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Cell-bound complement activation products associate with lupus severity in SLE

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ABSTRACT

Objectives To evaluate the association between lupus severity and cell-bound complement activation products (CB-CAPs) or low complement proteins C3 and C4.

Methods All subjects (n=495) fulfilled the American College of Rheumatology (ACR) classification criteria for SLE. Abnormal CB-CAPs (erythrocyte-bound C4d or B-lymphocyte-bound C4d levels >99th percentile of healthy) and complement proteins C3 and C4 were determined using flow cytometry and turbidimetry, respectively. Lupus severity was estimated using the Lupus Severity Index (LSI). Statistical analysis consisted of multivariable linear regression and groups comparisons.

Results Abnormal CB-CAPs were more prevalent than low complement values irrespective of LSI levels (62% vs 38%, respectively, p<0.0001). LSI was low (median 5.44, IQR: 4.77–6.93) in patients with no complement abnormality, intermediate in patients with abnormal CB-CAPs (median 6.09, IQR: 5.31–8.20) and high in the group presenting with both abnormal CB-CAPs and low C3 and/or C4 (median 7.85, IQR: 5.51–8.37). Odds of immunosuppressant use was higher in subjects with LSI ≥5.95 compared with subjects with LSI <5.95 (1.60 vs 0.53, p<0.0001 for both). Multivariable regression analysis revealed that higher LSI scores associated with abnormal CB-CAPs—but not low C3/C4—after adjusting for younger age, race and longer disease duration (p=0.0001), which were also independent predictors of disease severity (global R^2=0.145).

Conclusion Abnormalities in complement activation as measured by CB-CAPs are associated with increased LSI.

INTRODUCTION

SLE is a complex, chronic autoimmune disease characterised by autoantibody production and immune system dysregulation resulting in multiorgan inflammation and potentially damage.

Low complement levels, commonly found in patients with SLE, generally indicate complement activation, although they could represent decreased synthesis. Typical complement assessments include serum determinations of C3, C4 and in some cases CH50. Low complement is not included in the American College of Rheumatology (ACR) 1997 classification criteria[1]; however, it is a component of the criteria put forth by the Systemic Lupus Erythematosus International Collaborating Clinics[2] and the European League Against Rheumatism and ACR[3] because it is an important feature of dysregulated immunity in SLE.[4]

We and others have shown previously that complement activation can be measured by the accumulation of C4d on the surface of haematopoietic cells, including erythrocyte-bound C4d (EC4d) and B-lymphocyte-bound C4d (BC4d). These biomarkers, collectively known as cell-bound complement activation products (CB-CAPs),[5] have superior diagnostic accuracy for lupus[6 7] compared with low serum complement.[8] Beyond aiding in diagnosing SLE, CB-CAPs are useful for monitoring SLE disease activity as prognostic biomarkers, although their utility as predictive biomarkers awaits further study.[9]

Variability in disease manifestations and severity between different patients as well as within patients over their disease course is common. Although an instrument such as the Safety of Estrogen in Lupus National Assessment - Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI) reflects disease activity around the time of clinical evaluation, it does not measure cumulative disease burden. As cumulative disease severity can lead to organ damage, instruments that measure disease severity are important to identify subjects at risk of major organ involvement. The Lupus Severity Index (LSI) was

developed as a tool for scoring a subject's disease severity based on weighting of ACR classification criteria derived from one's treatment history.[10] The elements necessary to score the LSI do not require an in-person examination and are easily available in medical records and research datasets. Thus, the LSI can be calculated retrospectively for large subject cohorts.

In this cross-sectional study of patients with SLE, we correlated standard complement (C3 and C4) and CB-CAPs with LSI scores.

METHODS

Study population

This study reports a cross-sectional subanalysis of studies enrolling adult subjects with SLE (n=495) at 17 academic centres. Central or internal review boards approved the studies and subjects provided informed consent.

