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Research Article

Serum Ic3b/C3 Ratio As A Biomarker Of Complement Activation And Disease Activity In Systemic Lupus Erythematosus: A Cross-Sectional Study.

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Abstract

Background: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by fluctuating disease activity and complement-mediated immune injury. Conventional biomarkers, including serum C3, C4, and anti-double-stranded DNA (anti-dsDNA) antibodies, have limited sensitivity and specificity for accurately reflecting ongoing complement activation. The serum iC3b/C3 ratio has emerged as a promising biomarker that integrates complement activation with complement consumption and may provide a more reliable assessment of disease activity.

Objective: To evaluate the association between the serum iC3b/C3 ratio and disease activity in patients with SLE and to determine its value as an independent biomarker of moderate-to-severe disease activity.

Methods: This cross-sectional study included 100 patients fulfilling the 2019 European Alliance of Associations for Rheumatology/American College of Rheumatology (EULAR/ACR) classification criteria for SLE. Patients were classified into inactive/mild disease (SLEDAI-2K ≤5; n = 50) and moderate-to-severe disease (SLEDAI-2K ≥6; n = 50). Demographic characteristics, clinical manifestations, laboratory parameters, complement biomarkers, and treatment data were recorded. Serum iC3b concentrations were measured by enzyme-linked immunosorbent assay, and the serum iC3b/C3 ratio was calculated. Correlation analyses and logistic regression models were performed to evaluate the association between the serum iC3b/C3 ratio and disease activity.

Results: Patients with moderate-to-severe disease activity exhibited significantly higher anti-dsDNA antibody levels, inflammatory markers, proteinuria, and urine protein-to-creatinine ratios, together with significantly lower serum C3 concentrations and estimated glomerular filtration rate than patients with inactive or mildly active disease (all $P < 0.05$). Although absolute serum iC3b concentrations did not differ significantly between groups ($P = 0.325$), the serum iC3b/C3 ratio was significantly higher in patients with active disease (median, 7.03 vs. 4.30 μg/mg; $P = 0.001$). The serum iC3b/C3 ratio correlated positively with SLEDAI-2K score ($r = 0.382$, $P = 0.003$), anti-dsDNA antibody levels ($r = 0.398$, $P = 0.004$), 24-hour urinary protein excretion ($r = 0.393$, $P = 0.039$), and urine protein-to-creatinine ratio ($r = 0.383$, $P = 0.006$), while demonstrating inverse correlations with estimated glomerular filtration rate ($r = −0.406$, $P = 0.003$), platelet count ($r = −0.318$, $P = 0.024$), and serum C3 concentration ($r = −0.234$, $P = 0.019$). In multivariable logistic regression analysis, the serum iC3b/C3 ratio remained the strongest independent predictor of moderate-to-severe disease activity (adjusted OR = 110.31, 95% CI: 8.16–1491.94; $P < 0.001$), together with anti-dsDNA antibody level and serum C3 concentration.

Conclusions: The serum iC3b/C3 ratio is a robust biomarker of disease activity in SLE and independently predicts moderate-to-severe disease after adjustment for conventional clinical and laboratory variables. By simultaneously reflecting complement activation and complement consumption, the serum iC3b/C3 ratio may provide clinically meaningful information beyond traditional complement measurements and could serve as a valuable adjunct for disease monitoring and risk stratification in patients with SLE.

Keywords: Systemic lupus erythematosus; complement activation; iC3b/C3 ratio; disease activity; SLEDAI-2K; anti-double-stranded DNA; biomarkers; C3; autoimmune disease.

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic, multisystem autoimmune disease characterized by loss of immune tolerance, production of pathogenic autoantibodies, immune-complex deposition, and complement-mediated tissue injury, resulting in a broad spectrum of clinical manifestations and disease severity [1]. Despite substantial advances in diagnosis and treatment, SLE remains associated with recurrent disease flares, irreversible organ damage, impaired quality of life, and increased morbidity and mortality, emphasizing the need for accurate assessment of disease activity to guide therapeutic decisions and improve long-term outcomes [2].

Complement activation is a central component of SLE pathogenesis. Immune complexes formed by autoantibodies against nuclear antigens activate the classical complement pathway, leading to sequential cleavage of complement proteins, recruitment of inflammatory cells, amplification of cytokine production, and tissue injury. Although the complement system plays a physiological role in immune-complex clearance and removal of apoptotic cells, persistent complement activation contributes directly to the inflammatory cascade responsible for organ damage, particularly in lupus nephritis but also in hematologic, cutaneous, musculoskeletal, and neuropsychiatric manifestations of the disease [3-5].

