody to show a significant difference between normal and LN samples.

Cross-Sectional Analysis of Anti-C1q and Anti-C3b IgG Levels in Individuals without Lupus and Patients with SLE

Figure 2 shows the individual levels (normalized OD₄₅₀) of anti-C1q and anti-C3b IgG and compares normal controls with patients with SLE tested at cohort entry, compares nonrenal patients with SLE with patients with LN, and compares patients with LN who had at least one LN flare with patients with LN who never experienced an LN flare during the OSS. Table 3 summarizes these data and provides the statistical analyses between groups. As can be seen, both anti-C1q and anti-C3b IgG were significantly associated with SLE compared to the normal controls, and with LN compared to non-renal lupus. In both cases, anti-C3b IgG was less sensitive than anti-C1q IgG (24% versus 51% for all SLE and 36% versus 63% for LN). Anti-C3b IgG was more specific than anti-C1q IgG for LN (98% versus 71%), with only one of 41 nonrenal patients testing positive for anti-C3b IgG. Of note, 26 of 27 patients with anti-C3b IgG were also positive for anti-C1q IgG.

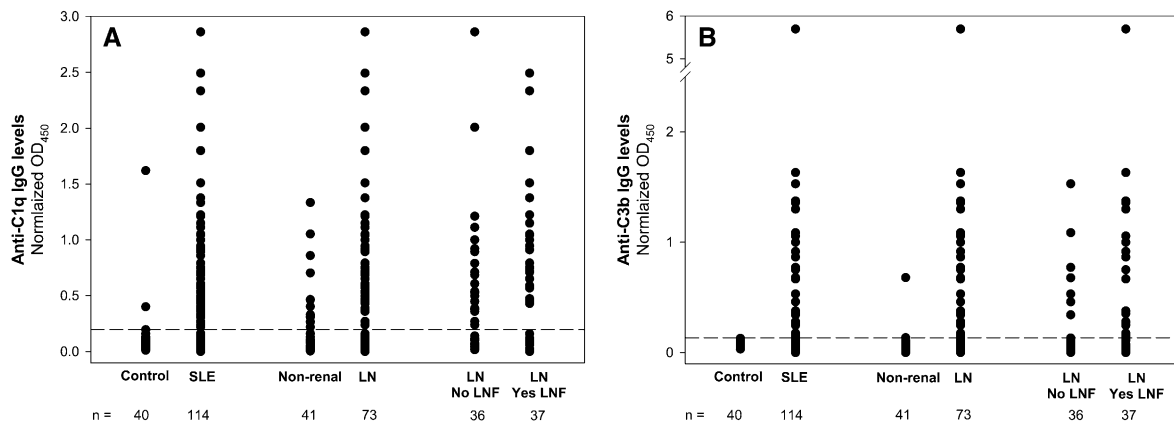


Figure 2. | Autoantibodies to complement C1q (anti-C1q) and anti-C3b IgG levels in normal controls and patients with SLE. (A) Anti-C1q IgG and (B) anti-C3b IgG levels, expressed as normalized OD₄₅₀ values, are shown for controls compared with patients in the Ohio SLE Study (OSS), nonrenal patients compared with patients in the OSS with lupus nephritis (LN), and patients in the OSS with LN who did not experience an LN flare (LNF; LN no LNF) compared with those who did (LN yes LNF) during the OSS. The horizontal dashed line represents the positive cutoff for each autoantibody. The number of patients tested in each category is shown at the bottom.

Of 73 patients with LN from the OSS included in this study, 37 experienced at least one LN flare during a median follow-up of 60 months in the OSS; 36 patients with LN from the OSS who did not experience an LN flare were followed for a median of 46 months. No difference was found in the proportion of patients who were positive for anti-C1q IgG between these two groups ($P=0.23$). In contrast, there was a higher prevalence of anti-C3b IgG in patients with LN who experienced an LN flare (51%) compared with those who did not (19%; $P<0.01$).

Longitudinal Analyses of Anti-C1q and Anti-C3b IgG Levels Versus LN Flare

To test the temporal relationship between anti-C1q or anti-C3b IgG levels and LN flare, the data were analyzed by repeated measures mixed effects multiple regression models as described above. Neither flare month ($P=0.37$) nor anti-C3b positivity ($P=0.10$) was found to be a significant predictor of anti-C1q IgG levels. However, an interaction between flare month and C3b positivity was observed ($P=0.03$), indicating that the profiles of anti-C1q IgG levels during the flare intervals from patients who were anti-C3b positive were different from those who were anti-C3b negative. Figure 3A shows the individual bimonthly anti-C1q IgG levels for LN flare intervals from patients who were anti-C3b positive ($n=16$ intervals; 76 bimonthly time points) or anti-C3b negative ($n=8$ intervals; 39 time points).