All subjects were diagnosed with SLE and fulfilled the 1997 ACR[1] classification criteria. Medication regimens were available for 438 subjects; 209 were on one or more immunosuppressants, including methotrexate (n=40), azathioprine (n=53), mycophenolate (n=105), belimumab (n=12), rituximab (n=2), oral ciclosporin (n=2), cyclophosphamide (n=3) or intravenous immunoglobulins (n=1).

Venous blood samples were tested at Exagen in our clinical laboratory accredited by the College of American Pathologists.

Lupus severity index

LSI was determined by weighting and summation of ACR criteria and subcriteria as previously described[10] (see online supplementary material).

Biomarker analysis

Serum C3 and C4 were determined by immunoturbidimetry (The Binding Site, San Diego, California, USA)[11] and were considered low if below the manufacturer's lower limits of normal (81.1 and 12.9 mg/dL for C3 and C4, respectively). Low complement status refers to low C3 and/or low C4.

Complement activation was determined using CB-CAPs measured by quantitative flow cytometry.[11] CB-CAPs were considered abnormal if levels of EC4d and/or BC4d were above the 99th percentile of a group of healthy individuals (>14 and >60 net mean fluorescence intensity, respectively).[7 8 11] Abnormal CB-CAPs status refers to abnormal EC4d and/or abnormal BC4d.

Anti-double-stranded DNA (dsDNA) antibodies were determined by ELISA (Quanta Lite, Inova Diagnostics, San Diego, California, USA). All serum samples above 301 IU/mL were further tested by indirect immunofluorescence assay (IFA) using the *Crithidia luciliae* assay (Nova-Lite, Inova Diagnostics). Anti-dsDNA antibodies were considered positive if confirmed by IFA.

Statistical analysis

Multivariable linear regression analysis was used to model the relative contributions of low complement and elevated CB-CAPs to LSI with race, gender, age and disease duration as covariates. Regression analysis of a subset of patients included medication and renal disease activity as additional covariates. McNemar's test was used to compare CB-CAPs to standard complement testing. Fisher's exact test, analysis of variance and Kruskal-Wallis tests were used for group comparisons as appropriate. Odds of immunosuppressant use were evaluated by binomial distribution analysis.

RESULTS

The demographic characteristics of the 495 subjects included in this study are reported in table 1.

Overall, per cent positivity was 62% for CB-CAPs and 38% for low complement (p<0.0001). Anti-dsDNA was positive in approximately a third of the population (table 1).

Median LSI was 5.95 (IQR (5.20–8.17)) and scores ranged from 3.27 to 9.38 (mean±SD=6.48±1.6). The majority of subjects were females (91%) and presented with LSI slightly lower (median 5.9 (5.2–8.2)) compared with males (median 7.5 (5.3–8.3)). LSI was highest in Asian subjects (7.1 (5.4–8.3)), followed by African-American/black (6.7 (5.4–8.2)), Latino/Hispanic (6.6 (5.4–8.2)), other races (5.8 (5.4–8.0)), and lowest in the Caucasian/white subjects (5.6 (4.9–8.0)) (p<0.001).

Among the 495 subjects, 153 had both low complement and abnormal CB-CAPs, 153 had abnormal CB-CAPs alone, 37 had low complement alone and 152 presented with no complement abnormalities (normal complement and normal CB-CAPs). LSI score was highest in the double positive group, intermediate in the subjects with low complement or abnormal CB-CAPs only and lowest in those with neither abnormality (p<0.001) (table 1).

As the LSI distribution across the entire patient population showed two peaks, similar to findings by Bello *et al*[10] (online supplementary figure 1), subjects were divided into two groups based on LSI: 247 subjects had low LSI (LSI <5.95, median 5.21 (4.66–5.49)) and 248 had high LSI (LSI ≥5.95, median 8.17 (7.54–8.51)). Both low complement and abnormal CB-CAPs were more prevalent in the high LSI group; interestingly, abnormal CB-CAPs was more prevalent than low complement in both groups (p<0.0001 for both) (figure 1).

Binomial distribution analysis showed that the odds of immunosuppressant use in subjects with LSI ≥5.95 was 1.60 while it was 0.53 in the subjects with LSI <5.95 (p<0.0001 for both).

