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Serum S100P and C4d proteins as markers for active proliferative lupus nephritis in Egyptian patients with systemic lupus erythematosus

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Abstract. *Lupus nephritis (LN), occurring in approximately 50% of individuals with systemic lupus erythematosus (SLE), significantly influences therapy and prognosis. Proliferative LN, more prevalent than non-proliferative LN, is associated with a poorer prognosis. This study aimed to investigate the potential correlation between serum levels of the pro-inflammatory proteins human calcium-binding S100P (S100P) and Human complement Fragment 4 (C4d) and confirmed proliferative LN through biopsy. The objective was to evaluate their reliability as biomarkers for disease activity in proliferative LN.*

Methods. Serum S100P and C4d were measured using enzyme-linked immunosorbent assay in 100 female patients with established SLE. Among these, 50 had confirmed proliferative LN through biopsy, and 50 did not. A control group of 50 healthy female donors' plasma samples was included.

Results. SLE patients with proliferative LN exhibited significantly higher serum levels of S100P ($P < 0.001$) and C4d ($P < 0.001$) compared to SLE patients without LN and controls. Serum S100P demonstrated a significantly higher positive correlation with the activity index in renal biopsies ($r = 0.784$) compared to serum C4d ($r = 0.416$). Additionally, serum S100P had a significantly higher positive correlation with SLEDAI-R than serum C4d ($r = 0.651$ vs. $r = 0.257$). Both serum S100P and C4d showed promise as reliable biomarkers for the diagnosis of active proliferative LN, as indicated by the ROC curve and AUC assessments.

Conclusion. Serum concentrations of S100P and C4d emerge as useful indicators for identifying SLE patients with active proliferative LN.

Keywords: *systemic lupus erythematosus, lupus nephritis, calcium-binding protein S100P, C4d fragment.*

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Протеїни сироватки S100P і C4d як маркери активного проліферативного люпус нефриту у пацієнтів з системним червоним вовчаком з Єгипту

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Резюме. *Люпус нефрит (ЛН) діагностується приблизно у 50% пацієнтів з системним червоним вовчаком (СЧВ) та суттєво впливає на вибір лікування і прогноз. Проліферативний ЛН є більш поширеним ніж непроліферативний та асоційований з гіршим прогнозом. Це дослідження мало на меті дослідити потенційний зв'язок прозапального протеїну S100P (S100P) і фрагменту комплемента людини 4 (C4d) з проліферативним ЛН, підтвердженим за допомогою біопсії та оцінити їх надійність як біомаркерів активності захворювання.*

Методи. Сироваткові S100P і C4d визначали за допомогою твердофазного імуноферментного аналізу у 100 пацієнтів із встановленим СЧВ. Серед включених у дослідження пацієнтів 50 хворих мали проліферативний ЛН, тоді як 50 не мали ураження нирок. Контрольну групу склали 50 умовно-здорових жінок.

Результати. Пацієнти з СЧВ та проліферативним ЛН продемонстрували значно вищі рівні S100P ($P < 0,001$) і C4d ($P < 0,001$) у порівняно з пацієнтами з СЧВ без ЛН та контрольною групою. Сироватковий S100P мав позитивний кореляційний зв язок з індексом гістологічної активності захворювання ($r = 0,784$) та був більш значущим у порівнянні з C4d ($r = 0,416$). Крім того, S100P мав більш значущий кореляційний зв'язок з SLEDAI-R, ніж сироватковий C4d ($r = 0,651$ проти $r = 0,257$). Обидва маркери продемонстрували статистично значущу надійність для діагностики активного проліферативного ЛН за результатами ROC аналізу.

Висновки. Концентрації S100P і C4d сироватки крові є корисними індикаторами для ідентифікації СЧВ пацієнтів з активним проліферативним ЛН.

Ключові слова: *системний червоний вовчак, вовчаковий нефрит, кальційзв язуючий білок S100P, C4d фрагмент.*

Introduction. Systemic lupus erythematosus (SLE) is the archetypal case of a systemic autoimmune disease, affecting multiple systems and producing a large variety of autoantibodies. Up to 50% of the SLE population may have lupus nephritis (LN) [1]. Despite significant advancements in the treatment of SLE patients, there is still a need for precise early diagnostic, sub-classifying, monitoring, and forecasting biomarkers for everyday clinical use [2]. One of the most often targeted organs is the kidney. In the US, LN is the main cause of morbidity and mortality [3]. End-stage kidney disease (ESKD), which requires dialysis or kidney transplantation, affects a sizable fraction of patients despite great advances in our understanding of the etiology of LN [4].

