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Complement Activation in Patients With Probable Systemic Lupus Erythematosus and Ability to Predict Progression to American College of Rheumatology-Classified Systemic Lupus Erythematosus

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Objective. To evaluate the frequency of cell-bound complement activation products (CB-CAPS) as a marker of complement activation in patients with suspected systemic lupus erythematosus (SLE) and the usefulness of this biomarker as a predictor of the evolution of probable SLE into SLE as classified by the American College of Rheumatology (ACR) criteria.

Methods. Patients in whom SLE was suspected by lupus experts and who fulfilled 3 ACR classification criteria for SLE (probable SLE) were enrolled, along with patients with established SLE as classified by both the ACR and the Systemic Lupus International Collaborating Clinics (SLICC) criteria, patients with primary Sjögren's syndrome (SS), and patients with other rheumatic diseases. Individual CB-CAPS were measured by flow cytometry, and positivity rates were compared to those of commonly assessed biomarkers, including serum complement proteins (C3 and C4) and autoantibodies. The frequency of a positive multianalyte assay panel (MAP), which includes CB-CAPS, was also evaluated. Probable SLE cases were followed up prospectively.

Results. The 92 patients with probable SLE were diagnosed more recently than the 53 patients with established SLE, and their use of antirheumatic medications was lower. At the enrollment visit, more patients with probable SLE were positive for CB-CAPS (28%) or MAP (40%) than had low complement levels (9%) (P = 0.0001 for each). In probable SLE, MAP scores of >0.8 at enrollment predicted fulfillment of a fourth ACR criterion within 18 months (hazard ratio 3.11, P < 0.01).

Conclusion. Complement activation occurs in some patients with probable SLE and can be detected with higher frequency by evaluating CB-CAPS and MAP than by assessing traditional serum complement protein levels. A MAP score above 0.8 predicts transition to classifiable SLE according to ACR criteria.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a clinically heterogeneous autoimmune disease characterized by the presence of diverse autoantibodies and activation of the complement system (1). The classification criteria for SLE by the American College of Rheumatology (ACR) (2) and more recently by the Systemic Lupus International Collaborating Clinics (SLICC) (3)—both developed...
for research purposes (3,4)—recognize this clinical and laboratory heterogeneity. Low levels of serum complement protein (C3 and C4) are included in the SLICC criteria as well as the classification criteria newly developed by the European League Against Rheumatism (EULAR) and the ACR (5), due to the relatively high specificity of complement activation leading to low serum complement in SLE (6).

Despite the specificity of hypocomplementemia, its frequency in SLE is low (1). We have previously shown that complement activation, measured reliably by assessing cell-bound complement activation products (CB-CAPs), especially C4d bound to erythrocytes (EC4d) and to B lymphocytes (BC4d), can be detected in SLE with greater frequency than by assessing high anti-double-stranded DNA (anti-dsDNA) and low serum complement proteins (7,8).

Many patients with suspected SLE who do not fulfill ACR criteria have been designated as having “probable, possible, latent, or incomplete” SLE (9–12). There is no consensus definition or nomenclature for these patients (13). However, some patients develop classifiable SLE over time (9–11). Currently, there are no biomarkers to reliably distinguish who, among patients with probable SLE, will develop SLE by classification criteria. However, early diagnosis and appropriate intervention may prevent lupus flares and more serious organ inflammation (9,14,15).

We hypothesized that probable SLE which ultimately develops into classifiable SLE may have detectable complement activation (1). Therefore, we conducted a cross-sectional and prospective study of patients with probable SLE to determine the frequency of elevated CB-CAPs in these patients and whether the presence of CB-CAPs, measured either directly or within a multianalyte assay panel (MAP), is predictive of development of classifiable SLE.

PATIENTS AND METHODS

Study populations. Adult patients were enrolled, in compliance with the Helsinki Declaration, from 2015 to 2017. Central or internal review boards at 7 academic institutions approved the study, and all subjects provided informed consent. Patients were recruited from lupus cohorts and faculty practices overseen by an experienced SLE investigator.