Univariate analysis results revealed that younger age at visit, younger age at diagnosis, low complement (C3 and/or C4), race, positive anti-dsDNA, use of corticosteroids or immunosuppressants and abnormal CB-CAPs (EC4d and/or BC4d) associated with higher LSI (table 2, top). Multivariable linear regression analysis revealed abnormal CB-CAPs, younger age at visit, longer disease duration, and race as the strongest predictors of current LSI (table 2, bottom); low complement (estimate=0.279±0.152,

Table 1 Subject characteristics

Characteristic	Total n=495		Both n=153		CB-CAPs Only n=153		Complement Only n=37		Neither n=152	
Gender										
Male	45	9.1	9	5.9	18	11.8	3	8.1	15	9.9
Female	450	91.0	144	94.1	135	88.2	34	91.9	137	90.1
Race/ethnicity										
Asian	38	7.7	16	10.5	9	5.9	3	8.1	10	6.6
Black/African-American	142	28.7	51	33.3	47	30.7	11	29.7	33	21.7
Hispanic/Latino	90	18.2	34	22.2	29	19.0	5	13.5	22	14.5
Other	13	2.6	4	2.6	5	3.3	1	2.7	3	2.0
White/Caucasian	212	42.8	48	31.4	63	41.2	17	45.9	84	55.3
Anti-dsDNA positivity	158	31.9	85	55.6	47	30.7	12	32.4	14	9.2
History of renal disease	196	39.6	83	54.2	64	41.8	14	37.8	35	23.0
Age at visit	39.31	29.26 to 50.93	31.89	24.82 to 41.90	41.06	30.20 to 52.17	38.03	30.37 to 51.05	45.69	35.03 to 57.39
Age at diagnosis	27.31	19.96 to 38.31	22.21	17.95 to 29.05	26.97	20.94 to 37.49	32.08	22.08 to 41.64	33.10	24.70 to 45.20
Time since diagnosis	8.53	3.45 to 16.34	8.60	4.00 to 15.04	8.11	2.82 to 19.08	8.05	2.37 to 13.02	8.88	4.03 to 16.34
Use of hydroxychloroquine	327	74.7	105	80.2	99	71.2	21	70.0	102	73.9
Use of corticosteroids	267	61.0	99	75.6	92	66.2	18	60.0	58	42.0
Use of immunosuppressants	209	47.7	72	55.0	66	47.5	13	43.3	58	42.0
Lupus Severity Index	5.95	5.20 to 8.17	7.85	5.51 to 8.37	6.09	5.31 to 8.20	5.61	4.71 to 7.87	5.44	4.77 to 6.93

Demographic information for the total group and each of the groups stratified by CB-CAPs and standard complement positivity (low complement proteins C3 and/or C4). CB-CAPs only group are subjects with positivity of CB-CAPs, but with normal serum complement proteins C3 and C4. Complement only group are subjects with low complement (C3, C4, or both), but normal CB-CAPs. Data are presented as number (per cent) or median (IQR). Medication information was available for 438 patients (both n=131; CB-CAPs only n=139; complement only n=30; neither n=138).

CB-CAPs, cell-bound complement activation products; dsDNA, double-stranded DNA.

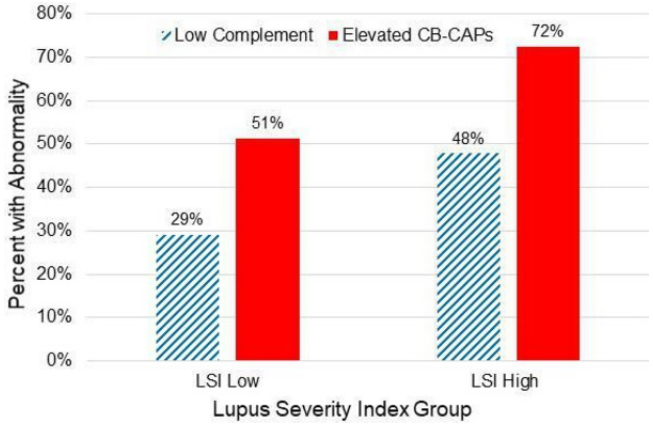

Figure 1 Comparison of low complement and elevated cell-bound complement activation products (CB-CAPs) by Lupus Severity Index (LSI) group. Percentage of subjects with low complement (low serum complement proteins C3 and/or C4) and elevated CB-CAPs (elevated EC4d and/or BC4d) by LSI groups.

