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Measurement of Cell-Bound Complement Activation Products Enhances Diagnostic Performance in Systemic Lupus Erythematosus

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Objective. **To determine the value of cell-bound complement activation products in combination with antinuclear antibody (ANA), anti–double-stranded DNA antibody (anti-dsDNA), and anti–mutated citrullinated vimentin antibody (anti-MCV) for the diagnosis of systemic lupus erythematosus (SLE).**

Methods. **This was a multicenter cross-sectional study in which 593 subjects were enrolled (210 SLE patients, 178 patients with other rheumatic diseases, and 205 healthy subjects). Complement receptor 1 levels on erythrocytes (ECR1) together with complement C4d levels on erythrocytes (EC4d), platelets (PC4d), and B cells (BC4d) were determined using fluorescence-activated cell sorting. Serologic markers were measured by enzyme-linked immunosorbent assay. Statistical analyses were performed using area under the curve (AUC), logistic regression, and calculations of diagnostic sensitivity and specificity.**

Results. **Anti-dsDNA was an insensitive (30%) but specific (>95%) marker for SLE. Levels of EC4d, BC4d, and PC4d were several times higher, and levels of ECR1 lower, in SLE patients compared to patients with other rheumatic diseases and healthy subjects. Among 523 anti-dsDNA–negative subjects, multivariate logistic regression analysis revealed that SLE was associated with ANA positivity (≥20 units), anti-MCV negativity (≤70 units), and elevated levels of both EC4d and BC4d (AUC 0.918, $P < 0.001$). A positive index score corresponding to the weighted sum of these 4 markers correctly categorized 72% of SLE patients. Specificity in relation to patients with other rheumatic diseases and healthy controls was >90%. The combination of anti-dsDNA and index score positivity yielded 80% sensitivity for SLE and 87% specificity against other rheumatic diseases.**

Conclusion. **An assay panel combining anti-dsDNA, ANA, anti-MCV, EC4d, and BC4d is sensitive and specific for the diagnosis of SLE.**

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that results in autoantibody-mediated tissue damage and potentially life-threatening multiorgan failure (1). This heterogeneous inflammatory disorder affects between 161,000 and 322,000 adults in the US, with the prevalence in women being 9 times

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Drs. Kalunian, Furie, Putterman, Ramsey-Goldman, Buyon, and Weinstein have received honoraria for serving on the Exagen Diagnostics advisory board (less than $10,000 each). Mr. Harris and Dr. Dervieux own stock or stock options in Exagen Diagnostics and are listed as named inventors on a patent held by Exagen Diagnostics for a diagnostic method for systemic lupus erythematosus.

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that in men (2). In addition, the prevalence rate is higher in African Americans and Hispanics compared to whites, and sociodemographic background is predictive of poor prognosis (3). The manifestations of SLE are diverse and include rash, arthritis, anemia, thrombocytopenia, serositis, nephritis, seizures, and psychosis. Because these symptoms are heterogeneous, nonspecific, evolutive, and often mimic those of other diseases, the diagnosis of SLE is complex and can be challenging to physicians. Diagnosis of SLE relies on a combination of the patient's medical history, current symptoms, and laboratory tests. The revised American College of Rheumatology (ACR) criteria set published in 1982 (4) requires the presence of 4 of 11 criteria to classify a patient as having SLE, but the use of these criteria in clinical practice is not uniform (5).

Prominent among the standard laboratory studies commonly used to support the diagnosis of SLE are antinuclear antibody (ANA) and anti–double stranded DNA (anti-dsDNA) antibody tests (6). Nonetheless, ANA and anti-dsDNA have limitations, and neither of these serologic markers provides adequate balanced sensitivity and/or specificity to diagnose SLE. It is well recognized that complement activation is central to the pathogenesis of SLE (7), and a decade of biomarker research has illustrated the potential usefulness of cell-bound complement activation products (CB-CAPs) to facilitate diagnosis of the disease (8,9). These CB-CAPs include complement C4–derived ligand deposited on erythrocytes (EC4d) (8,10), platelets (PC4d) (9), and B lymphocytes (BC4d)(11), and their relative increase in SLE compared to other diseases may be of diagnostic value. In addition, SLE patients have reduced levels of the CR1 receptor (CD35) (8) on erythrocytes (ECR1). Consequently, there is a decrease in the clearance of immune complexes with an increased likelihood of accumulation at sites such as the kidney.