Patients with SLE fulfilled both the ACR classification criteria (2) and the SLICC classification criteria (3) for SLE at enrollment. Patients with probable SLE were enrolled if they fulfilled 3 ACR criteria, irrespective of whether they fulfilled the SLICC criteria, and if the investigator had a high suspicion of the diagnosis of lupus. Patients with probable SLE could not be enrolled if they had proteinuria of >200 mg or biopsy-proven lupus nephritis. Investigators were asked to examine the historic electronic records for clinical, hematologic, and immunologic features. The date of diagnosis for probable SLE was the date on which the third ACR criterion was confirmed.

Patients with probable SLE were followed up prospectively, and 69 patients had a first follow-up visit 9–18 months after enrollment. Investigators determined whether patients met a fourth ACR criteria at the follow-up visit and the approximate date that classifiable SLE occurred, either at or prior to evaluation.

Disease activity was measured in SLE and probable SLE using the Safety of Estrogens in Lupus Erythematosus National Assessment (SELENA) version of the SLE Disease Activity Index (SLEDAI) (16). Low complement and anti-dsDNA levels were scored if they were shown to be abnormal in the central clinical laboratory (Exagen, Vista, CA). Nonserologic SELENA–SLEDAI was calculated by excluding the anti-dsDNA and complement components from the score.

This study also included patients with primary Sjögren’s syndrome (SS) and other well-defined rheumatic diseases, who served as controls. For the latter group (n = 51), clinical diagnosis was based on the expert opinion of the investigators, and the group included patients with rheumatoid arthritis (RA) (n = 31), psoriatic arthritis (n = 10), dermatomyositis (n = 4), juvenile idiopathic arthritis (n = 3), systemic sclerosis (n = 2), and ankylosing spondylitis (n = 1). Diagnosis of SS was based on a modification of the ACR criteria for SS (17), and patients were enrolled if positive for 2 of following 3 features: 1) current keratoconjunctivitis sicca in ≥1 eye with either an ocular staining score of ≥3 or a Schirmer’s test result of ≤5 mm in 5 minutes; 2) labial or salivary biopsy with a positive focus score (≥1 focus/4 mm²); 3) positive serum anti-SSA/Ro and/or anti-SSB/La and antinuclear antibody (ANA) titer of ≥1:80 determined by immunofluorescence assay (IFA).

Each of the 7 sites recruited patients with SLE, probable SLE, and other rheumatic diseases; patients with SS were recruited at 6 sites. Case report forms from the enrollment visit of all patients with probable SLE and 32 randomly selected patients with SLE were reviewed and adjudicated by a lupus expert clinician (KCK) not affiliated with any institution enrolling patients in the study. Case report forms from the probable SLE patient follow-up visits were adjudicated by 2 of the authors (KCK [n = 52] and AW [n = 17]) without knowledge of the results of the laboratory tests performed by Exagen (see below). The adjudicators assessed clinical features and routine laboratory tests, including complete blood cell counts and urinalyses, and the investigators were often asked to provide additional records as needed.

Patients provided venous blood samples that were collected in EDTA-containing tubes and serum separator tubes at all visits. Specimens were shipped overnight to Exagen for diagnostic immunology testing.

Biomarker analysis. ANA was measured using an enzyme-linked immunosorbent assay (ELISA) (QUANTA Lite; Inova Diagnostics) and indirect IFA (NOVA Lite; Inova Diagnostics) as described previously (8,18). Anti-dsDNA antibodies were also measured by ELISA and were confirmed using an IFA with Cricthidia luciliae (8,18). Autoantibodies to extractable nuclear antigens
(anti-Sm, anti-SSB/La, anti-SSA/Ro, anti-CENP, anti-Jo-1, and anti-Scl-70), anti-cyclic citrullinated peptide (anti-CCP) antibodies, and rheumatoid factor (RF) were measured using the EIA test on the Phadia 250 platform (ThermoFisher Scientific) (19). The IgG, IgM, and IgA isotypes of anticardiolipin and anti-β2-glycoprotein I, and the IgG isotype of anti-phosphatidylserine/prothrombin were measured using chemiluminescence immunoassay or ELISA.