Because of this fundamental role, serum complement proteins C3 and C4 have long been incorporated into routine clinical assessment and are included in disease activity monitoring strategies for patients with SLE. Reduced serum concentrations of C3 and C4 generally reflect complement consumption during active disease and have been associated with disease flares, especially renal flares. However, these conventional biomarkers have important biological limitations [6]. Serum C3 and C4 concentrations represent the net balance between hepatic synthesis, physiological turnover, and complement consumption rather than direct evidence of ongoing complement activation. Furthermore, both proteins behave as acute-phase reactants and may remain within normal ranges despite active immune-complex formation or increase in response to systemic inflammation, thereby reducing their sensitivity for detecting active disease [7].

Recognition of these limitations has stimulated growing interest in biomarkers that directly reflect complement activation rather than static complement concentrations. [8]. Among these, inactivated complement component 3b (iC3b) represents a stable degradation product generated following cleavage of activated C3b by complement regulatory proteins [9]. Because iC3b is produced only after activation of the complement cascade, circulating iC3b concentrations provide direct evidence of ongoing complement activation and may more accurately reflect the immunopathological processes underlying disease activity than measurements of intact C3 alone [10]. Previous studies have demonstrated significant associations between complement activation products including iC3b, C3dg, C4d, and cell-bound complement activation products and clinical disease activity in patients with SLE [11].

The serum iC3b/C3 ratio has emerged as a promising biomarker that integrates the concentration of a complement activation product with that of its precursor molecule. [12]. This ratio is biologically attractive because it normalizes complement activation to the available C3 pool, thereby minimizing interindividual variation in baseline complement concentrations and providing a dynamic measure of complement activation. Consequently, the iC3b/C3 ratio has the potential to distinguish active complement consumption from isolated reductions in serum C3 concentration and may better identify patients experiencing active immune-mediated disease. Initial clinical studies have suggested that the iC3b/C3 ratio may outperform conventional complement measurements in identifying active SLE and monitoring disease activity, although validation in independent patient cohorts remains limited [13].

Reliable biomarkers capable of accurately reflecting disease activity continue to represent an important unmet clinical need in SLE. Although composite indices such as the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) remain the standard for clinical assessment, they rely partly on laboratory markers that may not accurately capture ongoing complement activation. Biomarkers that directly quantify complement activation could improve disease stratification, facilitate earlier detection of disease flares, enhance treatment monitoring, and potentially support precision medicine approaches targeting dysregulated complement pathways [14].

The present study was undertaken to investigate the clinical utility of the serum iC3b/C3 ratio as a biomarker of complement activation in patients with systemic lupus erythematosus. Specifically, we evaluated the association between the serum iC3b/C3 ratio and disease activity measured by the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K), examined its relationship with established clinical and laboratory indicators of disease activity, and determined whether this complement activation index independently predicts moderate-to-severe disease activity. We hypothesized that the serum iC3b/C3 ratio would demonstrate a stronger association with disease activity than conventional complement measurements and could serve as a clinically useful biomarker for the assessment of active systemic lupus erythematosus [10-14].

MATERIALS AND METHODS

Study Design and Participants

This cross-sectional observational study enrolled 100 consecutive adult Egyptian patients with systemic lupus erythematosus (SLE) who attended the Internal Medicine and Rheumatology Unit of Ain Shams University Hospitals. All participants fulfilled the **2012 Systemic Lupus International Collaborating Clinics (SLICC) classification criteria** for systemic lupus erythematosus [15]."

Patients were recruited over a one-year period and underwent standardized clinical and laboratory assessment at the time of study enrolment. The primary objective was to evaluate the relationship between the serum iC3b/C3 ratio and disease activity in patients with SLE.

Eligibility Criteria

Inclusion Criteria

Eligible participants met all the following criteria:

- Age ≥18 years.
- Definite diagnosis of SLE according to the 2012 SLICC classification criteria [15].
- Availability of complete clinical and laboratory data at the time of evaluation.

Exclusion Criteria

Patients were excluded if they had:

- Other autoimmune diseases that could influence complement activation.
- Current or previous malignancy.
- Pregnancy.
- Blood-borne infectious diseases.
- End-stage renal disease.

These eligibility criteria were applied to minimize potential confounding factors affecting complement activation and circulating complement biomarkers.

Clinical Assessment

All participants underwent comprehensive clinical evaluation by experienced rheumatologists. Demographic variables included age, sex, body mass index (BMI), disease duration, smoking status, and current medications.