p=0.098) and gender (estimate=0.438±0.240, p=0.068) were not significantly associated with the LSI and, therefore, were not included in the model (see online supplementary table 1 for an additional analysis that included low complement and gender and online supplementary table 5 for an additional analysis that included positive anti-dsDNA, low complement and gender). Overall, the model accounted for 14.5% of the total variability of the LSI, and abnormal CB-CAPs alone remained a significant predictor of current LSI (estimate=0.697±0.148, p<0.0001) after adjusting for age at visit, time since diagnosis and race.

Analysis of a subset of patients for whom medication information was available (n=438) found that use of immunosuppressants correlated with higher LSI (online supplementary table 2) when adjusting for covariates of CB-CAPs, race, gender, age and disease duration (parameter estimate 0.727, p<0.0001). In a subset of patients for whom SELENA-SLEDAI renal activity information was available (n=446), renal activity correlated with LSI (parameter estimate 1.937, p<0.0001) after adjusting for age, disease duration and CB-CAPs (online supplementary table 3). In patients for whom both medication and renal activity information was available (n=402), CB-CAPs remained significant predictors of LSI (parameter estimate 0.760, p<0.0001) after controlling for immunosuppressant use, renal activity and age at visit (online supplementary table 4). Additionally, we show the progressive effect on the CB-CAPs' significance as predictors of LSI as additional variables are added to the final parsimonious model, and the resulting R^2 of each model are detailed in online supplementary table 6.

DISCUSSION

Complement activation has a central role in SLE.[5] In this study, we evaluated the contribution of complement activation and, more specifically, of the classical complement pathway, on SLE severity using the LSI.

In our cross-sectional, multicentre study of 495 well-characterised lupus subjects, we found that complement abnormalities (both low C3/C4 and abnormal CB-CAPs) were more prevalent in subjects with more severe disease based on their LSI score. When subjects were stratified by LSI into low and high groups, a higher percentage of individuals in both groups were CB-CAPs positive compared with having low complement. This is consistent with the higher sensitivity of CB-CAPs compared with low complement in SLE observed in this and other studies[6–8] and indicates that complement activation as measured by CB-CAPs reflects disease severity more accurately than low C3/C4. Consistent with these data, although low standard complement C3/C4 associated with elevated LSI, the association was no longer significant after adjusting for age and race. However, CB-CAPs correlated with LSI and remained significant after adjusting for race, age and time since diagnosis. LSI was highest in Asian subjects, followed by African-American/black subjects, Latino/Hispanic subjects, other and lowest in the Caucasian/white subjects. These racial differences in LSI are in agreement with previous data[10] and with the fact that SLE is more aggressive in non-white individuals.[12]

Younger age at diagnosis and younger age at visit were associated with higher LSI, as well as longer time between diagnosis and visit. Due to collinearity between age at diagnosis and age at visit, age at diagnosis was not included as a covariate.

Racial and age-related factors are known to have an impact on lupus severity.[12–14] We now show that abnormalities in the complement system and, in particular, classical complement activation as measured by CB-CAPs, are associated with increased LSI.

As the LSI was derived from immunosuppressant use as a proxy for disease severity,[10] it is not surprising that the odds of immunosuppressant use was high in the subjects with more severe disease (LSI ≥5.95). In addition, the analysis of a subset of patients with available medication information showed that use of immunosuppressants correlated (but was not collinear) with LSI scores as determined by stepwise multivariable analysis.

LSI also correlated with SELENA-SLEDAI renal activity in a subset analysis. Though these data were not available for all patients in our dataset, these additional analyses demonstrated that CB-CAPs remained significant predictors of LSI when adjusting for significant covariates.