To clarify the influence of anti-C3b positivity on anti-C1q levels, the LN flare intervals from anti-C3b-positive patients were analyzed separately from those from anti-C3b-negative patients. As can be seen in Figure 3B, for flare intervals in which anti-C3b IgG was present, anti-C1q IgG increased significantly at the time of LN flare ($P=0.02$). In contrast, for flare intervals in which anti-C3b IgG was absent, there was no significant change in anti-C1q IgG levels ($P=0.12$).

For the anti-C3b-positive group, *post hoc* testing revealed that anti-C1q IgG levels were higher at LN flare than at -6 and -4 months (38% and 41% higher, respectively). No other differences were found. Of the covariates

tested as predictors of anti-C1q IgG, only the mycophenolate mofetil dose was a significant covariate ($P<0.01$).

Anti-C3b IgG levels were measured in all 24 flare intervals, including eight flare intervals from patients who were anti-C3b negative during the cross-sectional analysis. None of the months in these eight flare intervals showed measurable anti-C3b IgG levels, indicating that these patients remained anti-C3b negative at least during the 8 months leading to LN flare. Accordingly, these eight flare intervals were excluded from the anti-C3b IgG regression models. Of the remaining 16 flare intervals, two exhibited increases in anti-C3b IgG levels from -4 to -2 or 0 months that were well above the other 14 flare intervals (Figure 3C). Because these data were not representative of the other data points, these intervals were excluded from the regression model. The final analysis showed an apparent trend to increase at LN flare (Figure 3D) that did not reach statistical significance ($P=0.07$). The profile for anti-C3b shown in Figure 3D followed closely the profile for anti-C1q in patients who were anti-C3b positive shown in Figure 3B. No other covariates were identified as predictors of anti-C3b IgG levels.

Relationship of Serum C4 and C3 Levels to Anti-C1q and Anti-C3b during LN Flare

To assess the relationship between complement levels and anticomplement levels, serum C4 or C3 levels were tested as predictors of anti-C1q or anti-C3b IgG levels. Both C4 and C3 levels predicted anti-C1q levels ($P<0.001$ for both), and both predicted anti-C3b levels ($P<0.001$ for C4 and $P=0.02$ for C3). In all analyses, lower C3/C4 levels correlated with higher antibody levels.

In assessing the role of anti-C3b positivity in influencing the relationship between changes in C4 or C3 levels and LN flare, no relationship was found in anti-C3b-negative patients between C4 levels and LN flare ($P=0.97$) or between C3 levels and LN flare ($P=0.83$). In contrast, in anti-C3b-positive patients, although again, there was no association between C4 levels and LN flare ($P=0.30$), C3 levels were