Clinical assessment focused on SLE-related manifestations, including mucocutaneous involvement, arthritis, serositis, neurological manifestations, hematological abnormalities, and lupus nephritis. Information regarding corticosteroid exposure, cumulative steroid dose, hydroxychloroquine use, and immunosuppressive therapy was also recorded.

Disease Activity Assessment

"Disease activity was assessed using the **Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K)**, a validated instrument for quantifying global disease activity in SLE [16]."

For the primary analysis, patients were classified into two predefined groups according to SLEDAI-2K score:

- **Active disease:** moderate, high, or very high disease activity (SLEDAI-2K ≥6).
- **Inactive or mildly active disease:** remission or mild disease activity (SLEDAI-2K ≤5).

Accordingly, 50 patients were included in the active disease group and 50 in the inactive/mild disease group.

Laboratory Investigations

Venous blood samples were obtained from all participants under standardized conditions. Approximately 5 mL of peripheral venous blood was collected from each participant. One aliquot was transferred into EDTA-containing tubes for complete blood count analysis, while the remaining sample was allowed to clot, centrifuged to separate serum, aliquoted, and stored at −20°C until biochemical analysis.

Routine laboratory investigations included:

- Complete blood count.
- Erythrocyte sedimentation rate (ESR).
- Blood urea nitrogen.
- Serum creatinine.
- Estimated glomerular filtration rate (eGFR).
- Twenty-four-hour urinary protein excretion.
- Urine protein-to-creatinine ratio.
- Antinuclear antibodies (ANA).
- Anti-double-stranded DNA (anti-dsDNA) antibodies.
- Serum complement C3.
- Serum complement C4.

Measurement of Serum iC3b and Calculation of the iC3b/C3 Ratio

Serum iC3b concentrations were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. Serum C3 concentrations were determined using routine laboratory methods employed at the participating institutions.

To estimate the degree of complement activation, the serum iC3b/C3 ratio was calculated for each participant by dividing the serum iC3b concentration by the corresponding serum C3 concentration. This ratio was selected because it integrates complement activation (iC3b generation) with complement availability (C3 concentration), thereby providing a biologically meaningful indicator of ongoing complement consumption.

Study Outcome

The primary outcome was **moderate-to-severe SLE disease activity**, defined as a SLEDAI-2K score ≥6.

The principal exposure variable was the serum iC3b/C3 ratio. Secondary analyses evaluated associations between the iC3b/C3 ratio and conventional laboratory markers of disease

activity, including anti-dsDNA antibodies, serum C3, serum C4, ESR, hematological parameters, renal function indices, and proteinuria.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 20 (IBM Corp., Armonk, NY, USA). Continuous variables were assessed for normality before analysis and are presented as mean ± standard deviation (SD) or median with interquartile range (IQR), as appropriate. Categorical variables are expressed as frequencies and percentages.

Comparisons between two independent groups were performed using the independent-samples t test for normally distributed variables and the Mann–Whitney U test for non-normally distributed variables. Categorical variables were compared using the χ^2 test or Fisher's exact test when appropriate. Correlations between continuous variables were assessed using correlation analysis according to data distribution. Logistic regression analysis was performed to identify independent predictors of active disease. Variables with significant associations in univariable analyses were entered into multivariable logistic regression models to determine independent predictors after adjustment for potential confounders.

All statistical tests were two-sided, and a P value <0.05 was considered statistically significant.

RESULTS

Baseline Characteristics According to Disease Activity

The study included 100 patients with systemic lupus erythematosus (SLE), who were equally divided into two groups according to disease activity as assessed by the SLE Disease Activity Index 2000 (SLEDAI-2K): 50 patients with inactive or mildly active disease and 50 patients with moderate-to-severe disease activity.

There were no significant differences between the two groups regarding age (34.3 ± 8.0 vs. 36.8 ± 10.0 years, $P = 0.160$), female predominance (76.0% vs. 60.0%, $P = 0.086$), disease duration (5.66 ± 1.97 vs. 5.08 ± 1.54 years, $P = 0.103$), or body mass index (26.62 ± 3.50 vs. 25.87 ± 3.90 kg/m², $P = 0.316$).