It follows that measurements of C4d deposition on cells together with determination of ECR1 density may improve the performance of SLE diagnostic procedures compared to conventional serologic profiling (e.g., ANA, anti-dsDNA). In addition, the potential contribution of CB-CAPs to disease activity has been established (12,13), and their measurement may help in the clinical management of SLE. In the present study we evaluated the contribution of CB-CAPs to the differential diagnosis of SLE. Our data indicate that an assay panel combining ANA and anti-dsDNA together with EC4d and BC4d offers an SLE diagnostic tool with enhanced sensitivity and specificity.

PATIENTS AND METHODS

Patients and study protocol. This study (the CAPITAL study [Study of Complement Activation Products in the Assessment of Lupus]) was a multicenter cross-sectional investigation and required 1, or at most 2, subject visits for screening and blood sample collection. There were no followup visits required. We enrolled adult patients (≥18 years) who were classified as having SLE according to the ACR 1982 classification criteria (4) updated in 1997 (14), patients with other well-defined rheumatic diseases, and healthy volunteers. All patients with rheumatic diseases were enrolled as part of their routine care. The study was approved by an internal review board at each site, and all participants provided informed consent. Each subject's medical history related to the diagnosis of rheumatologic conditions was obtained and reviewed for inclusion/exclusion criteria. Disease activity was measured in all SLE patients at the time of the study visit, using the Safety of Estrogens in Lupus Erythematosus National Assessment (SELENA) version of the SLE Disease Activity Index (SLEDAI) (15). Active disease was defined as a SELENA-SLEDAI score of ≥6 (16). Blood was collected in EDTA-containing tubes for analysis of CB-CAPs, and serum was collected for analysis of autoantibodies. The blood was shipped overnight from the participating center to a central clinical laboratory at Exagen Diagnostics. Within 24 hours of receipt at the clinical laboratory, CB-CAPs were analyzed by fluorescence-activated cell sorting (FACS). Serum was stored at −80°C prior to enzyme-linked immunosorbent assay (ELISA) measurements. Throughout the study, all laboratory scientists involved in FACS and ELISA testing were blinded with regard to the subjects' diagnoses.

FACS measurements. EC4d, BC4d, PC4d, and ECR1 were measured using a validated FACS assay. All FACS analyses were performed using an FC500 cytometer and CXP software (Beckman Coulter). The mean fluorescence intensity (MFI) of the isotype background control and each complement protein (C4d, CR1) was obtained, and the net MFI was then determined by subtracting nonspecific MFI from the specific MFI results. Median interday (5 consecutive days) coefficients of variation (CVs) for EC4d and ECR1 levels at low, medium, and high intensity were established using blood from 44 patients with rheumatic diseases and ranged from 3.3% to 9.6% for EC4d and from 4.4% to 4.9% for ECR1. BC4d interday CVs at low, medium, and high intensity in 34 patients with rheumatic diseases ranged from 5.3% to 12.1%. The interday CV for PC4d in 12 patients with rheumatic diseases was 15.7%. In all FACS experiments, control procedures to establish proper calibration, compensation, and linearity of the flow cytometer were included.

EC4d and ECR1. Whole blood (50 μl) was washed with Dulbecco's phosphate buffered saline and centrifuged for 5 minutes at 800*g*, and erythrocyte pellets were resuspended with 500 μl of a 1% normal goat serum solution (Jackson ImmunoResearch). A 10-μl erythrocyte suspension was subsequently stained for 45 minutes at 4°C with purified mouse monoclonal antibodies against human C4d (mouse anti-human C4d; Quidel) or human CR1 (mouse anti-human antibody; Taconic) or, alternatively, using nonspecific mouse anti-human IgG1κ antibody (MOPC-21; BD Biosciences). Samples were then washed as described above. Erythrocyte pellets were

resuspended for 45 minutes at 4°C in the dark, in a solution (25 μl) containing fluorescein isothiocyanate (FITC)–conjugated goat anti-mouse antibody (Jackson ImmunoResearch). Following staining, washing, and resuspension with 250 μl of cold 1% normal goat serum solution the erythrocytes were subjected to FACS analysis for detection of C4d or CR1 on the cell surface.