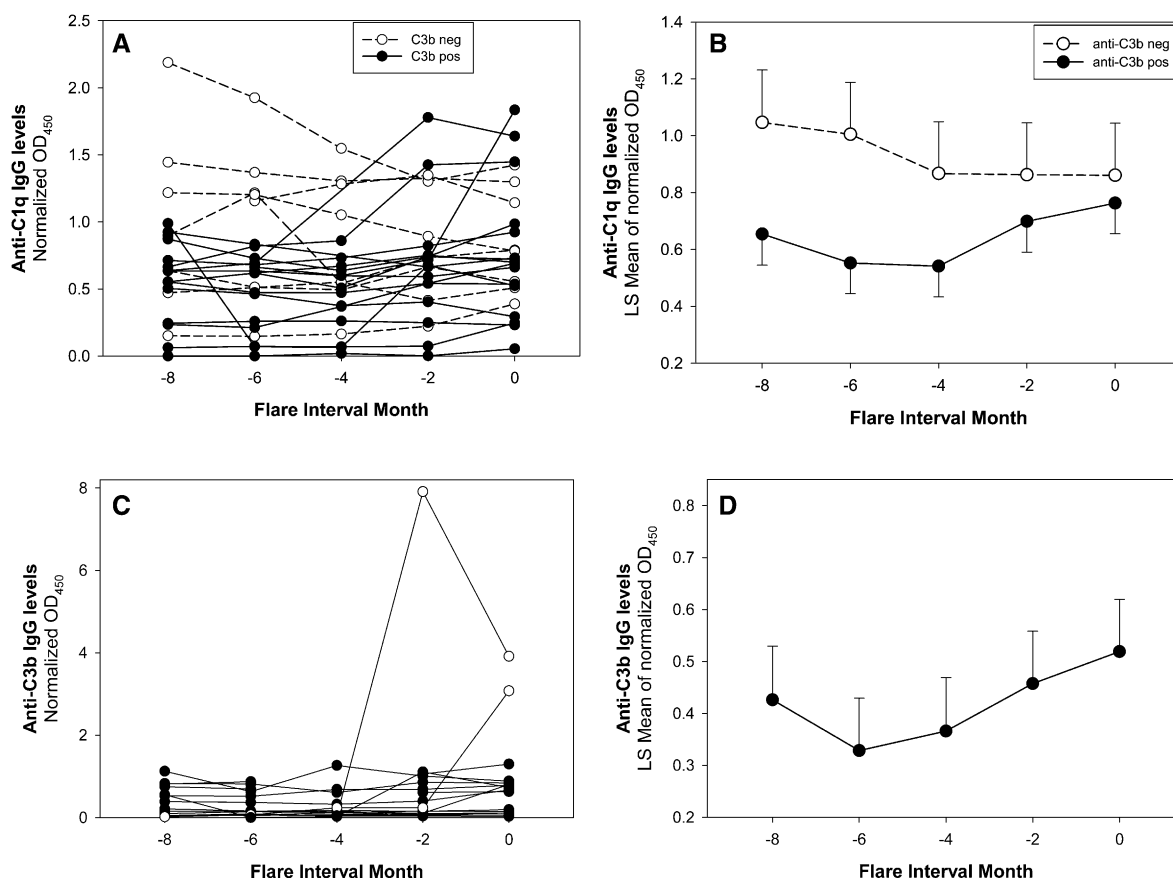


Figure 3. | Temporal relationship between autoantibodies to complement C1q (anti-C1q) or anti-C3b IgG levels and onset of lupus nephritis (LN) flare. Individual levels (normalized OD₄₅₀) and least squares (LS) means are shown for (A and B) anti-C1q IgG and (C and D) anti-C3b IgG for five flare interval months (-8, -6, -4, -2, and 0). In A and B, the anti-C1q levels are shown for both C3b-negative patients with LN (white circles) and C3b-positive patients with LN (black circles). The two intervals excluded from the anti-C3b regression model are shown in C as white circles. $P=0.02$. Neg, negative; pos, positive.

found to significantly change during the flare interval ($P=0.02$), specifically decreasing at LN flare.

Discussion

In this study, compared with anti-C1q IgG, anti-C3b IgG was found to be less sensitive but more specific for LN and uniquely specific for patients with LN who experienced at least one LN flare. The longitudinal analysis of serial bimonthly serum samples revealed that, although neither anti-C1q nor anti-C3b IgG alone was a biomarker of LN flare, the presence of anti-C3b IgG identified patients with LN in whom anti-C1q could serve this function.

There was a trend for anti-C3b IgG levels to increase at flare ($P=0.07$) that followed closely the profile for anti-C1q IgG levels in anti-C3b-positive patients. The reason that this did not reach significance was because of higher overall variation in anti-C3b IgG levels. This was observed even after excluding the two flare intervals from the regression model because they were not representative of the other flare intervals. These two intervals actually exhibited large increases in anti-C3b IgG levels 2 months before or at LN flare (Figure 3C). Therefore, under different circumstances (*e.g.*, different follow-up frequency), anti-C3b

alone may indeed serve as a useful biomarker of LN flare. Nevertheless, under the bimonthly testing schedule of this study, in anti-C3b IgG-positive patients, anti-C1q IgG performed better than anti-C3b IgG as a biomarker of LN flare.

