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Activity of Protein S-C4b Binding Protein and Total TFPI Levels in Egyptian SLE Patients: A Cross-Sectional Study

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Abstract

BACKGROUND: Systemic lupus erythematosus (SLE) is an immune disorder with alternating active and remission phases. Cardiovascular diseases and thrombosis are the major causes of mortality in SLE. The anticoagulant activity of Protein S (PS) is complemented by C4 binding protein (C4BP) and tissue factor pathway inhibitor (TFPI).

AIM: This study aims to determine the extent of change in the levels of PS activity, C4BP, and total TFPI in active SLE in comparison to the SLE remission phase and their association with thrombosis during SLE flare.

METHODS: The study included 180 Egyptian SLE patients who were classified into two groups: 100 SLE cases as the active group and 80 SLE cases as the remission group. The PS activity levels were processed on automated coagulation analyzers, whereas the C4BP and total TFPI levels were measured via enzyme-linked immunosorbent assay.

RESULTS: The PS activity and C4BP levels were lower in the active SLE cases than in the remitted ones ($p < 0.05$). The levels of PS activity and C4BP were revealed to be independent predictors of SELENA-SLEDAI flare scores. In active SLE cases, the PS activity and C4BP levels were rated as excellent and fair classifiers of thrombotic risk in SLE flare, respectively. The total TFPI levels showed no association with SLE activity or its thrombotic consequences.

CONCLUSIONS: The levels of PS activity and C4BP act as important biomarkers for SLE activity. Both can be implanted as predictive tools for thrombosis during activity.

Introduction

Systemic lupus erythematosus (SLE) is a chronic multisystem immune disorder with alternating active and remission phases. It is one of the first 20 predisposing risk factors for mortality in females aged 5–64 years [1]. The prevalence of SLE ranges from 9 to 241/100,000 person-years, with an incidence rate ranging from 0.3 to 23.2/100,000 person-years [2]. The SLE incidence in Arabs was found to be two-fold higher than that in non-Arabs [3], where the female-to-male incidence was 1:1 in the first decade of life and 9:1 in the fourth decade of life [4].

The pathogenesis of SLE is unclear, complex, and multifactorial, characterized by microvasculature inflammation and autoantibody production [5]. Venous and arterial thrombosis are frequent complications in SLE that are not necessarily linked to antiphospholipid syndrome (APS) sequels but have a significant effect on the clinical course and prognosis of SLE. Cardiovascular diseases and thrombosis are the major causes of mortality in SLE patients [6], [7].

The disruption of the coagulation and complement pathways is a contributing factor to the pathogenesis of SLE and SLE flare [8]. Although each of the coagulation and complement pathways has its own definite components, many interactivities have been found between both pathways [9].

Protein S (PS) is a Vitamin K-dependent factor with two forms: a free (active) form that constitutes 40% of the total PS and a bound inactive form that binds to the β-chain of C4BP [10]. PS has an essential antithrombotic function, acting as a cofactor for the activated protein C (APC) in the suppression of activated factor V and FVIIIa [11]. PS also functions as a cofactor for the anticoagulation function of tissue factor pathway inhibitor (TFPI), dampening FXa activation [12]. TFPI is a serine protease inhibitor that inactivates two coagulation complexes: TF-VIIa with subsequent FXa inhibition and the prothrombinase complex that is involved in thrombin formation [13]. Therefore, PS deficiency has a thrombotic risk, including venous and arterial thrombosis as well as pulmonary thromboembolism [14].

SLE flare is characterized by complement activation with its subsequent tissue deposition and consumption leading to organ damage. Complement plays a pivotal role in SLE activity and is therefore used in several researches as a point of study in relation to many other factors to improve SLE disease monitoring [15]. C2 and C4 are components of the C3 convertase

complex of the classical pathway and are regulated by the C4-binding protein (C4BP). The serum levels of C4BP were found to be reduced and correlated to decreased levels of C2 and C4 in SLE [16]. C4BP is also an acute-phase reactant and its level can increase in tissue inflammation that may overcome its consumption in SLE [17].

The definitions of SLE flare are dependent on one or more of the following items: (a) increase in disease activity scores estimated by confirmed indexes, (b) criteria for the emergence of new disease or worsening of disease, (c) trend of the physician's global assessment scale to more active/severe grade, and (d) requirement for treatment intensification [18].

SLE flares are attributed to a 124% increase in healthcare costs per year. The identification of patients who are at risk for developing SLE in the active phase is important to apply the necessary preventive measures. African–American ethnicity, male sex, major organ affections, especially nephritis, cytopenia, and persistent disease activity are among the predictors of SLE flare [18].

The purpose of the research was to study the link between PS activity, C4BP, total TFPI, and SLE phases. As well, if they could be used as predictors of SLE activity and its related thrombotic complications in an attempt to reduce the morbidity and mortality outcomes of SLE disease and the associated healthcare budget.