BC4d levels. Following lysis of erythrocytes from whole blood (700 μl) using an ammonium chloride–based reagent (BD Pharm Lyse; BD Biosciences) and centrifugation for 5 minutes at 800*g*, cell pellets were resuspended in 500 μl of a 1% normal goat serum solution and stained for 45 minutes at 2–8°C using C4d monoclonal antibody as described above. A 25-μl cell suspension was subsequently stained for 45 minutes at 4°C using purified mouse monoclonal antibodies against human C4d or nonspecific mouse anti-human IgG1κ antibody as above. Cell surface C4d staining was detected by addition of FITC-conjugated goat anti-mouse antibody for 45 minutes at 2–8°C in the dark. An R-phycoerythrin (R-PE)–conjugated monoclonal antibody against human CD-19 (a 95-kd type I transmembrane glycoprotein expressed during all stages of B cell differentiation and maturation) was used to detect the C4d complement activation–derived fragment specific to B lymphocytes.

PC4d levels. C4d monoclonal antibody was used to measure cell surface levels of C4d in platelet cells by FACS, as described above. Whole blood samples (50 μl) were diluted and stained for 45 minutes at 2–8°C with the C4d monoclonal antibody, followed by staining with FITC-conjugated goat anti-mouse antibody for 45 minutes at 2–8°C in the dark. An R-PE–conjugated monoclonal antibody against human CD42b was used to identify the C4d activation–derived fragment specific to the platelets.

ELISA measurements. ANA, anti-dsDNA, and anti–mutated citrullinated vimentin antibodies (anti-MCV, an anti-citrullinated peptide antibody) (17) were measured by ELISA. All ELISA methods used have been approved by the US Food and Drug Administration as safe and effective for in vitro diagnostic uses. ANA and anti-dsDNA were from Inova, and anti-MCV was from Orgentec Diagnostika. Intraday and interday CVs for all methods were established at the clinical laboratory and were <20%. Appropriate positive and negative controls were included for all ELISA experiments.

Statistical analysis. Statistical analysis was conducted using R software. Receiver operating characteristic (ROC) curves were used as appropriate (18) for each of the markers (univariate analysis) and also following the determination of an index value as the output of a multivariate logistic regression equation. As measures of performance, sensitivity and specificity (1 − false-positive rate) were computed. Ninety-five percent confidence intervals (95% CIs) were calculated by estimating the asymptotic standard error for a binomial proportion; these were confirmed using the Agresti-Coull CI (19), with very similar results (within 1%; data not shown). Group comparisons were performed using Mann-Whitney or chi-square tests as appropriate. Analyses evaluating the contributions of CB-CAPs to disease activity (SELENA-SLEDAI) were exploratory.

The reported performance statistics (sensitivity, specificity, and ROC area under the curve [AUC]) were calculated using apparent validation, also known as resubstitution validation, in which model performance is assessed directly from the samples used to derive the model. This validation strategy is known to yield a biased or optimistic estimate of model performance. Therefore, the size of this bias was estimated using a bootstrapping procedure to quantify the optimism correction and obtain an "optimism-corrected performance estimate" (20). Using this resampling procedure, with 5,000 sampling draws, the optimism bias for sensitivity and specificity of the models (both with and without anti-dsDNA) was consistently <1%, thereby indicating that the performance estimates calculated using apparent validation were very close to the more complex optimism-corrected performance estimates.

RESULTS

A total of 593 individuals were enrolled at 14 participating sites across the US between April 2010 and August 2010. The study population consisted of 210 SLE patients (90.5% female, 36% white, mean age 41 years), 178 patients with other rheumatic diseases (80.3% female, 63% white, mean age 57 years), and 205 healthy individuals (65.9% female, 56% white, age 41 years). Characteristics of the 210 SLE patients are presented in Table 1. Disease activity scores were assessed using the SELENA-SLEDAI and were available for 209 SLE patients. Diagnoses in the group of 178 patients with other rheumatic diseases included rheumatoid arthritis (RA) (n = 120), systemic sclerosis (SSc) (n = 21), dermatomyositis (DM) (n = 9), vasculitis (n = 8), Sjögren's syndrome (SS) (n = 8), polymyositis (PM) (n = 7), granulomatosis with polyangiitis (Wegener's) (GPA) (n = 2), fibromyalgia (n = 2), and SS with fibromyalgia (n = 1).