Antibodies to C3 and its fragments have long been recognized as part of a group of anticomplement antibodies known as immunoglobulins (14). They have been reported to occur in high levels in patients with SLE (15–17). No reports have shown a specific association of anti-C3b antibody with LN. However, our finding of such a relationship is not unexpected. Antibody produced to C3b likely reflects an autoantibody response to C3b neoepitopes formed on C3 activation (18,19). Indeed, in this study, C3 levels were inversely correlated with anti-C3b IgG levels. The relationship of anti-C3b IgG to LN suggests that the C3b neoepitopes are forming at sites of kidney damage. We have previously shown using a much larger sample size from the OSS cohort that C3 levels (but not C4 levels) predicted (decreased at) LN flare, and this relationship was particularly strong in patients with defective C3 regulation at tissue surfaces (20). In the smaller OSS sample size of this study, C3 levels (but again, not C4 levels) predicted LN flare only in anti-C3b-positive patients. Together these observations are consistent with a model where C3 activation at the kidney surface causes tissue damage, resulting in C3b

deposited in a way that presents neoepitopes that drive anti-C3b IgG production. These observations also support the hypothesis that the clinical use of other indicators of complement activation in the management of patients with LN will be dictated by the presence of anti-C3b IgG.

This study does not address whether anti-C3b IgG is simply a biomarker of LN flare or also contributes to the pathogenesis of LN flare. It is plausible that anti-C3b antibody, by binding to deposited C3b in the kidney, focuses additional complement activation to the kidney, which has been previously proposed for anti-C1q antibodies (21). Other possible contributions to LN flare pathogenesis include interfering with regulation of C3 activation by anti-C3b (16) and interfering with complement-mediated disposal of apoptotic cells (22).

Other limitations of this study include the inability to assess the influences of WHO classification at the time of flare because biopsies were not performed at that time, and flare severity because almost all were moderate proteinuric flares. Also, the sample size for the longitudinal analysis was somewhat small, with 24 flares available for study for anti-C1q and 16 flares available for study for anti-C3b. Clearly, verification in an independent cohort will be needed.

In conclusion, this study shows that, compared with anti-C1q, anti-C3b IgG is more specific for LN (approaching 100%) and uniquely associated with patients with LN who experience LN flare. The presence of anti-C3b IgG identifies patients with LN in whom anti-C1q IgG and likely other complement-related markers may serve as a biomarker of LN flare. We propose that the presence of anti-C3b IgG signifies a level of complement activation sufficient to initiate and accelerate kidney damage in lupus and as such, could prove to be an important consideration in the management of LN.

Acknowledgments

This work was supported by National Institutes of Health Grants P01 DK55546 (to L.A.H.) and UL1 RR025755 (to The Ohio State University), the Hernandez Nephrology Fellowship Research Fund, and the James D. Casto Research Fund.

Disclosures

None.