The cross-sectional evaluation of a time-expanded concept like the LSI is a limitation of this study. In addition, disease severity in SLE can be influenced by multiple factors, while the LSI is based only on weighted ACR criteria and subcriteria. In the original validation study, the LSI predicted early mortality, however, it may not capture all the elements that contribute to disease severity in SLE.[10] Calculation of the Katz Lupus Severity of Disease index,[15] which reflects disease damage, was not

Table 2 Top: Association of LSI with low complement, elevated CB-CAPs, race/ethnicity, gender, age, age at diagnosis and disease duration (time since diagnosis) as determined by univariate analysis. These variables, except age at diagnosis due to collinearity with age at visit, were included in a stepwise model building analysis. Bottom: Association of LSI with elevated CB-CAPs, race/ethnicity, age and disease duration (time since diagnosis), as determined by stepwise multivariable analysis. The model found that gender and low complement (low C3 and/or C4) were not significant when controlling for the other variables (p=0.068 and p=0.098, respectively); therefore, they were not included in the final model (R^2=0.145)

Top: Univariate analysis

Factor	Parameter estimate	SE	95% CI	P value
Gender				
Male	0.440	0.256	−0.062 to 0.942	0.0859
Female	Ref			
Race/ethnicity				
Asian	0.709	0.284	0.151 to 1.268	0.0129
Black/African-American	0.660	0.175	0.316 to 1.003	0.0002
Hispanic/Latino	0.600	0.203	0.201 to 0.999	0.0033
Other	0.602	0.461	−0.303 to 1.508	0.1920
White/Caucasian	Ref			
Age at visit	−0.025	0.005	−0.035 to −0.015	<0.0001
Age at diagnosis	−0.035	0.005	−0.045 to −0.024	<0.0001
Time since diagnosis	0.011	0.008	−0.005 to 0.027	0.1793
Low C3 and/or C4	0.648	0.149	0.356 to 0.940	<0.0001
Elevated CB-CAPs	0.958	0.145	0.672 to 1.244	<0.0001
Anti-dsDNA positivity	0.548	0.156	0.241 to 0.855	0.0005
Use of hydroxychloroquine	−0.221	0.180	−0.575 to 0.134	0.2221
Use of corticosteroids	0.641	0.158	0.330 to 0.952	<0.0001
Use of immunosuppressants	0.893	0.151	0.595 to 1.190	<0.0001

CB-CAPs, cell-bound complement activation products; dsDNA, double-stranded DNA; LSI, Lupus Severity Index.

Bottom: Final multivariable model

Factor	Parameter estimate	SE	95% CI	P value
Race/ethnicity				
Asian	0.646	0.272	0.112 to 1.179	0.0177
Black/African-American	0.466	0.173	0.127 to 0.805	0.0071
Hispanic/Latino	0.395	0.197	0.008 to 0.781	0.0456
Other	0.435	0.441	−0.432 to 1.301	0.3246
White/Caucasian	Ref			
Age at visit	−0.024	0.006	−0.035 to −0.013	<0.0001
Time since diagnosis	0.033	0.008	0.016 to 0.049	0.0001
Elevated CB-CAPs	0.697	0.148	0.406 to 0.987	<0.0001

possible in this study as some of the elements of this index were not collected for all patients in the dataset.

The findings of this study expand on our previous work that shows association of CB-CAPs and, in particular, EC4d, with SLE disease activity.[9] Taken together, our data suggest that complement activation as measured by CB-CAPs parallels disease activity and associates with disease severity, especially in younger or non-white subjects and in those with long-standing disease. However, data need to be interpreted with caution as the model accounted for a small fraction (14.5%) of the total variability of the LSI.

Evaluation of the ability of CB-CAPs to predict future lupus severity through inception and longitudinal studies will be important to the field.

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Competing interests RVA, JC, TO and TD are current or former employees of Exagen. AW is a consultant to Exagen and chief medical officer. CA, SN, AS, CEC, DJW, EM, KCK, CP, RR-G, JPB, AA, RF, SM, JA have received research support from Exagen.

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