Contribution of serologic and CB-CAP biomarkers as assessed by univariate analysis. Levels of serologic markers (anti-dsDNA, ANA, anti-MCV) and CB-CAPs were determined in all subjects (n = 593). Using assay manufacturers' cutoffs for positivity, anti-dsDNA was shown to be insensitive for SLE (29.5% [positive results in 62 of 210 SLE patients]) yet specific against other rheumatic diseases (96.1% [false-positive results in 7 patients with other rheumatic diseases: 6 with RA and 1 with SSc]) and healthy individuals (99.5% [1 − false-positive]) (Table 2). In contrast, ANA was a highly sensitive marker (89% of SLE patients tested positive) but largely nonspecific against other rheumatic diseases (59.0%). ANA specificity against normal healthy individuals was 90.7% (Table 2).

Table 1. Characteristics of the 210 SLE patients enrolled in the study*

Female	190 (90)
Age, median (range) years	41 (19–81)
Ethnicity	
White	75 (36)
African American	76 (36)
Asian	16 (8)
Hispanic	40 (19)
Other	3 (1)
Malar rash	91 (43)
Discoid rash	29 (14)
Photosensitivity	76 (36)
Oral ulcers	59 (28)
Arthritis	154 (73)
Serositis	59 (28)
Pleuritis	40 (19)
Pericarditis	26 (12)
Renal disorder	86 (41)
Proteinuria 0.5 gm/day	80 (38)
3+ cellular casts	9 (4)
Neurologic disorder	15 (7)
Seizures	14 (7)
Psychosis without other causes	2 (1)
Hematologic disorder	113 (54)
Hemolytic anemia	8 (4)
Leukopenia (<4,000/liter)	59 (28)
Lymphopenia (<1,500/liter)	53 (25)
Thrombocytopenia (<100,000/liter)	29 (14)
Immunologic disorder	171 (81)
Anti-dsDNA	140 (67)
Anti-Sm	47 (22)
Antiphospholipid antibody	57 (27)
Antinuclear antibodies	205 (98)
SELENA-SLEDAI score†	
Median (range)	2 (0–22)
≥6	41 (20)

* Data were obtained from the patients' medical records. Except where indicated otherwise, values are the number (%). SLE = systemic lupus erythematosus; anti-dsDNA = anti–double-stranded DNA; SELENA-SLEDAI = Safety of Estrogens in Lupus Erythematosus National Assessment version of the SLE Disease Activity Index.
† Assessed within 30 days of blood withdrawal for the present study. Data available on 209 patients.

CB-CAP analysis revealed that the levels of C4d deposited on erythrocytes, B lymphocytes, and platelets were several times higher in SLE patients than in patients with other rheumatic diseases (2.8-fold, 3.2-fold, and 4.5-fold, respectively; all $P < 0.001$). Significantly higher C4d levels were also observed in SLE patients compared to healthy volunteers ($P < 0.001$). Levels of CR1 on erythrocytes were lower in SLE patients compared to patients with other rheumatic diseases or healthy subjects (both $P < 0.01$). ROC analysis comparing SLE and other rheumatic diseases revealed that EC4d (AUC 0.825) and BC4d (AUC 0.822) were the best predictors, followed by PC4d (AUC 0.739) and ECR1 (AUC 0.625). Table 2 highlights the performance characteristics of CB-CAPs at optimal cutoffs, with sensitivity ranging from 46.2% (PC4d) to 73.8% (EC4d) and specificity against other rheumatic diseases ranging from 48.9% (ECR1) to 92.7% (PC4d). In relation to the 205 healthy subjects, specificity of the CB-CAPs was >90%, with the exception of ECR1 (69.8%).