S100 proteins are being investigated as potential diagnostic and prognostic indicators for a range of inflammatory diseases and cancers. The S100A protein family is a huge calcium-binding protein subfamily that controls many physiological processes. Members of this family act as damage-associated molecular patterns (DAMPs), which are largely engaged in innate immune responses. Many different types of cells and tissues contain these small, acidic proteins (10–12 kDa). Through interactions with various effector proteins, they control homeostasis, the cell cycle, migration, phosphorylation, and the release of a range of proteins [5]. Among additional S100 proteins that bind calcium are S100 A4, A7, A9, A11, A12, B, and S100P. S100 proteins control a variety of biological processes, including calcium homeostasis, cell division and apoptosis, cell invasion and motility, cancer metastasis, angiogenesis, cytoskeleton connections, protein phosphorylation, transcription factor regulation, autoimmune, chemotaxis, and inflammation [6, 7]. A distinctive characteristic of S100 proteins is their capacity to form dimers. Many of these proteins' biological activities seem to be dependent on dimerization, which may be because it interferes with their ability to bind to target structures [8, 9]. The prior investigation of S100A8/A9 and S100A12 in SLE and glomerulonephritis (GN) has been documented by Tyden et al. In that paper, the levels of S100 proteins were found to exhibit no disparity based on the presence or absence of nephritis, while variations were observed contingent only on the existence of immunosuppression [10]. Also, Davies and his colleague investigated S100A8/A9 and S100A12 with Real disease activity based on BILAG-BR scores not confirmed by renal

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biopsy [11]. S100P is expressed in various normal tissues including the placenta, lung, heart, kidney, bladder, skeletal muscle, and other organs, and has been associated with migration, invasion, proliferation, and progression of several types of cancer including pancreatic, prostate, non-small cell lung, breast, and colorectal cancer. S100 P role has been investigated in the pathogenesis of malignancy and response to treatment but not investigated before in SLE patients. The pathogenesis of LN involves immune complex deposition, which strongly activates complement and thus incites inflammatory mechanisms contributing to the severity of the disease [12]. Complement component 4 (C4) is a protein that is derived from the human leukocyte antigen (HLA) system and is a component of the complex complement system in humans. It carries out several crucial functions in immunity, tolerance, and autoimmunity with the assistance of other numerous components. It's crucial for linking the other innate immune response effector proteins to the recognition pathways that are activated by antibody-antigen (Ab-Ag) complexes throughout the whole system [13].

C3 and C4 levels can be affected by inflammation and don't reflect the activation of complement accurately in SLE patients.C3 and C4 have low sensitivity in the follow-up of SLE patients and were unable to predict the recurrence of LN as stand-alone markers [14]. Plasma levels of C4d, which is the final cleavage fragment of C4 exclusively arise from complement activation [15].

Martin and his colleagues detected that the plasma C4d level is negligible in healthy control, and C4d levels were significantly higher in LN patients than in patients without renal involvement, good correlation with anti. dsDNA antibodies, and can distinguish between remission and flare, as well as predict future LN flares in relapsing patients in SLE [16].

Martin et al, found that the C4d plasma levels are higher in SLE with LN than in SLE patients with non-renal manifestations, which may be a better measure for active LN than for chronic presentation. However, he did not analyze associations between specific non-renal manifestations and C4d findings [30].

To our knowledge, serum levels of S100P in active SLE patients with proliferative LN have not been investigated. This investigation aimed to assess serum levels of S100P and C4d in relation to renal disease activity with an emphasis on biopsy-proven proliferative LN and their correlation with the NIH lupus activity index in renal biopsy and proteinuria. Also, analyze associations between non-renal SLE manifestations and both S100P and C4d.

Patient and methods. This cross-sectional study included 100 female patients diagnosed with systemic lupus erythematosus (SLE) based on The Systemic Lupus International Collaborating Clinics (SLICC) classification criteria. The participants were recruited from the rheumatology and nephrology clinics at Cairo University hospitals over a six-month period. Group I comprised 50 SLE patients without lupus nephritis (LN), and Group II comprised 50 SLE patients with biopsy-proven LN (ISN/PRS class III and IV), all testing positive for antinuclear antibody (ANA) and anti-double-stranded DNA (anti-dsDNA). A control group (Group III) consisted of 50 age-matched healthy female participants. The study adhered to ethical standards set by the Internal Medicine department's ethical committee (Cairo University) and followed the 1975 Declaration of Helsinki, as revised in 2008. Informed consent was obtained from all participants. SLE patients with proliferative LN were not on immunosuppressive drugs (only receiving anti-malarial treatment with Hydroxychloroquine) when blood samples were collected, while SLE patients without LN were on anti-malarial treatment (Hydroxychloroquine) and small doses of oral prednisolone ranging from 5 to 10 mg per day.

The Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) was utilized to assess disease activity. SLEDAI-2K items on the involvement of specific organs or tissues were consolidated into distinct domains, including neuropsychiatric features (seizure, psychosis, organic brain syndrome, cranial nerve disorder, lupus headache, cerebrovascular accident), renal features (hematuria, proteinuria, pyuria, urinary casts), serositis (pleurisy, pericarditis), and hematological features (thrombocytopenia, leukopenia) [18].

LN activity was also assessed using the SLE Disease Activity Index Renal Domain (SLEDAI-R). Each of the four SLEDAI-R items obtains a score of 4 for the SLEDAI-R score (range 0-16; 0 indicates inactive LN): cellular casts, hematuria, and pyuria (both > 5 cells/high power field) [19]. All SLE patients' demographic and clinical data, including age at diagnosis, duration of the disease, SLE disease activity as determined by the SLEDAI-2K, SLEDAI-R score for LN, and individual organ-specific disease involvement, laboratory data, and current medications, were gathered at the time of visits. Renal biopsy was performed for SLE patients with proteinuria greater than 0.5 g/day or active urine sediment. Additionally, nrSLEDAI-2K, or non-renal SLEDAI-2K, was estimated. NIH Lupus Activity Index (NIH-AI) (score of 24) and NIH Lupus Chronicity Index (NIH-CI) (score of 12), both of which were evaluated by a renal pathologist, were modified National Institutes of Health activity and chronicity indices [20].

All SLE patients and healthy controls had their blood drawn, centrifuged, and stored at – 20 °C until analysis. Enzyme-linked immunosorbent assays (ELISA) were used to measure serum S100 P and C4d levels per the manufacturer's instructions (Shanghai Sunred Biological Technology Co., Ltd.). The AU system with reagents (Beckman Coulter, Brea, CA, USA) was used to measure complement levels. The reference range for serum C3 was 90–120 mg/dl, while serum C4's range was 10–20 mg/dl.

Statistical analysis. The statistical analysis was conducted using IBM SPSS version 26 (IBM SPSS,

Armonk, NY, USA). Data normality was assessed using the Shapiro-Wilk test with a significance threshold of 5%. Continuous variables were presented using the interquartile range (IQR) and median value. Categorical data were summarized using absolute frequencies and percentages. Correlations between nonparametric data were analyzed using Spearman's rank-order correlation test. Mann–Whitney U and Wilcoxon signed-rank tests were applied for statistical analyses. The chi-squared test was employed for comparing qualitative data. Spearman and Pearson correlation analyses were used to assess linear relationships between parameters, with correlation coefficients (r) interpreted as very weak (<0.2), weak (0.2–0.4), moderate (0.4–0.6), high (0.6–0.8), and very high (>0.8). Receiver operating characteristic (ROC) curve analysis determined the accuracy of distinguishing SLE patients with LN from those without LN. The significance threshold was set at a two-tailed p-value of 0.05 or less.

Results. The demographic, clinical, and laboratory characteristics of the patients and healthy controls are summarized in Tables 1 and 2.

Table 1

Studied participants' demographics and clinical data

Variables	SLE without LN (n = 50)	SLE with proliferative LN (n = 50)	Age-Matched control (n = 50)	*P*-value
Age (Years)	22 (8)	23.5 (8)	22 (10)	0.5839
Weight (kg)	65 (13)	65 (14)	64 (10)	0.7776
Duration of SLE (months)	6.5 (6)	7 (8)	-	0.4514
Duration of LN (months)	-	4 (3)	-	-
SLEDAI-2K score	16 (3)	10 (7)	-	< 0.001
SLEDAI-R score	0	8 (8)	-	< 0.001
nrSLEDAI-2K	16 (3)	2.5 (2)	-	< 0.001
Seizure (N,%)	0 (0 %)	0 (0 %)	-	-
Psychosis (N,%)	0 (0 %)	0 (0 %)	-	-
Organic brain syndrome(N,%)	0 (0 %)	0 (0 %)	-	-
Visual disturbance (N,%)	0 (0 %)	0 (0 %)	-	-
Cranial nerve damage (N,%)	0 (0 %)	0 (0 %)	-	-
Lupus headache (N,%)	0 (0 %)	0 (0 %)	-	-
Cerebrovascular accident (N,%)	14 (28 %)	0 (0 %)	-	-
Vasculitis (N,%)	44 (88 %)	0 (0 %)	-	-
Arthritis (N,%)	13 (26 %)	0 (0 %)	-	-
Myositis (N,%)	16 (32 %)	0 (0 %)	-	-
Kidney involvement (N,%)	0 (0%)	50 (100%)	-	-
Rash (N,%)	13 (26 %)	1 (2 %)	-	-
Alopecia (N,%)	11 (22 %)	1 (2 %)	-	-
Oral Ulcer (N,%)	14 (28 %)	0 (0%)	-	-
Pleurisy (N,%)	12 (24 %)	8 (16 %)	-	-
Pericarditis (N,%)	13 (26 %)	8 (16 %)	-	-
Low complement C3or C4 (N,%)	17 (34 %)	50 (100 %)	-	-
Increase DNA binding (N,%)	50 (100%)	50 (100%)	-	-
Fever (N,%)	24 (48 %)	3 (6 %)	-	-