Serum complement proteins C3 and C4 were measured by standard immunoturbidimetry assay (The Binding Site) (19) and were considered low if they were below the manufacturer’s cutoff levels (81.1 mg/dl and 12.9 mg/dl, respectively). Individual CB-CAPs (EC4d and BC4d) were measured by quantitative flow cytometry and expressed as net mean fluorescence intensity (MFI), as previously described (8,19). CB-CAP positivity was determined by a net MFI of >14 for EC4d and/or a net MFI of >60 for BC4d. Assessment of CB-CAPs was not available for 3 patients (2 SLE and 1 SS) at enrollment. These patients were included in all other analyses.

The MAP with algorithm, which included EC4d, BC4d, ANA, anti-dsDNA, anti-Sm, as well as other lupus and non-lupus autoantibodies, was evaluated as described in detail elsewhere (8,18,19). MAP assessment was not available for 3 patients (1 SLE, 1 probable SLE, and 1 SS) at enrollment; these subjects were included in all other analyses. For 1 of the follow-up visits, BC4d and MAP determination was not available; this visit was included in all other analyses.

**Statistical analysis.** Statistical comparisons were conducted using unpaired t-test, Mann-Whitney test, Fisher’s exact test, chi-square test, or McNemar’s test, as appropriate (GraphPad). Sensitivity, specificity, positive and negative likelihood

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**Figure 1.** American College of Rheumatology (ACR) criteria for systemic lupus erythematosus (SLE) at enrollment. Clinical and immunologic 1997 ACR criteria were evaluated in the entire population of patients with SLE and patients with probable SLE (pSLE) (A) and in the probable SLE subgroups fulfilling or not fulfilling the 2012 Systemic Lupus International Collaborating Clinics (SLICC) criteria (B) at enrollment. All criteria, including antinuclear antibody (ANA) and immunologic criteria, refer to historical positivity. The average number of ACR criteria fulfilled by the patients with SLE was 5.3, while those with probable SLE fulfilled 3 ACR criteria (per study protocol). Statistically significant differences are indicated with P values, obtained by Fisher’s exact test.
Calc Software), for time to fulfillment of the fourth ACR criterion. For statistical analysis, MAP-indeterminate assessments (14 subjects) and equivocal assessments (8 subjects) at enrollment were considered positive or negative based on their actual MAP score value.

Follow-up data on the patients with probable SLE were analyzed using Fisher’s exact test and a Kaplan-Meier curve with log rank test and Cox proportional hazards model (MedCalc Software), for time to fulfillment of the fourth ACR criterion. Analyse-it software was used for the receiver operating characteristics curve and decision plot analyses.

RESULTS

Study populations. Baseline. A total of 246 patients were included in this study: 53 patients with SLE, 92 with probable SLE, 50 with SS, and 51 with other rheumatic diseases. Of the 92 patients with probable SLE, 35 (38%) met the SLICC classification criteria at enrollment. Patients who did not meet the criteria for study enrollment or for whom the adjudicator could not make a definite determination were excluded from the study, and follow-up visits were not required (see Supplementary Table 1, on the Arthritis & Rheumatology web site at http://onlinelibrary.wiley.com/doi/10.1002/art.41093/abstract). A higher percentage of patients with probable SLE were white compared to patients with SLE (P = 0.002). The mean number of years since diagnosis of probable SLE was lower than that of SLE (3.6 ± 4.9 years and 9.6 ± 9.4 years, respectively; P = 0.0001), which is consistent with the possibility that patients with probable SLE were enrolled early in their disease. The demographic characteristics of patients with probable SLE who fulfilled the SLICC criteria were similar to those of patients who did not.

The ACR criteria and the SLICC criteria fulfilled by the SLE and probable SLE patients at enrollment are reported in Figure 1 and Figure 2. According to study protocol, all subjects fulfilled the criterion of historical ANA positivity, and none of the patients with probable SLE had renal proteinuria.

Fulfillment rates of individual ACR criteria (Figure 1B) and SLICC criteria (Figure 2B) were similar among patients with probable SLE who did or did not satisfy SLICC classification criteria, apart from historical low complement levels and alopecia. The presence of historical anti-Sm antibodies approached significance (P = 0.052) (Figure 2B).