Contribution of serologic and CB-CAP biomarkers as assessed by multivariate analysis. Because dsDNA positivity was highly specific for SLE (>95%), we next evaluated the predictive values of CB-CAPs, ANA, and anti-MCV and their capacity to improve diagnostic sensitivity while maintaining adequate specificity (low false-positive rates). Among the 523 anti-dsDNA–negative subjects (148 patients with SLE, 171 patients with other rheumatic diseases, and 204 healthy controls), multivariate logistic regression analysis revealed that the stepwise addition of ANA positivity (≥20 units/liter) (*P*

Table 2. Diagnostic assay results among patients with SLE, patients with other rheumatic diseases, and healthy subjects*

	Healthy subjects (n = 205)	Other rheumatic diseases (n = 178)	SLE (n = 210)
ANA, units/liter			
Mean ± SEM	13 ± 1	40 ± 4	88 ± 3
% ≥20†	9.3	41.0	89.0
Anti-dsDNA, units/liter			
Mean ± SEM	40 ± 4	61 ± 8	228 ± 17
% >301†	0.5	3.9	29.5
Anti-MCV, units/liter			
Mean ± SEM	2 ± 0.6	160 ± 21	9 ± 2
% >70‡	0.5	36.0	1.9
EC4d, net MFI			
Mean ± SEM	5.3 ± 0.4	6.3 ± 0.3	17.6 ± 1.2
% >9§	7.3	16.9	70.0
BC4d, net MFI			
Mean ± SEM	23.5 ± 1.1	34.9 ± 3.4	110.4 ± 7.2
% >48§	4.4	13.5	65.7
PC4d, net MFI			
Mean ± SEM	2.0 ± 0.4	3.6 ± 0.3	16.3 ± 2.2
% >7§	0.5	7.3	46.2
ECR1, net MFI			
Mean ± SEM	20.7 ± 0.5	16.1 ± 0.5	13.3 ± 0.4
% <16§	30.2	51.1	73.8

* Percent values for the healthy subjects and the patients with other rheumatic diseases are the percent false-positive (1 − specificity). SLE = systemic lupus erythematosus; ANA = antinuclear antibody; anti-dsDNA = anti–double-stranded DNA; anti-MCV = anti–mutated citrullinated vimentin; EC4d = erythrocyte C4d; MFI = mean fluorescence intensity; BC4d = B cell CD4d; PC4d = platelet CD4d; ECR1 = erythrocyte CR1.
† Manufacturer-recommended cutoff.
‡ Optimal cutoff with high specificity against rheumatoid arthritis.
§ Cutoff established by receiver operating characteristic analysis (18).

Table 3. Improvement of diagnostic performance among anti-dsDNA–negative subjects by stepwise addition of ANA, EC4d, BC4d, and anti-MCV measurements*

	ANA ≥20 units/ml	ANA ≥20 units/ml + logEC4d net MFI	ANA ≥20 units/ml + logEC4d net MFI + logBC4d net MFI	ANA ≥20 units/ml + logEC4d net MFI + logBC4d net MFI + anti-MCV >70 units/ml
Sensitivity for SLE	84.5 (125/148)	65.5 (97/148)	68.2 (101/148)	71.6 (106/148)
Specificity against other rheumatic diseases	60.8 (104/171)	80.1 (137/171)	86.0 (147/171)	90.1 (154/171)
Specificity against healthy controls	90.7 (185/204)	97.5 (199/204)	99.0 (202/204)	98.0 (200/204)
ROC AUC, mean ± SEM	0.808 ± 0.0185	0.887 ± 0.0165	0.903 ± 0.0159	0.918 ± 0.0146

* For each model, an index score corresponding to a weighted sum of the markers (as appropriate) was calculated, and the receiver operating characteristic area under the curve (ROC AUC) for clinical sensitivity and specificity of an index score of ≥0 was determined. Sensitivity and specificity are shown as the % (no. of subjects with the criterion/total no. of subjects). See Table 2 for other definitions.