Patients with moderate-to-severe disease activity exhibited a significantly greater frequency of major clinical manifestations than those with inactive or mildly active disease. Lupus nephritis was present in 86.0% of patients with active disease but was absent in the inactive/mild disease group ($P < 0.001$). Similarly, arthritis (48.0% vs. 4.0%, $P < 0.001$), malar rash (38.0% vs. 6.0%, $P < 0.001$), oral ulcers (34.0% vs. 10.0%, $P = 0.004$), alopecia (28.0% vs. 12.0%, $P = 0.046$), serositis (16.0% vs. 0%, $P = 0.003$), and neurological manifestations (10.0% vs. 0%, $P = 0.020$) were significantly more common among patients with active disease.

Compared with patients with inactive or mildly active SLE, those with moderate-to-severe disease activity demonstrated significantly lower white blood cell counts (median, 3.95 vs. 9.50 ×10³/mm³, $P < 0.001$), lower hemoglobin concentrations (9.95 ± 2.01 vs. 11.20 ± 2.33 g/dL, $P = 0.005$), and impaired renal function, as reflected by higher serum creatinine concentrations (median, 1.75 vs. 1.30 mg/dL, $P < 0.001$), higher blood urea levels (median, 54 vs. 39 mg/dL, $P = 0.001$), and lower estimated glomerular filtration rate (61.66 ± 14.99 vs. 82.26 ± 7.37 mL/min/1.73 m², $P < 0.001$). They also exhibited markedly higher inflammatory activity, with significantly elevated erythrocyte sedimentation rate (median, 99 vs. 27 mm/h, $P < 0.001$).

Markers of renal involvement were also significantly increased in patients with active disease, including 24-hour urinary protein excretion (414.9 ± 110.2 vs. 246.4 ± 55.3 mg/day, $P < 0.001$) and urine protein-to-creatinine ratio (3.17 ± 0.86 vs. 1.25 ± 0.18, $P < 0.001$).

Regarding serological markers, patients with moderate-to-severe disease activity had significantly higher anti-double-stranded DNA (anti-dsDNA) antibody levels (117.1 ± 34.4 vs. 65.5 ± 19.2 U/mL, $P < 0.001$) together with significantly lower serum C3 concentrations (89.4 ± 19.0 vs. 112.6 ± 16.0 mg/dL, $P = 0.043$). Although serum C4 concentrations tended to be lower in the active disease group, the difference did not reach statistical significance ($P = 0.052$). Likewise, antinuclear antibody (ANA) levels and absolute serum iC3b concentrations were comparable between the two groups ($P = 0.339$ and $P = 0.325$, respectively).

Despite the absence of a significant difference in absolute serum iC3b concentrations, the serum iC3b/C3 ratio was significantly higher in patients with moderate-to-severe disease activity than in those with inactive or mildly active disease (median, 7.03 vs. 4.30 μg/mg, $P = 0.001$), indicating enhanced complement activation relative to circulating C3 levels.

Regarding treatment, all patients received corticosteroids and hydroxychloroquine. Additional immunosuppressive therapy was used exclusively in the moderate-to-severe disease group, including mycophenolate mofetil (28.0%), azathioprine (26.0%), and cyclophosphamide (34.0%) (all $P < 0.001$). Patients with active disease also received significantly higher cumulative corticosteroid doses than those with inactive or mildly active disease (31.2 ± 11.0 vs. 18.5 ± 7.0 mg/day, $P < 0.001$) (**Table 1**).

Table 1. Baseline demographic, clinical, laboratory, biomarker, and treatment characteristics according to disease activity.