Continuation of Table 1

Variables	SLE without LN (n = 50)	SLE with proliferative LN (n = 50)	Age-Matched control (n = 50)	*P*-value
Thrombocytopenia (N,%)	4 (8 %)	5 (10 %)	-	-
Leukopenia (N,%)	0 (0%)	0 (0%)	-	-
Renal biopsy class III (N,%)	-	31 (62%)	-	-
Renal biopsy class IV (N,%)	-	19 (38%)	-	-
Activity index (/24)	-	13 (2)	-	-
Chronicity index (/12)	-	0 (1)	-	-

All table values are expressed as median (IQR) for continuous variables and number (percent) for categorical variables.

Abbreviations: SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000; SLEDAI-R: SLE Disease Activity Index Renal Domain; SLE: systemic lupus erythematosus; LN: lupus nephritis. nrSLEDAI-2K: non-renal Systemic Lupus Erythematosus Disease Activity Index 2000

Table 2

Studied participants' laboratory data

Variables	All SLE patients (n = 100)	Control (n = 50)	*P*-value	SLE without LN (n = 50)	SLE with proliferative LN (n = 50)	*P*-value
Urea, mg/dL	27.5 (20)	13.5 (5)	< 0.001	24 (13)	32 (31)	0.076
Creatinine, mg/dL	0.9 (0.4)	0.8 (0.1)	< 0.001	0.8 (0.2)	1.05 (0.95)	< 0.001
Uric Acid, mg/dl	4.9 (1)	4.5 (1.1)	0.26	4.9 (2)	4.9 (1)	0.722
ALT, IU//L	20.5 (11)	18.5(6)	0.0609	21 (9)	20.5 (15)	0.344
AST, IU//L	22 (11)	20.2(8)	0.0445	21.5 (9)	22.5 (12)	0.893
Hemoglobin, g/dL	11.9 (3.53)	12.9 (0.5)	< 0.001	12 (2.55)	11 (4.75)	0.213
WBCs, $\times 10^9$/ml	7.60 (3.65)	6.4 (1.3)	< 0.001	7.80(3.38)	6.6 (4.13)	0.702
Platelet count, $\times 10^9$/ml	220 (167)	325 (87)	< 0.001	172 (34)	305 (134)	< 0.001
Corrected Ca, mg/dL	8.4 (0.9)	9.1(0.6)	< 0.001	8 (0.52)	8.9 (0.93)	< 0.001
Phosphorus, mg/dL	4.0 (0.68)	3.6(0.4)	< 0.001	4.4 (1.2)	4 (0.52)	< 0.001
ESR, mm/h	53 (50)	4 (2)	< 0.001	66 (44)	50 (47)	0.72
CRP, mg/dl	1.65 (3.78)	0.8 (0.8)	0.0059	1.20(2.17)	1.95 (4.47)	0.332
Urinary protein, g/24 h	1.05 (2.8)	-	-	0.2 (0.2)	3 (1.05)	< 0.001
Serum C3, mg/dL	98.15 (45)	-	-	113 (44)	84 (35.5)	< 0.001
Serum C4, mg/dL	14 (16)	-	-	16 (2.6)	9.1 (17)	0.636
S100P, 0.05-10ng/m	13.15(10.2)	0.06(0.02)	< 0.001	10.90(1.3)	20.8 (5.2)	< 0.001
C4d, 0.05-15U/L	17.55(2.67)	0.06(0.02)	< 0.001	16.65(2.6)	18.45(3.175)	< 0.001

All values are expressed as median (IQR) for continuous variables and number (percent) for categorical variables.

Abbreviations: ALT: alanine transaminase; AST: aspartate transaminase; WBC: white blood cell; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; Corrected Ca: Corrected calcium; Serum C3: serum complement 3; Serum C4: serum complement 4; S100P: Human calcium-binding S100P protein, C4d: Human complement Fragment 4.