References

- Siebert C, Daha M, Westedt ML, van der Voort E, Breedveld F: IgG autoantibodies against C1q are correlated with nephritis, hypocomplementemia, and dsDNA antibodies in systemic lupus erythematosus. *J Rheumatol* 18: 230–234, 1991
- Yin Y, Wu X, Shan G, Zhang X: Diagnostic value of serum anti-C1q antibodies in patients with lupus nephritis: A meta-analysis. *Lupus* 21: 1088–1097, 2012
- Rovin BH, Tang Y, Sun J, Nagaraja HN, Hackshaw KV, Gray L, Rice R, Birmingham DJ, Yu CY, Spetie DN, Aziz A, Hebert LA: Clinical significance of fever in the systemic lupus erythematosus patient receiving steroid therapy. *Kidney Int* 68: 747–759, 2005
- Rovin BH, Song H, Birmingham DJ, Hebert LA, Yu CY, Nagaraja HN: Urine chemokines as biomarkers of human systemic lupus erythematosus activity. *J Am Soc Nephrol* 16: 467–473, 2005
- Birmingham DJ, Nagaraja HN, Rovin BH, Spetie L, Zhao Y, Li X, Hackshaw KV, Yu CY, Malarkey WB, Hebert LA: Fluctuation in self-perceived stress and increased risk of flare in patients with lupus nephritis carrying the serotonin receptor 1A-1019 G allele. *Arthritis Rheum* 54: 3291–3299, 2006
- Birmingham DJ, Hebert LA, Song H, Noonan WT, Rovin BH, Nagaraja HN, Yu CY: Evidence that abnormally large seasonal declines in vitamin D status may trigger SLE flare in non-African Americans. *Lupus* 21: 855–864, 2012
- Seelen MA, Roos A, Wieslander J, Mollnes TE, Sjöholm AG, Wurzner R, Loos M, Tedesco F, Sim RB, Garred P, Alexopoulos E, Turner MW, Daha MR: Functional analysis of the classical, alternative, and MBL pathways of the complement system: Standardization and validation of a simple ELISA. *J Immunol Methods* 296: 187–198, 2005
- Sinico RA, Rimoldi L, Radice A, Bianchi L, Gallelli B, Moroni G: Anti-C1q autoantibodies in lupus nephritis. *Ann N Y Acad Sci* 1173: 47–51, 2009
- Kohro-Kawata J, Wener MH, Mannik M: The effect of high salt concentration on detection of serum immune complexes and autoantibodies to C1q in patients with systemic lupus erythematosus. *J Rheumatol* 29: 84–89, 2002
- Antes U, Heinz HP, Loos M: Evidence for the presence of autoantibodies to the collagen-like portion of C1q in systemic lupus erythematosus. *Arthritis Rheum* 31: 457–464, 1988
- Wisniewski JJ, Jones SM: Comparison of autoantibodies to the collagen-like region of C1q in hypocomplementemic urticarial vasculitis syndrome and systemic lupus erythematosus. *J Immunol* 148: 1396–1403, 1992
- Vanhecke D, Roumenina LT, Wan H, Osthoff M, Schaller M, Trendelenburg M: Identification of a major linear C1q epitope allows detection of systemic lupus erythematosus anti-C1q antibodies by a specific peptide-based enzyme-linked immunosorbent assay. *Arthritis Rheum* 64: 3706–3714, 2012
- Mannik M, Wener MH: Deposition of antibodies to the collagen-like region of C1q in renal glomeruli of patients with proliferative lupus glomerulonephritis. *Arthritis Rheum* 40: 1504–1511, 1997
- Lachmann PJ: Conglutinin and immunoconglutinins. *Adv Immunol* 6: 479–527, 1967
- Durand CG, Burge JJ: A new enzyme-linked immunosorbent assay (ELISA) for measuring immunoconglutinins directed against the third component of human complement. Findings in systemic lupus erythematosus. *J Immunol Methods* 73: 57–66, 1984
- Nilsson B, Ekdahl KN, Svarvare M, Bjelle A, Nilsson UR: Purification and characterization of IgG immunoconglutinins from patients with systemic lupus erythematosus: Implications for a regulatory function. *Clin Exp Immunol* 82: 262–267, 1990
- Nilsson B, Ekdahl KN, Sjöholm A, Nilsson UR, Sturfelt G: Detection and characterization of immunoconglutinins in patients with systemic lupus erythematosus (SLE): Serial analysis in relation to disease course. *Clin Exp Immunol* 90: 251–255, 1992
- Iseman DE, Cooper NR: The structure and function of the third component of human complement–I. The nature and extent of conformational changes accompanying C3 activation. *Mol Immunol* 18: 331–339, 1981
- Alsenz J, Becherer JD, Nilsson B, Lambris JD: Structural and functional analysis of C3 using monoclonal antibodies. *Curr Top Microbiol Immunol* 153: 235–248, 1990
- Birmingham DJ, Irshaid F, Nagaraja HN, Zou X, Tsao BP, Wu H, Yu CY, Hebert LA, Rovin BH: The complex nature of serum C3 and C4 as biomarkers of lupus renal flare. *Lupus* 19: 1272–1280, 2010
- Trouw LA, Groeneveld TW, Seelen MA, Duijts JM, Bajema IM, Prins FA, Kishore U, Salant DJ, Verbeek JS, van Kooten C, Daha MR: Anti-C1q autoantibodies deposit in glomeruli but are only pathogenic in combination with glomerular C1q-containing immune complexes. *J Clin Invest* 114: 679–688, 2004
- Kenyon KD, Cole C, Crawford F, Kappler JW, Thurman JM, Bratton DL, Boackle SA, Henson PM: IgG autoantibodies against deposited C3 inhibit macrophage-mediated apoptotic cell engulfment in systemic autoimmunity. *J Immunol* 187: 2101–2111, 2011

Received: April 10, 2015 Accepted: October 1, 2015

Published online ahead of print. Publication date available at www.cjasn.org.

See related editorial, “Lupus Nephritis in the Era of Biomarkers,” on pages 4–5.

This article contains supplemental material online at <http://cjasn.asnjournals.org/lookup/suppl/doi:10.2215/CJN.03990415/-/DCSupplemental>.