Disease activity in both patients with SLE and patients with probable SLE was mild at enrollment (see Supplementary Data and Supplementary Table 3, http://onlinelibrary.wiley.com/doi/10.1002/art.41093/abstract). Hydroxychloroquine (HCQ) and prednisone use were significantly lower in patients with probable SLE compared to patients with SLE, despite the physician’s opinion that the patients with probable SLE likely had SLE (Supplementary Figures 1A and 1B, http://onlinelibrary.wiley.com/doi/10.1002/art.41093/abstract).

Follow-up. Sixty-nine patients with probable SLE completed a follow-up visit 9–18 months after enrollment. Follow-up visits were conducted at all sites, and there were no differences in age, sex, time since diagnosis, race/ethnicity, or fulfillment of SLICC criteria or individual ACR criteria between the group of patients who had a follow-up visit in this time frame (n = 69) and those who did not (n = 23) (data not shown). Disease activity was slightly higher in the group that did not attend a follow-up visit (nonserologic SELENA–SLEDAI 2.09 versus 1.09; P = 0.04).

Of the 69 patients who completed a follow-up visit, 20 (29%) fulfilled ≥1 additional ACR criterion. Hematologic disorder was the criterion fulfilled with highest frequency (50%), followed by oral ulcers (19%), immunologic disorder (8%), serositis (8%), arthritis (8%), photosensitivity (4%), and rash (4%). Of the 13 patients who fulfilled an additional hematologic criterion at follow-up, 10 had lymphopenia as their sole feature. Of these, 3 were receiving medications that might have affected lymphocyte counts: azathioprine 50 mg/day, methotrexate 15 mg/week, and/or mycophenolate 2,000 mg/day plus prednisone 2.5 mg/day. Investigators concluded that the lymphopenias were due to SLE.

Fulfillment of the SLICC criteria at enrollment did not predict fulfillment of the ACR criteria 18 months later. Of the 69 patients with probable SLE who completed a follow-up visit, 26 (38%) had fulfilled SLICC criteria at enrollment and 43 (62%) had not. Ten of the 26 patients with probable SLE who had fulfilled SLICC at enrollment (38.5%) acquired additional ACR classification criteria versus 10 of the 43 (23.3%) who had not fulfilled SLICC criteria at enrollment (P = 0.27). In addition, fulfillment of the SLICC criteria at baseline did not lead to faster fulfillment of the ACR criteria (P = 0.19 by Cox regression) (Supplementary Table 4, http://onlinelibrary.wiley.com/doi/10.1002/art.41093/abstract). Six patients converted from SLICC criteria–negative to SLICC criteria–positive at follow-up, 5 of whom also transitioned to ACR-classifiable SLE.

The number of organ manifestations at enrollment did not contribute to progression. Among the 69 patients with probable
SLE who had a follow-up visit, 41 (59%) had 1 organ manifestation (including hematologic, excluding immunologic), and 28 (41%) had 2. Of the 41 patients with 1 organ involved, 14 (34%) transitioned to classifiable SLE, whereas of the 28 patients with 2 organs involved, 6 (21%) transitioned. This difference was not significant ($P = 0.29$).

At follow-up, HCQ use increased from 63% to 74%. Among the 20 patients who converted to classifiable SLE, HCQ use increased from 75% to 80% (data not shown).

**Biomarker analysis at baseline and follow-up.**

Most patients with SLE and probable SLE were ANA-positive on the day of the enrollment visit (Table 1). The ANA IFA assay used in this study was more sensitive than an ELISA in most cases, although ANA positivity is known to vary greatly depending on the assay platform or kit used (25,26). Specificity of ANA for SLE was low, while anti-dsDNA had a high specificity for SLE, as 2% of the patients with SS and none of the patients with other diseases were anti-dsDNA-positive.
Anti-dsDNA sensitivity was low for SLE (38%) and even lower for probable SLE (11%), although among patients with probable SLE, positivity for anti-dsDNA was more frequent in those who fulfilled the SLICC criteria than in those who did not (20% versus 5%; \( P = 0.039 \)). Unsurprisingly, anti-SSA and anti-SSB positivity rates were highest among SS patients [27], whereas RF and anti-CCP positivity was observed mainly in RA patients [28] (Table 1).