One hundred SLE female patients with a disease duration of 1 to 28 months were split into two groups according to the presence or absence of proliferative LN as determined by a biopsy. With LN durations ranging from 1 to 9 months, 50 of them had active proliferative LN (focal or diffuse proliferative LN; ISN/PRS classes III and IV), 31 of whom had Class III, and 19 of whom had Class IV. The median activity index score for the kidney biopsy was 13 (range: 8–16), and the median chronicity index score was 0 (range: 0–4). With a statistically significant difference (p <0.001), the median SLEDAI-2K score for SLE patients without LN was 16 (range: 12-26), while the median score for SLE patients with active proliferative LN was 10 (range: 6–17). For an SLE with an active proliferative LN, the median SLEDAI-R score was 8, with a range of 4 to 12. Serum S100P levels were significantly different between SLE with active proliferative LN and SLE without LN, as shown by Table 2 and Fig. 1 (median 20.8 ng/ml [range between 12.8–45] versus 10.9 ng/ml [range between 9.5-13.5]; P < 0. 001).

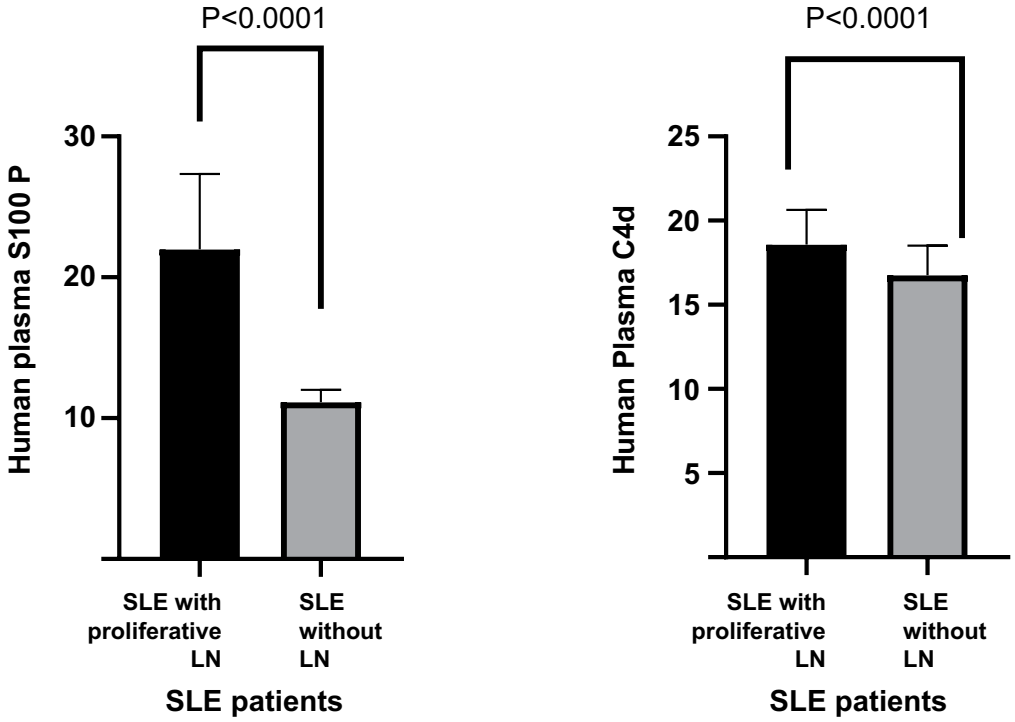

Fig. 1. Difference in serum level of S100P and serum level of C4d in SLE patients with and without LN.

Abbreviations: SLE: systemic lupus erythematosus, LN: lupus nephritis, S100P: Human calcium-binding S100P protein, C4d: Human complement Fragment 4.