< 0.001), log-normalized EC4d and BC4d net MFI ($P < 0.001$), and anti-MCV negativity (positivity for anti-MCV [>70 units/liter] has a negative coefficient) ($P < 0.001$) increased the ROC AUC from 0.808 to 0.918 (Table 3). ECR1 and PC4d did not significantly contribute to the model ($P < 0.10$). The model yielding the highest sensitivity and specificity for SLE corresponded to an index score (weighted sum of these 4 markers) of 1.20 (95% CI 0.86, 1.53) in patients with SLE, −2.54 (95% CI −2.83, −2.24) in patients with other rheumatic diseases, and −2.74 (95% CI −2.89, −2.59) in healthy subjects (Figure 1A). Estimates and odds ratios are provided in Table 4.

Using an index cutoff of 0, sensitivity for identification of SLE among anti-dsDNA–negative SLE patients was 71.6% (106 of 148) (95% CI 64.4, 78.9), specificity against anti-dsDNA–negative patients with other diseases was 90.1% (154 of 171) (95% CI 85.6, 94.5) and specificity against anti-dsDNA–negative healthy subjects was 98.0% (200 of 204) (95% CI 96.1, 99.9). Figure 1B illustrates the sensitivity and specificity obtained with various index scores.

Together, the combination of anti-dsDNA positivity and a positive index score yielded 80.0% sensitivity for SLE (95% CI 74.6, 85.4) (168 of 210 SLE patients, including 62 anti-dsDNA–positive patients and 106 patients with an index score of >0), and 86.5% specificity against other rheumatic diseases (95% CI 81.5, 91.5). Specificity was 92.5% against RA (9 false-positives), 76.2% against SSc (5 false-positives), 66.7% against DM (3 false-positives), 62.5% against vasculitis (3 false-positives), 75.0% against SS (2 false-positives), and 71.4% against PM (2 false-positives). None of the patients with GPA (2 patients), fibromyalgia (2 patients), or SS with fibromyalgia (1 patient) were positive (100% specificity). Overall specificity against healthy subjects was 97.6% (200 of 205) (95% CI 95.4, 99.7). We also determined the performance characteristics when anti-dsDNA and ANA were combined. Sensitivity was 89.0% (187 of 210 SLE patients), while specificity against other rheumatic diseases was only 58.4% (negative results in 104 of 178).

Effect of SLE disease activity on CB-CAP levels and index score. A total of 41 SLE patients (19.6%) presented with active disease as defined by a SELENA-SLEDAI score of ≥6. As seen in Table 5, patients presenting with active disease had elevated levels of ANA, EC4d, BC4d, and PC4d and reduced levels of ECR1 ($P < 0.005$). ROC analysis indicated that ANA ≥90 units/liter (AUC 0.694) was associated with a 3.9-fold increased likelihood of active disease (95% CI 1.7, 9.8). Similarly, EC4d MFI >14.8 (AUC 0.646), BC4d MFI >71.5 (AUC 0.643), and PC4d MFI >6.3 (AUC 0.718) were associated with a 3.4-fold (95% CI 1.6, 7.4), a 4.3-fold (95% CI 1.9, 10.8), and a 5.3-fold (95% CI 2.3, 13.5) greater likelihood of active disease, respectively. Conversely, ECR1 MFI <10.2 (AUC 0.694) was associated with a 4.2-fold increased likelihood of active disease (95% CI 1.9, 9.3).

Moreover, the index score calculated to differentiate SLE from other diseases was significantly higher in SLE patients with active disease compared to those with inactive disease (Table 5). Among the 14 anti-dsDNA–negative patients with active disease, 13 (92.9%) had a positive index score, whereas among the 133 anti-dsDNA–negative patients with inactive disease 92 (69.2%) had a positive index score. Finally, a positive index score was observed in 46 of the 71 patients with a SELENA-SLEDAI score of <2 (64.8%).