Although a high percentage of patients with SLE and probable SLE had historically low complement levels (64% and 36%, respectively) (Figure 2), complement protein levels were low on the day of enrollment in a minority of patients with SLE and probable SLE (23% and 9%, respectively) (Table 1), consistent with findings from previous studies [8,18]. At baseline, anti-C1q antibodies were present in 34% of patients with SLE and 13% of patients with probable SLE. Other plasma complement-related markers were not measured. The positivity rate for antiphospholipid antibodies with isotypes was 25% in SLE, 17% in probable SLE, 10% in SS, and 8% in other diseases. None of these biomarkers individually or in combination were significantly associated with transition of probable SLE to classifiable SLE (Supplementary Table 4, http://onlinelibrary.wiley.com/doi/10.1002/art.41093/abstract).

The frequency of CB-CAP positivity was higher than that of low complement levels in both SLE and probable SLE \( (P = 0.0001 \) for both) (Table 1). In probable SLE, this was true whether or not patients fulfilled the SLICC criteria. The difference between the rate of low complement levels and the rate of CB-CAP positivity in probable SLE was especially large in the subgroup not fulfilling the SLICC criteria (2% and 18% respectively; \( P < 0.008 \)). In addition, CB-CAP positivity demonstrated higher sensitivity than anti-dsDNA positivity in probable SLE \( (P < 0.0005) \).

MAP positivity, unlike CB-CAP positivity, was equally distributed between the subgroups fulfilling or not fulfilling the SLICC criteria (Table 1). The MAP algorithm includes biomarkers (in addition to CB-CAPs) that increase its diagnostic sensitivity for SLE, but may not correlate as closely with low serum complement levels as with CB-CAPs alone.

More patients with probable SLE were white than black (61% versus 16%), while patients with SLE were more racially diverse (34% white versus 38% black) (Supplementary Table 2, http://onlinelibrary.wiley.com/doi/10.1002/art.41093/abstract). Positivity for CB-CAPs was more common in black patients with SLE and probable SLE (SLE: white 56%, black 68%; probable SLE: white 21%, black 33%), but this difference was not statistically significant.

The MAP demonstrated higher sensitivity at enrollment than low complement levels in SLE (77% versus 23%) and in probable SLE (40% versus 9%) \( (P = 0.0001 \) for both) and higher positive LRIs for SLE and probable SLE (Tables 1 and 2). The MAP was also more sensitive \( (P < 0.05 \) and more specific than positive CB-CAPs in probable SLE (Tables 1 and 2). Specificity of the MAP versus all patients with other autoimmune rheumatic diseases \( (n = 101, \) including SS) was 85% (data not shown).

Since the specificity of anti-dsDNA for SLE compared to the group of patients with other diseases, excluding SS, was 100% (leading to infinite positive LRIs for SLE and probable SLE), an adjusted specificity of 97.5% was used for the calculation of these LRIs [18]. Although, as expected, the positive LR of anti-dsDNA was strong for SLE (>15.1), it was moderate for probable SLE (>4.35), due to its low sensitivity (11%). The positive LR of the MAP
was higher than that of low complement and anti-dsDNA for both SLE and probable SLE (Table 2), indicating that a positive MAP increases the posttest probability of lupus more than the positivity of any individual biomarker.

The MAP was associated with a moderate negative LR (0.24 in SLE and 0.63 in probable SLE), indicating that a negative test has moderate ability to reduce the likelihood of the diagnosis of SLE or probable SLE. Nonetheless, the MAP yielded a negative LR lower than that obtained with complement and anti-dsDNA levels in both SLE and probable SLE. Therefore, the MAP and positive CB-CAPs had greater diagnostic accuracy as demonstrated by a higher Youden index (J score) than did low complement levels and anti-dsDNA in SLE and probable SLE (Table 2).