Additionally, there was a significant difference between SLE with active proliferative LN and SLE without LN in terms of serum C4d levels (median 18.45 U/L [range: 15.9–24] versus 16.6 U/L [range: 10.6–20.6]; P < 0.001). In SLE patients with active proliferative LN, serum levels of S100P protein and C4d protein had a positive correlation with the SLEDAI-R score (r = 0.651 and r = 0.257, respectively; P < 0.001), a strong positive correlation with 24-hour urinary protein (for S100P is r= 0.755, P < 0.001; for C4d is r = 0.386, P < 0.001) and positive correlation with anti-DNA (for S100P is r= 0.543, P < 0.001; for C4d is r = 0.397, P < 0.001). While there were substantial negative associations with SLEDAI-2K (for S100P is r = – 0.564, P < 0.001; for C4d is r = – 0.387, P < 0.001), nrSLEDAI-2K (for S100P is r = – 0.763, P < 0.001; for C4d is r = – 0.409, P < 0.001). Additionally, the serum S100P level showed a weak negative correlation with C3 (r = – 0.319, P < 0.001), a moderate positive correlation with LN Duration (r = 0.643, P < 0.001), a high positive correlation with the NIH-lupus activity index score in renal biopsy (r =0.784, P < 0.001), and no correlation with C4. Serum C4d protein levels were moderately positively correlated with LN duration (r = 0.443, P < 0.001), and NIH-lupus activity index score in renal biopsy (r = 0.416, P < 0.001), but not with C3 or C4 levels (r = – 0.0913, P = 0.368) (Table 3).

Table 3

Correlation between S100P, C4d, and different variables in SLE patients with active proliferative nephritis

Variables	S100P		C4d	
	r	*P*-value	r	*P*-value
Age (Years)	0.180	0.105	– 0.034	0.814
Body weight	0.112	0.76	– 0.021	0.885
Duration of LN (months)	0.643	< 0.001	0.443	0.659
SLEDAI-2K	– 0.564	< 0.001	– 0.387	< 0.001
nrSLEDAI-2K	– 0.763	< 0.001	0.409	< 0.001
SLEDAI-R	0.651	< 0.001	0.257	< 0.001
24-hour urinary protein (g/day)	0.755	< 0.001	0.386	< 0.001
Anti-DNA	0.543	< 0.001	0.397	< 0.001
NIH-lupus activity index score in Biopsy	0.784	< 0.001	0.416	< 0.001
Complement C3	– 0.319	< 0.001	– 0.091	0.368
Complement C4	0.187	0.263	0.265	0.29

P-value< 0.05 was considered statistically significant.

Abbreviations: SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000; SLEDAI-R: SLE Disease Activity Index Renal Domain; SLE: systemic lupus erythematosus; LN: lupus nephritis. nrSLEDAI-2K: non-renal Systemic Lupus Erythematosus Disease Activity Index 2000, S100P: Human calcium-binding S100P protein, C4d: Human complement Fragment 4.

In SLE without LN, only S100P had a statistically significant weak correlation with SLEDAI-2K and nrSLEDAKI (r = 0.372, P =0.008), C4d had a negative correlation with 24 h urine protein (r = – 0.421, P =0.02). Both S100P and C4d had a significant correlation with anti-dsDNA antibodies (for S100P is r = 0.279, P = 0.029; for C4d is r = – 0.115, P = 0.048) (Table 4).

Table 4

Correlation between S100P, C4d, and different variables in SLE patients without LN

Variables	S100P		C4d	
	r	*P*-Value	r	*P*-Value
Age (Years)	– 0.341	0.115	0.135	0.352
Body weight	– 0.110	0.446	0.125	0.388
Duration of LN (months)	0.058	0.689	– 0.026	0.858
SLEDAI-2K	0.372	0.008	0.095	0.511
nrSLEDAI-2K	0.372	0.008	0.095	0.511
24-hour urinary protein (g/day)	0.143	0.323	– 0.421	0.02
Anti-DNA	0.279	0.029	– 0.115	0.048
Complement C3	– 0.152	0.292	0.060	0.676
Complement C4	– 0.132	0.360	0.115	0.427

P-value< 0.05 was considered statistically significant.

Abbreviations: SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000; SLEDAI-R: SLE Disease Activity Index Renal Domain; SLE: systemic lupus erythematosus; LN: lupus nephritis. nrSLEDAI-2K: non-renal Systemic Lupus Erythematosus Disease Activity Index 2000, S100P: Human calcium-binding S100P protein, C4d: Human complement Fragment 4.

S100P and C4d proteins were investigated for their potential as diagnostic biomarkers to forecast active proliferative LN using ROC curve analysis. The optimal cutoff for the S100P levels was 13.15 ng/ml, and the area under the curve (AUC) was 0.999 with a sensitivity and specificity of 98% and 98%, respectively (Fig. 2A).