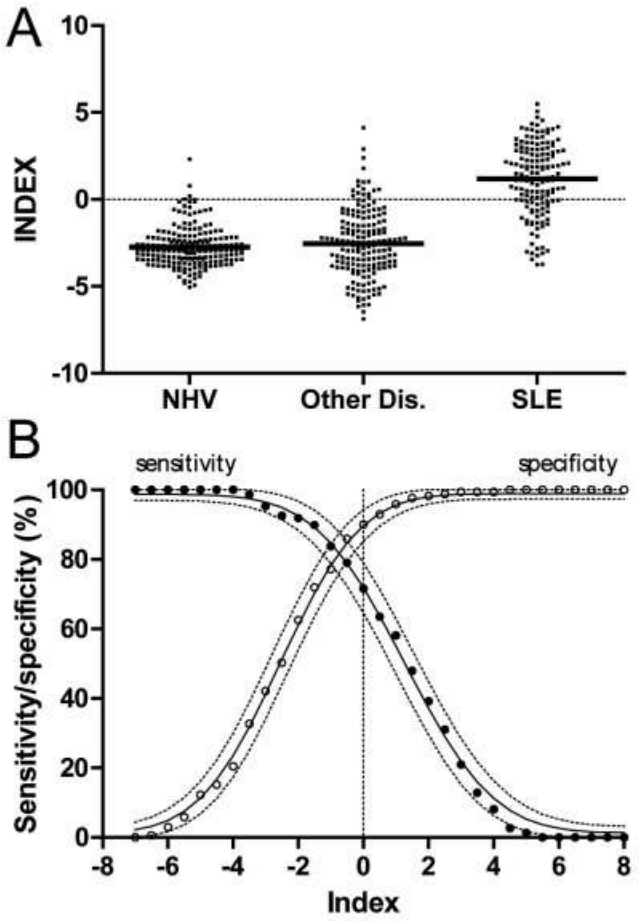

Figure 1. Index score for the differential diagnosis of systemic lupus erythematosus (SLE). **A,** Index scores of 523 anti–double-stranded DNA–negative subjects (204 normal healthy volunteers [NHV], 171 patients with other rheumatic diseases [Dis.], and 148 SLE patients). The index score was calculated as the weighted sum of antinuclear antibody (ANA) positivity, log-normalized erythrocyte C4d (EC4d) and B cell C4d (BC4d) net mean fluorescence intensity (MFI), and anti–mutated citrullinated vimentin (anti-MCV) negativity (estimates are provided in Table 4). For example, a patient with positive ANA (≥20 units), anti-MCV <70 units, an EC4d net MFI of 37, and a BC4d net MFI of 102 would have an index score of 3.53, i.e., $-8.080 + [2.2833 \times 1] - [2.6575 \times 0] + [1.1526 \times \log 37] + [1.1165 \times \log 102]$. Symbols represent individual subjects; horizontal lines show the mean. **B,** Sensitivity and specificity (against other rheumatic diseases) of the index score among anti-dsDNA–negative individuals. Circles represent the actual performance (sensitivity [solid circles] and specificity [open circles]). Dotted lines represent the 95% confidence intervals (20).

DISCUSSION

The primary objective of this large multicenter cross-sectional study was to evaluate the contribution of CB-CAPs to SLE diagnosis. Our data confirm that C4d complement deposition on erythrocytes, B cells, and platelets is generally several times higher, and expression of CR1 on erythrocytes is lower, in SLE compared to other diseases (8–10). These findings are consistent with the notion that complement activation through the classical pathway together with decreased CR1-mediated clearance of immune complexes is implicated in the pathogenesis of SLE.

However, significant overlap in the expression of these markers between SLE and other rheumatic diseases was observed, and therefore none of the individual CB-CAPs achieved adequate balanced clinical sensitivity and specificity alone, in accordance with previously

Table 4. Multivariate logistic regression analysis among anti-dsDNA–negative subjects*

	Estimate, mean ± SEM	Odds ratio (95% CI)	*P*
Intercept	−8.08 ± 0.84	–	$<2 \times 10^{-16}$
ANA ≥20 units/liter	2.28 ± 0.30	9.81 (5.46–17.62)	2.17×10^{-14}
Anti-MCV >70 units/liter	−2.66 ± 0.64	0.07 (0.02–0.25)	3.40×10^{-5}
LogEC4d net MFI	1.15 ± 0.28	3.17 (1.85–5.43)	2.85×10^{-5}
LogBC4d net MFI	1.12 ± 0.24	3.05 (1.92–4.86)	2.42×10^{-6}

* ECR1 and PCd4 did not contribute significantly ($P = 0.09$ and $P = 0.34$, respectively). Log corresponds to the natural log of the net MFI for EC4d and BC4d. For each subject, the index score corresponded to the weighted sum of each of the components including intercept. 95% CI = 95% confidence interval (see Table 2 for other definitions).