### Table 2. Performance of biomarkers for SLE and probable SLE at enrollment*

|                      | SLE (n = 53) |                      | Probable SLE (n = 92) |                      |
|----------------------|--------------|----------------------|-----------------------|----------------------|
|                      | Sensitivity, % | LR+ (95% CI)         | LR− (95% CI)          | J                     |
| Anti-dsDNA†          | 38           | >15.1 (5.34–∞)       | 0.64 (0.49–0.74)      | 0.38                  |
| Low complement levels‡| 23           | 11.5 (2.06–68.2)     | 0.79 (0.66–0.90)      | 0.21                  |
| CB-CAPs§             | 61           | 4.43 (2.26–6.18)     | 0.45 (0.31–0.63)      | 0.47                  |
| MAP                  | 77           | 19.6 (5.73–71.54)    | 0.24 (0.12–0.38)      | 0.73                  |
|                      |              |                      |                        |                      |
|                      | Sensitivity, % | LR+ (95% CI)         | LR− (95% CI)          | J                     |
| Anti-dsDNA†          | 11           | >4.35 (1.52–∞)       | 0.91 (0.81–0.98)      | 0.11                  |
| Low complement levels‡| 9            | 4.43 (0.76–27.05)    | 0.93 (0.85–1.02)      | 0.07                  |
| CB-CAPs§             | 28           | 2.06 (1.0–4.42)      | 0.83 (0.70–1.0)       | 0.15                  |
| MAP                  | 40           | 10.1 (2.91–37.25)    | 0.63 (0.52–0.74)      | 0.36                  |

* Specificity of biomarkers for SLE and probable SLE was calculated versus the group of patients with other rheumatic diseases (n = 51). Specificity of anti-dsDNA was estimated at 97.5% for calculation of likelihood ratios (LRs). The upper limit of the 95% confidence interval (95% CI) of anti-dsDNA (infinity) indicates 100% predictability. J = Youden index (see Table 1 for other definitions).
† Measured by ELISA and confirmed by IFA.
‡ C3 and/or C4.
§ Elevated EC4d (MFI >14) and/or BC4d (MFI >60).

At follow-up, positivity for CB-CAPs (n = 69) decreased from 30% to 17% (P = 0.01) and positivity for the MAP (n = 68) decreased from 41% to 32% (P = 0.05). Nonsignificant decreases in disease activity were seen in mean SELENA–SLEDAI scores (from 1.63 to 1.21; P = 0.54) and in mean nonserologic SELENA–SLEDAI scores (from 1.1 to 0.8; P = 0.65).

Only 2% of SS patients had low complement levels, while CB-CAPs were elevated in 10% of these patients, and 27% had a positive MAP score (Table 1). In patients with SS, positive and negative LRs for the MAP were 6.77 and 0.76, respectively, and the Youden index was 0.23 (data not shown).

We evaluated whether positivity for CB-CAPs, MAP, or other biomarkers at enrollment predicted fulfillment of the ACR classification criteria in the probable SLE population at
DISCUSSION

We assessed the frequency of positive CB-CAPs measured directly or within the MAP algorithm in patients with probable SLE compared to those with SLE and other autoimmune rheumatic diseases, and we evaluated the usefulness of these measures of complement activation as predictors of the evolution of probable SLE to classifiable SLE (according to ACR criteria). Our data suggest that CB-CAPs, measured directly or within the MAP algorithm, perform well as a potential test to support the diagnosis of SLE in patients with probable SLE. In particular, CB-CAPs and the MAP performed better than standard testing, which includes assessing antibodies to dsDNA or low serum complement levels. In addition, a MAP score of >0.8 was the best predictor that a patient with probable SLE would acquire a fourth ACR criterion within 18 months postenrollment.

It is well recognized that diagnosis relates to the probability of an illness in a specific symptomatic individual, in contrast to classification criteria, which are based on performance characteristics, such as sensitivity and specificity (29,30). However, in the absence of diagnostic SLE guidelines, the classification criteria are often used to diagnose SLE (31). In a recent study of newly diagnosed lupus patients, with physician diagnosis as the gold standard, only 66.1% met ACR criteria and 83.5% met SLICC criteria; 89 patients (23%) fulfilled only 3 ACR criteria (32). Furthermore, while the SLICC criteria are more sensitive than the ACR criteria, they are somewhat less specific (33). This demonstrates a need for better diagnostic biomarkers.