Variable	Inactive/Mild SLE (n=50)	Moderate/Severe SLE (n=50)	P value
Demographic characteristics			
Age (years), mean ± SD	36.8 ± 10.0	34.3 ± 8.0	0.160
Female sex, n (%)	30 (60.0)	38 (76.0)	0.086
Disease duration (years), mean ± SD	5.08 ± 1.54	5.66 ± 1.97	0.103
BMI (kg/m²), mean ± SD	25.87 ± 3.90	26.62 ± 3.50	0.316
Clinical manifestations, n (%)			
Lupus nephritis	0 (0.0)	43 (86.0)	<0.001
Arthritis	2 (4.0)	24 (48.0)	<0.001
Malar rash	3 (6.0)	19 (38.0)	<0.001
Oral ulcers	5 (10.0)	17 (34.0)	0.004
Alopecia	6 (12.0)	14 (28.0)	0.046
Serositis	0 (0.0)	8 (16.0)	0.003
Neurological manifestations	0 (0.0)	5 (10.0)	0.020
Laboratory findings			
WBCs (×10³/mm³), median (IQR)	9.5 (7.2–11.0)	3.95 (1.0–6.9)	<0.001
Lymphocyte count (×10³/mm³), median (IQR)	1.0 (0.6–1.4)	0.8 (0.4–1.2)	0.325
Hemoglobin (g/dL), mean ± SD	11.20 ± 2.33	9.95 ± 2.01	0.005
Platelet count (×10³/mm³), median (IQR)	254 (140–365)	193.5 (100–365)	0.301
ESR (mm/h), median (IQR)	27 (18–33)	99 (76–115)	<0.001
Serum creatinine (mg/dL), median (IQR)	1.30 (1.0–1.4)	1.75 (1.3–4.5)	<0.001
Blood urea (mg/dL), median (IQR)	39 (35–45)	54 (36–92)	0.001
24-hour urinary protein (mg/day), mean ± SD	246.4 ± 55.3	414.9 ± 110.2	<0.001
Urine protein/creatinine ratio, mean ± SD	1.25 ± 0.18	3.17 ± 0.86	<0.001
eGFR (mL/min/1.73 m²), mean ± SD	82.26 ± 7.37	61.66 ± 14.99	<0.001
Anti-dsDNA (U/mL), mean ± SD	65.5 ± 19.2	117.1 ± 34.4	<0.001
ANA (U/mL), median (IQR)	67.4 (60–80)	55.5 (18–90.5)	0.339
C3 (mg/dL), mean ± SD	112.6 ± 16.0	89.4 ± 19.0	0.043
C4 (mg/dL), mean ± SD	21.0 ± 6.59	15.9 ± 8.49	0.052
Complement biomarkers			
Serum iC3b (µg/dL), mean ± SD	523.7 ± 90.0	536.3 ± 120.0	0.325
iC3b/C3 ratio (µg/mg), median (IQR)	4.3 (3.8–5.5)	7.03 (3.5–8.5)	0.001
Treatment			
Corticosteroids + hydroxychloroquine	50 (100)	50 (100)	—
Mycophenolate mofetil	0	14 (28.0)	<0.001
Azathioprine	0	13 (26.0)	<0.001
Cyclophosphamide	0	17 (34.0)	<0.001
Cumulative steroid dose (mg), mean ± SD	18.5 ± 7.0	31.2 ± 11.0	<0.001

Data are presented as mean ± SD, median (IQR), or n (%), as appropriate. BMI, body mass index; ESR, erythrocyte sedimentation rate; eGFR, estimated glomerular filtration rate; ANA, antinuclear antibodies; anti-dsDNA, anti-double-stranded DNA antibodies.

Correlation Between the Serum iC3b/C3 Ratio and Clinical and Laboratory Indicators of Disease Activity

Among patients with moderate-to-severe SLE, the serum iC3b/C3 ratio demonstrated significant correlations with several markers of disease activity and organ involvement (**Table 2**). The ratio showed a moderate positive correlation with SLEDAI-2K score ($r = 0.382$, $P = 0.003$), indicating that higher levels of complement activation were associated with greater disease activity. Significant positive correlations were also observed with anti-dsDNA antibody levels ($r = 0.398$, $P = 0.004$), 24-hour urinary protein excretion ($r = 0.393$, $P = 0.039$), and urine protein-to-creatinine ratio ($r = 0.383$, $P = 0.006$).

Conversely, the serum iC3b/C3 ratio was inversely correlated with estimated glomerular filtration rate ($r = −0.406$, $P = 0.003$), platelet count ($r = −0.318$, $P = 0.024$), and serum C3 concentration ($r = −0.234$, $P = 0.019$), suggesting that increasing complement activation was associated with declining renal function, thrombocytopenia, and greater complement consumption.

No statistically significant correlations were observed between the serum iC3b/C3 ratio and white blood cell count, lymphocyte

count, hemoglobin concentration, erythrocyte sedimentation rate, ANA level, serum C4 concentration, or cumulative corticosteroid dose (all P > 0.05).

Table 2. Correlation between the serum iC3b/C3 ratio and clinical and laboratory indicators of disease activity in patients with active SLE (n = 50).

Variable	Spearman's r	P value
White blood cell count	−0.062	0.670
Lymphocyte count	−0.052	0.607
Hemoglobin	−0.024	0.867
Platelet count	−0.318	0.024
ESR	0.108	0.454
24-hour urinary protein	0.393	0.039
Urine protein/creatinine ratio	0.383	0.006
eGFR	−0.406	0.003
ANA	0.115	0.428
Anti-dsDNA	0.398	0.004
C3	−0.234	0.019
C4	−0.073	0.617
SLEDAI-2K	0.382	0.003
Cumulative steroid dose	0.126	0.382

Spearman's rank correlation coefficient was used for correlation analyses. Positive values indicate direct associations, whereas negative values indicate inverse associations. Statistically significant correlations are shown in bold.