Table 5. Levels of ANA and cell-bound complement activation products, and results obtained with the index developed in the present study, in SLE patients with inactive versus active disease*

	Inactive disease (n = 168)	Active disease (n = 41)	*P*
ANA, units/liter			
Median (IQR)	87 (33, 131)	126 (98, 140)	<0.001
% ≥90	47.6	78.0	<0.001
EC4d, net MFI			
Median (IQR)	11.4 (7.4, 19.5)	16.6 (11.3, 26.0)	<0.004
% >14.8	33.9	63.4	<0.001
BC4d, net MFI			
Median (IQR)	66.7 (35.1, 130.0)	117.0 (75.2, 188.6)	<0.005
% >71.5	45.2	78.0	<0.001
PC4d, net MFI			
Median (IQR)	5.2 (2.4, 10.8)	13.9 (7.3, 43.4)	<0.001
% >6.3	39.9	78.0	<0.001
ECR1, net MFI			
Median (IQR)	13.5 (9.2, 17.9)	9.3 (6.7, 12.4)	<0.001
% >10.2	67.9	34.1	<0.001
Index			
Median (IQR)	1.75 (−0.02, 2.92)	2.85 (1.81, 3.63)	<0.001
% ≥0	74.4	97.6	<0.001

* Active disease was defined as a score of ≥6 on the Safety of Estrogens in Lupus Erythematosus National Assessment version of the SLE Disease Activity Index. IQR = interquartile range (see Table 2 for other definitions).

reported findings (21). Anti-dsDNA was an insensitive (29.5%) yet specific (>95%) marker, whereas ANA was a sensitive but poorly specific marker. We relied on the high anti-dsDNA antibody specificity (low false-positive rate) to evaluate the incremental diagnostic value of CB-CAPs, ANA, and anti-MCV. Among anti-dsDNA–negative individuals, the stepwise addition of log-normalized EC4d and BC4d markers significantly increased sensitivity (Table 3) while maintaining adequate specificity. The gain in specificity further contributed by anti-MCV was dependent on RA (specificity against RA was 87.7% without anti-MCV versus 97.4% with anti-MCV) and enabled the selection of a more optimal cutoff, increasing overall SLE sensitivity.

An index score (combining the weighted sum of ANA, EC4d, and BC4d together with anti-MCV) of >0 was sensitive (71.6%) and specific (90.1%) for SLE. The combination of anti-DNA and the index score improved the clinical sensitivity versus that achieved with anti-DNA alone, from 29.5% to 80.0%. This 50.5% improvement in clinical sensitivity largely outweighed the 9.6% loss in specificity (from 96.1% to 86.5%). Our data clearly demonstrate the value of combining serologic and CB-CAP markers to achieve adequate balanced sensitivity and specificity for the diagnosis of SLE.

A secondary objective was to evaluate the effect of disease activity as assessed using the SELENA-SLEDAI on the performance of these diagnostic biologic markers. Our preliminary results confirm that elevated EC4d, BC4d, and PC4d and reduced ECR1 levels are associated with active disease, as previously suggested (12,13). However, our data were cross-sectional, and prospective longitudinal studies will be essential to establish the true value of CB-CAP measurements for monitoring disease activity in SLE. Our analysis also revealed that the index score yielded lower clinical sensitivity among anti-dsDNA–negative patients presenting with inactive disease (69.2%) compared to those presenting with active disease (92.9%). However, among patients with a SELENA-SLEDAI score of <2, the index score yielded a sensitivity of 64.8%. Taken together, these results illustrate the capability of the index score to classify SLE among anti-dsDNA–negative patients with mild symptoms.

In conclusion, our data establish the value of CB-CAPs for the differential diagnosis of SLE. An assay panel combining anti-dsDNA, ANA, and anti-MCV antibodies together with cell surface EC4d and BC4d is sensitive and specific for SLE.

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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. Dervieux 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.

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ROLE OF THE STUDY SPONSOR

Exagen facilitated the study design and reviewed and approved the manuscript prior to submission. The authors independently collected the data, interpreted the results, and had the final decision to submit the manuscript for publication.

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