Probable, incomplete, or latent lupus has been a diagnostic construct applied to patients who do not fulfill ACR classification criteria for SLE (34). Cohort studies have shown that in up to 20% of patients, probable SLE may progress to SLE that fulfills ACR classification criteria over a period of 2–5 years, and some may develop organ damage (9,10,35). If patients who are more likely to have true incipient SLE can be identified at an early stage, decision-making regarding therapeutic intervention may be improved. For example, James et al demonstrated that HCQ therapy delayed the onset of complete SLE in patients who had not yet been diagnosed (36). The patients with probable SLE in the current study were less likely to be treated with HCQ or prednisone compared to patients with SLE, despite fulfilling 3 ACR criteria and the belief by a physician experienced in lupus that SLE was a likely diagnosis.

Since we recruited patients from academic sites where large lupus cohorts were being followed up, the likelihood of an SLE diagnosis was based on expert opinion. We surmised that a higher percentage of these selected patients with probable SLE would be more likely to develop a fourth ACR criterion over 2–3 years than those in a more loosely defined probable SLE group. This is supported by the observation that 35 of 92 patients with probable SLE (38%) who did not meet the ACR classification for SLE did meet the SLICC classification criteria at enrollment. This suggests that the investigators considered ≥1 SLICC feature in their evaluations to not be definitely related to SLE, since those patients could have been included in the SLE group instead of the probable SLE group. This also highlights an important difference between classification criteria and diagnosis. A similar percentage...

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**Table 3.** HRs of biomarkers predicting fulfillment of ACR classification criteria by 18 months in patients with probable SLE*  

| Biomarker                | HR     | 95% CI     | P       |
|--------------------------|--------|------------|---------|
| Anti-dsDNA†              | 2.97   | 0.98–8.99  | 0.043   |
| Low complement levels‡   | 1.93   | 0.44–8.53  | 0.375   |
| CB-CAPs§                 | 1.66   | 0.67–4.09  | 0.275   |
| EC4d >20 MFI             | 2.61   | 0.99–6.88  | 0.053   |
| MAP >0.8                 | 3.11   | 1.26–7.69  | 0.0097  |

* Hazard ratios (HRs) of the biomarkers were calculated by Cox regression. Data on 69 follow-up visits (n = 68 for MAP) that occurred 9–18 months after enrollment were analyzed. ACR = American College of Rheumatology (see Table 1 for other definitions).† Measured by ELISA and confirmed by IFA.‡ Elevated EC4d (MFI >14) and/or BC4d (MFI >60).
of SLICC criteria fulfillment was found in a recently described cohort of patients who fulfilled 3 ACR criteria (37). Furthermore, in the present study, 29% of the 69 patients with a follow-up visit within 18 months acquired a fourth ACR criterion. This is a much higher rate in a shorter period of time than shown in prior studies (13,24).

We have confirmed findings of previous studies (7,8), demonstrating that positive CB-CAPs measured directly and within the MAP algorithm are more sensitive biomarkers than low serum complement levels or anti-dsDNA antibodies in SLE. We report positive CB-CAPs in a significant number (28%) of patients with probable SLE, whereas low serum complement levels were present in only 9% and anti-dsDNA in 11%. Furthermore, the MAP algorithm was positive in 77% of patients with SLE and in 40% of patients with probable SLE. This confirms our hypothesis and observations that complement activation is characteristic of SLE and can be detected more reliably by measuring activation products than serum complement component levels (1,8). We and others have shown that the presence of complement activation products closely correlates with active SLE (38,39).

The clinical and laboratory (immunologic) individual ACR and SLICC criteria in the probable SLE group versus the SLE group showed, as expected, a numerical and statistically significant lower incidence of many features, including arthritis, rashes, alopecia, serositis, and leukopenia. Conversely, there were few differences between the probable SLE subsets fulfilling SLICC criteria or not, except for alopecia and a history of low complement levels. However, C3 and C4 lack sensitivity as markers of complement activation in probable SLE, with only 9% showing hypocomplementemia at baseline. While 83% of the patients with probable SLE who fulfilled SLICC criteria had historically low complement levels, only 20% presented with low levels at the time of testing. Even within the SLICC-positive subset, positive CB-CAPs and MAP were significantly more frequent than low serum complement levels.