Univariable Logistic Regression Analysis of Predictors of Moderate-to-Severe Disease Activity

Univariable logistic regression analysis identified several variables significantly associated with moderate-to-severe SLE disease activity (**Table 3**). Higher ESR, elevated anti-dsDNA antibody levels, lower serum C3 and C4 concentrations, increased serum iC3b/C3 ratio, greater 24-hour urinary protein excretion, and higher cumulative corticosteroid dose were all significantly associated with increased odds of moderate-to-severe disease activity (all $P < 0.01$). In contrast, the urine protein-to-creatinine ratio was not significantly associated with disease activity ($P = 0.053$).

Among all evaluated variables, the serum iC3b/C3 ratio demonstrated one of the strongest associations with disease activity, supporting its potential role as a biomarker of complement activation in active SLE.

Table 3. Univariable logistic regression analysis for predictors of moderate-to-severe disease activity in patients with systemic lupus erythematosus.

Variable	Odds Ratio (OR)	95% Confidence Interval	P value
ESR (mm/h)	117.00	103.20–134.28	<0.001
Anti-dsDNA (U/mL)	52.39	15.00–182.99	<0.001
C3 (mg/dL)	8.1	2.57–14.73	<0.001
C4 (mg/dL)	8.6	0.57–16.73	0.003
iC3b/C3 ratio	16.00	5.38–47.57	<0.001
24-hour urinary protein (mg/day)	1.033	1.018–1.048	<0.001
Urine protein/creatinine ratio	0.979	7.68–61.74†	0.053
Cumulative steroid dose (mg/day)	12.25	4.65–32.26	<0.001

Abbreviations: ESR, erythrocyte sedimentation rate; anti-dsDNA, anti-double-stranded DNA antibodies; OR, odds ratio; CI, confidence interval.

Multivariable Logistic Regression Analysis of Independent Predictors of Moderate-to-Severe Disease Activity

Variables demonstrating significant associations in the univariable analysis were subsequently entered into a multivariable logistic regression model (**Table 4**). After adjustment for potential confounding variables, the serum iC3b/C3 ratio remained the strongest independent predictor of moderate-to-severe disease activity (adjusted OR = 110.31, 95% CI: 8.16–1491.94; P < 0.001).

Anti-dsDNA antibody level also remained independently associated with disease activity (adjusted OR = 1.37, 95% CI: 1.05–1.80;

P = 0.022), while serum C3 concentration retained independent predictive significance (P = 0.030). In contrast, ESR, serum C4 concentration, 24-hour urinary protein excretion, urine protein-to-creatinine ratio, and cumulative corticosteroid dose were no longer independently associated with disease activity after multivariable adjustment (all P > 0.05).

Overall, these findings indicate that the serum iC3b/C3 ratio represents a robust independent indicator of disease activity in SLE, outperforming conventional inflammatory markers after adjustment for established clinical and laboratory predictors.

Table 4. Multivariable logistic regression analysis for independent predictors of moderate-to-severe disease activity.

Variable	Adjusted OR	95% Confidence Interval	P value
ESR (mm/h)	10.32	1.37–177.88	0.360
Anti-dsDNA (U/mL)	1.37	1.05–1.80	0.022
C3 (mg/dL)	564	1.27–1127.00	0.030
C4 (mg/dL)	56.2	0.88–111.71	0.052
iC3b/C3 ratio	110.31	8.16–1491.94	<0.001
24-hour urinary protein (mg/day)	0.179	0.019–1.693	0.133
Urine protein/creatinine ratio	0.820	0.129–15.199	0.833
Cumulative steroid dose (mg/day)	0.282	0.019–14.243	0.360

Abbreviations: OR, odds ratio; CI, confidence interval; ESR, erythrocyte sedimentation rate; anti-dsDNA, anti-double-stranded DNA antibodies.

DISCUSSION

The present study investigated the clinical utility of the serum iC3b/C3 ratio as a biomarker of disease activity in patients with systemic lupus erythematosus (SLE). The principal findings demonstrate that the serum iC3b/C3 ratio was significantly elevated in patients with moderate-to-severe disease activity compared with those with inactive or mildly active disease. Furthermore, the ratio correlated significantly with established indicators of disease activity, including SLEDAI-2K score, anti-double-stranded DNA (anti-dsDNA) antibody levels, proteinuria, urine protein-to-creatinine ratio, and estimated glomerular filtration rate (eGFR). Importantly, multivariable logistic regression analysis identified the serum iC3b/C3 ratio as the strongest independent predictor of moderate-to-severe disease activity after adjustment for conventional clinical and laboratory variables. These findings support the hypothesis that the iC3b/C3 ratio reflects ongoing complement activation more accurately than measurement of individual complement components alone.