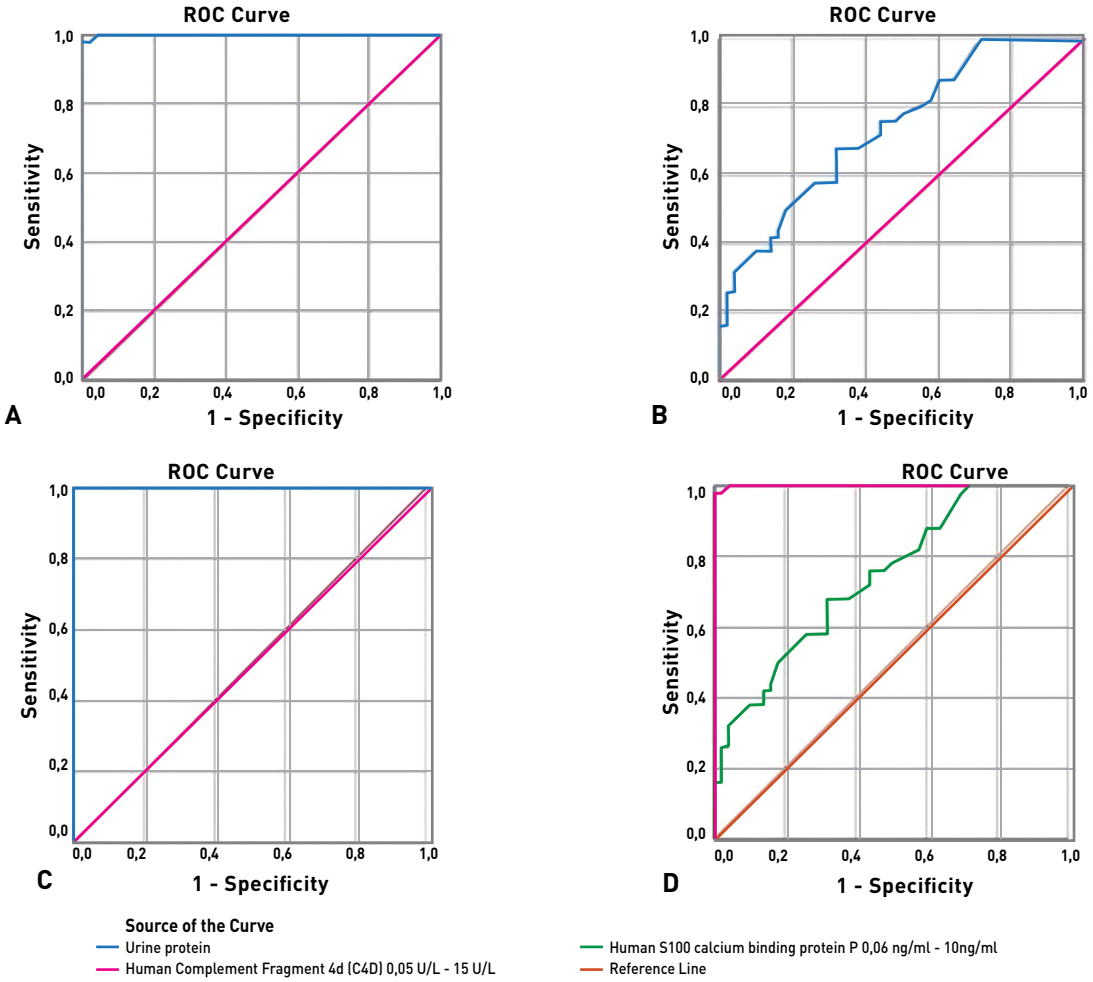

Fig. 2. ROC curve analyses for (A) S100P, (B) C4d protein, (C) 24-h urine protein, and (D) S100P and C4d protein and 24-h urine protein.

Abbreviations: Human calcium-binding S100P protein, C4d: Human complement Fragment 4.

The optimum cutoff was 17.05 U/L, and the AUC for the serum C4d level was 0.741 with a sensitivity of 76% and a specificity of 56% (Fig. 2B). Compared the S100P ROC curve to the 24-hour urinary protein ROC curve which showed that the optimal cutoff was 1.05 g/day, and AUC was 1 with 100% sensitivity and 100 % specificity for diagnosing active proliferative LN (Fig. 2C). This finding shows that S100P is promising for clinical application to predict active proliferative LN.