In this study, the presence of CB-CAPs (BC4d and/or EC4d) alone did not predict progression to classifiable SLE. However, a MAP score of >0.8 did predict progression, as did an EC4d MFI of >20 and anti-dsDNA positivity, although with lower hazard ratios. This may be related to the greater frequency of MAP positivity in probable SLE, since the cohort of patients followed up is relatively small. A higher cutoff for the MAP index (0.8 versus 0.1) was needed to show prediction of developing ACR-classifiable SLE. This may be related to the lack of specificity of a lower index in this small cohort of patients with probable SLE who converted. EC4d alone (at a higher cutoff than what is used routinely [MFI of 20 versus 14]) also showed a correlation with conversion, suggesting that complement activation is one key pathogenetic element in the evolution of SLE and is better detected by measurement of CB-CAPs directly or within the MAP than by low serum complement levels. We suggest that a longer follow-up, with potential evolution from probable SLE to SLE in more patients, might reveal that EC4d alone and the MAP would correlate with conversion even more significantly.

We also studied patients with SS, since they can exhibit numerous autoantibodies and low serum complement levels (40). We observed an increased frequency of positive CB-CAPs compared to low serum complement levels (10% versus 2%) in SS patients, but these were not significantly different from frequencies in the control group with other rheumatic diseases. However, the MAP did show a higher positivity rate than in the control group (27% versus 4%), possibly because of the high titers of ANA found in our SS cohort (data not shown), which can influence the results of the algorithm. Since only 2% of the SS patients exhibited low serum C3 or C4 levels, it is possible that our group of 50 patients is not representative of the large cohorts that have been studied in the past (40).

A limitation of this study is the small cohort size of patients with probable SLE for whom we have follow-up data, as well as the relatively short follow-up period. However, 20 of the 69 patients (29%) who had a follow-up visit within 18 months fulfilled a fourth ACR criterion in this time frame, confirming our expectation that this cohort from academic lupus centers might be more likely to progress to classifiable SLE than prior studies have suggested. Another limitation is that retrospective determination of whether patients fulfill classification criteria is dependent on a comprehensive review of prior medical records and laboratory test results (including the presence of leukopenia or lymphopenia), and these may not have been available for all patients at every site. The adjudicators often requested and received additional records. However, the records we received may have been incomplete, as data on the lupus anticoagulant test, for example, were rarely reported. One strength of our study is that the probable SLE group is well defined and not classified by physician judgment alone.

In summary, our data show that positive CB-CAPs alone or in the MAP algorithm are present in a higher percentage of patients with SLE and patients with probable SLE compared to antibodies to dsDNA or low serum complement levels. The relatively high positivity rate of CB-CAPs and the MAP in the probable SLE group suggests that complement activation might occur early in the evolution of SLE and may be a feature in patients with suspected lupus. In addition, patients with probable SLE who had a MAP score of >0.8 or an EC4d MFI of >20 at enrollment were more likely to fulfill a fourth ACR criterion during a relatively brief follow-up; these biomarkers predicted the transition to SLE better than other clinical and laboratory parameters. The detection of complement activation in patients with probable lupus who do not fulfill ACR criteria or even SLICC criteria could have implications with regard to treatment, as early appropriate therapy in these patients may potentially slow the rate of disease progression (36,41).
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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Weinstein had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Ramsey-Goldman, Alexander, Massarotti, Wallace, Kalunian, Dervieux, Weinstein.

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Analysis and interpretation of data. Ramsey-Goldman, Alexander, Kalunian, O’Malley, Dervieux, Weinstein.

ROLE OF THE STUDY SPONSOR

Exagen, Inc. facilitated the design of the study along with the investigators. The investigators independently collected the data. Exagen, Inc. analyzed the data and, with the investigators, interpreted the results. The paper was primarily written by the senior investigator and the principal investigator, and the decision to submit the manuscript was made by all the authors and approved by Exagen, Inc.

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