Complement activation is a central pathogenic mechanism in SLE, contributing to immune complex deposition, inflammation, and tissue injury. Activation of the classical complement pathway leads to cleavage of C3 into biologically active fragments, including iC3b, while progressive consumption of native C3 accompanies increasing disease activity. Consequently, simultaneous assessment of complement activation products and complement consumption may provide a more dynamic evaluation of disease activity than static measurement of serum C3 or C4 concentrations alone. Previous investigations have shown that complement activation fragments, including iC3b, C3dg, and C4d, increase during disease flares and may correlate more closely with clinical activity than conventional complement measurements [5,8,11].

In the current study, absolute serum iC3b concentrations did not differ significantly between patients with active and inactive disease. In contrast, the serum iC3b/C3 ratio was significantly higher in patients with moderate-to-severe disease activity, suggesting that normalization of iC3b to circulating C3 enhances the sensitivity of complement assessment by simultaneously accounting for complement activation and complement consumption. This observation supports previous reports indicating that complement activation indices outperform isolated complement measurements for monitoring lupus activity and identifying patients with active immune complex-mediated inflammation [12-15].

The serum iC3b/C3 ratio also demonstrated significant positive correlations with SLEDAI-2K score and anti-dsDNA antibody levels, two widely accepted indicators of disease activity in SLE. Higher ratios were additionally associated with greater proteinuria and higher urine protein-to-creatinine ratios, whereas inverse correlations with eGFR and serum C3 concentrations suggest that increasing complement activation accompanies progressive renal impairment and complement consumption. These findings are biologically plausible because lupus nephritis is characterized by intrarenal complement activation and immune complex deposition, processes that simultaneously consume circulating complement components while generating activation fragments detectable in peripheral blood. The absence of a significant correlation with serum C4 may reflect the greater variability of C4 concentrations and the influence of inherited C4 copy number variation, which limits its sensitivity as an isolated biomarker of disease activity.

One of the most important findings of the present study is that the serum iC3b/C3 ratio remained independently associated with moderate-to-severe disease activity after adjustment for conventional laboratory markers. Although anti-dsDNA antibody levels and serum C3 also retained independent significance, inflammatory markers such as erythrocyte sedimentation rate (ESR), proteinuria, urine protein-to-creatinine ratio, and cumulative corticosteroid dose lost significance in the multivariable model. These observations suggest that the iC3b/C3 ratio provides complementary information beyond traditional biomarkers and may better reflect the underlying immunopathological processes driving disease activity.

The findings of the present study are consistent with an expanding body of evidence supporting the use of complement activation products as biomarkers of disease activity in SLE. Several investigations have demonstrated that activation fragments generated during complement cascade activation correlate more closely with disease flares than measurements of intact complement proteins. Unlike serum C3 and C4, which are influenced by both synthesis and consumption, activation products directly reflect ongoing complement activation and immune complex-mediated inflammation. Consequently, biomarkers such as iC3b, C3dg, and cell-bound complement activation products (CB-CAPs) have attracted increasing interest as more sensitive indicators of disease activity and treatment response.

Previous studies have reported that serum iC3b concentrations increase during active SLE and correlate with disease activity indices. However, considerable overlap in absolute iC3b concentrations between active and inactive disease has limited its clinical applicability when used as a standalone biomarker. Our findings provide additional evidence that expressing iC3b relative to its precursor protein C3 substantially improves its discriminatory ability. Although absolute serum iC3b concentrations did not differ significantly between disease activity groups, the serum iC3b/C3 ratio demonstrated significant differences between patients with active and inactive disease, supporting the concept that simultaneous assessment of complement activation and complement consumption provides a more comprehensive evaluation of complement system dynamics.