Discussion. One of the worst clinical manifestations of SLE is lupus nephritis, which affects 30–50% of patients with the condition [21]. In a single study, LN was discovered in 60% of lupus patients [22]. Immune complex deposition, which aggressively activates complement and results in high amounts of C4d, plays a role in the pathogenesis of LN [12]. Patients with SLE who have previously experienced LN are more likely to experience renal flares again and typically have a worse prognosis [14]. Renal biopsy is the gold standard for LN activity diagnosis and surveillance, with results interpreted in accordance with the 2003 International Society of Nephrology/Renal Pathology Society(ISN/RPS) classification [23]. The National Institutes of Health activity index (NIH-AI), with a range of 0-24 and 0 denoting no active LN characteristics, classifies active signs of inflammation on biopsy [24]. Noninvasive clinical and laboratory LN activity markers are neither sensitive nor specific enough to accurately track the progression of LN [25]. Therefore, precise noninvasive biomarkers are required to identify LN activity. Although they were proposed as biomarkers for the activity of renal illness, anti-dsDNA, and anti-nucleosome antibodies failed to convince as a solitary biomarker [26]. In contrast to healthy controls, SLE patients had significantly higher levels of S100P protein, according to our study analysis. Additionally, it was positively correlated with the NIH-lupus activity index in biopsy-proven proliferative LN, positively correlated with SLEDAI-R, and negatively correlated with disease activity as determined by SLEDAI-2K in SLE with proliferative LN as compared to SLE patients without LN.

S100P is involved in many biological processes, however, its precise function or mode of action is still completely unclear. It has also been researched as a biomarker and its function in some tumors' aggressive characteristics, tumor growth, and treatment response or resistance [27, 28]. As far as we are aware, serum S100P has never been used to forecast active proliferative LN in SLE patients. SLEDAI-2K and lupus nephritis have been linked to other S100 proteins from the S100 family, including S100A4, S100A6, S100A8/9, and S100A12. These proteins have been examined in the blood of SLE patients. These calcium-binding S100 proteins are a varied category that is mostly expressed by phagocytes. SLEDAI-R score was used by Jessica et al. to test the serum of patients with child onset SLE and LN for the presence of the five types of S100 proteins (S100A4, S100A6, S100A8/9, and S100A12), and they discovered that these patients had higher serum levels of these proteins than healthy controls and that these levels did not correlate with disease activity [29].

According to our findings, the C4d protein level was also significantly higher in SLE patients compared to healthy controls ($P < 0.001$; see Table 2). Additionally, it was favorably linked with the NIH-lupus activity index in proliferative LN and statistically substantially greater in SLE patients with active proliferative LN compared to SLE patients without LN ($P < 0.001$). The optimum cutoff was 17.05 U/L, and the AUC for the serum C4d protein level was 0.741 with a sensitivity of 76% and specificity of 56%. This is consistent with the findings of Martin et al, who discovered that serum C4d levels were minimal in healthy control subjects and significantly elevated in SLE patients ($P < 0.0001$) [30], as well as Petri et al, who discovered that serum C4d levels can distinguish between LN and SLE without LN ($P = 0.003$), with a sensitivity of 79% and specificity of 58% to detect patients with nephritis [17]. In the follow-up of SLE, particularly in the LN, C4d, a proteolytic fragment of C4 produced exclusively upon complement activation, appears to be a useful marker. In line with Senaldi et al. and Petri et al., who discovered that serum C4d levels correlate with disease activity and rise before renal flares so they have the potential to predict the recurrence of LN, we also discovered that there was a positive correlation with SLEDAI-R and a negative correlation with SLEDAI-2K [17, 31]. When SLE patients are followed up on, C3 and C4 levels show limited sensitivity with wide reference intervals for healthy people [32]. Martin et al. discovered a negative link between C4d and both serum C3 and C4 while we found no association between serum C4d protein and either serum C3 or C4 [17]. C4d deposition in glomerular capillaries of LN does not indicate the current disease activity but may be a useful marker to predict the prognosis of LN, according to research by Tahereh Malakoutian and colleagues who examined the staining of C4d positivity in renal biopsy of 72 patients with different classes of LN. Additionally, there was no connection seen between the low complement levels and C4d positive [32].

The modest number of participants in our study is one of its drawbacks. Also, Our study had primarily focused on a relatively youthful demographic and exclusively targeted female participants since SLE is more common in females. We need a bigger, longitudinal, and prospective cohort of SLE patients with various types of biopsy-proven LN to confirm our findings.

Conclusions. When compared to healthy people, SLE patients had statistically significantly higher levels of the serum proteins C4d and S100P. Serum concentrations of S100 P and C4d seem to show promise for clinical application for identifying SLE patients with active, biopsy-proven proliferative LN.

Conflict of interest statement. The authors declare that there are no competing interests associated with the manuscript.

Data availability statement. The data sets analyzed during the current study are available from the corresponding author upon reasonable request.

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The authors' contributions.

Mahmoud M. Elnokeety: Study design, writing the manuscript, data interpretation, drafting, and revising of the work;

Noha Mohamed Khalil: Samples collection and data curation;

Ahmed Fayed: Study design, writing the manuscript, data interpretation, drafting, and revising of the work.

All authors read and approved the final manuscript.

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