The observed association between the serum iC3b/C3 ratio and lupus nephritis is particularly noteworthy. Patients with moderate-to-severe disease activity exhibited substantially higher rates of renal involvement, greater proteinuria, higher urine protein-to-creatinine ratios, elevated serum creatinine concentrations, and reduced eGFR. Furthermore, the iC3b/C3 ratio correlated positively with proteinuria and negatively with renal function, suggesting that increased complement activation parallels the severity of renal involvement. These findings are consistent with current understanding of lupus nephritis, in which persistent activation of the classical complement pathway contributes to glomerular inflammation, endothelial injury, and progressive renal dysfunction. Although renal biopsy remains the diagnostic gold standard for lupus nephritis, circulating biomarkers capable of reflecting ongoing complement-mediated injury may provide valuable adjunctive information during disease monitoring and follow-up.

An important strength of the present study is the comprehensive evaluation of the iC3b/C3 ratio in relation to both conventional laboratory biomarkers and a validated clinical disease activity index. By integrating demographic characteristics, clinical manifestations, inflammatory markers, renal parameters, serological biomarkers, and multivariable regression analysis, the study provides a robust assessment of the independent relationship between complement activation and SLE disease activity. The inclusion of multivariable logistic regression further strengthens the findings by demonstrating that the serum iC3b/C3 ratio remained independently associated with disease activity after adjustment for established clinical and laboratory predictors.

The present study also has several limitations. First, its cross-sectional design precludes assessment of temporal changes in the serum iC3b/C3 ratio during disease flares and remission, limiting conclusions regarding its ability to predict future disease activity or therapeutic response. Second, the study was conducted at a single tertiary referral center with a relatively modest sample size, which may limit the generalizability of the findings to broader SLE populations. Third, serial measurements of complement activation products were not available; therefore, the value of longitudinal monitoring could not be evaluated. Finally, additional complement activation biomarkers, including C3dg, C4d, Bb, soluble C5b-9, and cell-bound complement activation products, were not measured, precluding direct comparison of their diagnostic performance with that of the serum iC3b/C3 ratio.

Despite these limitations, the present study provides clinically relevant evidence supporting incorporation of the serum iC3b/C3 ratio into the laboratory assessment of SLE disease activity. Because conventional biomarkers such as serum C3, C4, and anti-dsDNA antibodies do not consistently reflect ongoing disease activity in all patients, a biomarker that simultaneously captures complement activation and complement consumption may improve disease monitoring and facilitate earlier recognition of disease exacerbation. The serum iC3b/C3 ratio may therefore represent a useful adjunct to existing clinical assessment tools rather than a replacement for established biomarkers.

Future multicenter prospective studies involving larger and more ethnically diverse patient populations are warranted to validate these findings and establish standardized reference

ranges and optimal clinical cut-off values for the serum iC3b/C3 ratio. Longitudinal studies evaluating changes in the ratio before, during, and after disease flares will be particularly valuable in determining its utility for predicting relapse, monitoring therapeutic response, and guiding individualized treatment strategies. Comparative studies evaluating the serum iC3b/C3 ratio alongside emerging complement biomarkers, including cell-bound complement activation products and terminal complement activation markers, may further define its role within multimarker approaches for precision medicine in SLE.

CONCLUSIONS

The serum iC3b/C3 ratio was significantly associated with disease activity in patients with systemic lupus erythematosus and demonstrated superior clinical utility compared with measurement of absolute serum iC3b concentrations alone. The ratio correlated with established indicators of disease activity, including SLEDAI-2K score, anti-dsDNA antibody levels, proteinuria, and renal function, while remaining an independent predictor of moderate-to-severe disease activity after multivariable adjustment. These findings indicate that the serum iC3b/C3 ratio reflects ongoing complement activation and complement consumption simultaneously, providing a more comprehensive assessment of disease activity than conventional complement measurements. Incorporation of the serum iC3b/C3 ratio into routine clinical evaluation may improve disease monitoring and risk stratification in patients with SLE. Nevertheless, larger prospective multicenter studies are required to validate its prognostic value, determine standardized diagnostic thresholds, and establish its role in guiding personalized therapeutic decision-making.

Declarations

Ethics Approval and Consent to Participate

The study protocol was reviewed and approved by the Research Ethics Committee, Faculty of Medicine, Ain Shams University, Cairo, Egypt.(IRB number : FMASU M S 722/2021) The study was conducted in accordance with the ethical principles of the Declaration of Helsinki [17]. and its subsequent amendments. Written informed consent was obtained from all participants prior to enrolment in the study.

Consent for Publication

Not applicable.

Availability of Data and Materials

The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

All authors made substantial contributions to the conception and design of the study, data acquisition, analysis, and interpretation. All authors participated in drafting the manuscript and critically revising it for important intellectual content. All authors reviewed and approved the final version of the manuscript before submission and agree to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the study are appropriately investigated and resolved.

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