Materials and Methods

This cross-sectional study included 180 adult Egyptian SLE patients diagnosed according to the 2012 Systemic Lupus International Collaborating Clinics (SLICC) criteria [19]. They were divided into two groups: the active group, which involved 100 SLE patients who were followed up for thrombosis occurrence during the period of activity for 6 months, and the remission group, which included 80 SLE patients in the remission phase. Pregnancy, liver disease, deficiency of either PS, PC or antithrombin III at the time of SLE diagnosis, anticoagulant medication administration [oral anticoagulants, unfractionated heparin (UFH), and low-molecular-weight heparin (LMWH)], and oral contraceptive drugs were all exclusion criteria. They were recruited from the Rheumatology and Rehabilitation Department, Faculty of Medicine, Cairo University, from January 2019 to December 2019.

The patients were subjected to a full history and proper clinical examination according to the rheumatological disorders' standard sheet of the Rheumatology and Rehabilitation Department. The score of SLE activity was calculated using the SELENA-SLEDAI flare index (no flare present, ≤3; mild or moderate flare, >3–12; and severe flare, >12) [20].

Moreover, laboratory investigations were conducted, including complete blood count, coagulation studies, liver and kidney functions, hepatitis markers, complete urine analysis, and 24 h urinary protein, as well as immunological tests for SLE diagnosis: C3, C4, ANA, anti-DNA, and antiphospholipid (APL) antibodies (Abs) [e.g., anticardiolipin (ACL), anti-B2 glycoprotein I (anti-B2GPI), and lupus anticoagulant (LAC)].

The activity case group was sampled upon the onset of activity before the start of SLE flare management. Thrombosis on follow-up during SLE flare was diagnosed through color Doppler ultrasonography using the iU22 ultrasound machine with a C5-1 convex probe (Philips Healthcare-Imaging Systems, Bothell, WA, USA).

The study was accepted by the ethical committee of the Faculty of Medicine, Cairo University, with a reference number (I-251016). It was managed in agreement with the Declaration of Helsinki (ethical medical research principles, including human subjects). A written informed consent was taken from each contributor.

Sample collection

Three milliliters of venous blood were withdrawn into a sodium citrate vacutainer with a 9:1 blood to citrate ratio. The samples were doubly centrifuged at 2500 RPM for 20 min. The supernatants were collected carefully and stored at −20°C for a maximum of 1 month until use.

PS activity assay

The PS activity level was measured as it is considered as an initial step for screening PS deficiency and recommended by some experts on the condition to avoid confounding factors in its measurement, such as anticoagulant drugs, oral contraceptives, variability of coagulation factors quantity in the patient sample, elevated FVIII, and APC resistance [21]. Avoidance of these factors was achieved by the installation of the exclusion criteria for our patients as well as by the selection of an efficient method of PS activity assay [Siemens PS activity (Ac) assay, Sysmex Health Care Diagnostic Products GmbH, Marburg, Germany]. Since it contains a) Protein S Ac deficient reagent, which contains enough FV, fibrinogen, and other required coagulation factors to eliminate reliance on variable patient coagulation factors; b) PS Ac reagent containing APC, which reduces the effect of APC resistance on the results; and c) PS Ac Activator, which includes vipera russelli venom, which activates directly FX independent of the presence of other factors.

By this method, Heparin (UFH and LMWH) of up to 3 U/ml and FVIII activity of up to 400% do not interfere with the assay. The PS activity assay was performed on the Siemens automated coagulation analyzer (CS-5100) according to the Siemens Healthcare Diagnostic

Company procedure instructions. The results were obtained using a reference curve prepared beforehand by serially diluting the standard human plasma with PS-deficient plasma. The reference intervals were as follows: males (75% to > 130%) and females (59%–118%).

Assay for C4BP and total TFPI

The enzyme-linked immunosorbent assay (ELISA) was used for C4BP and total TFPI. The kit of C4BP assay (Catalog no: E0388Hu) was supplied by the Bioassay Technology Laboratory, Shanghai Crystal Day Biotech Co. While the kit for the total TFPI assay (Catalog no. SG-11467) was supplied by SinoGeneClon Biotech Co.

The plasma samples were added to pre-coated 96-well microplates. After sample incubation, detection antibodies were added, along with streptavidin-HRP and substrate. A microplate reader (Stat Fax-2100) was used to read the optical density (OD) of each well at a 450 nm wavelength. According to the standard concentrations and the corresponding OD values, the concentration of the corresponding sample was calculated. The assay range of the C4BP level was 1–400 ng/ml, whereas the assay range of the TFPI level was 0.6 to 20 ng/ml.

Statistical analysis

Data were analyzed using IBM SPSS version 26 (IBM Corp., Armonk, NY, USA) and expressed as frequency and percentage for the categorical variables and as mean, standard deviation (SD) or median, and minimum and maximum for the quantitative variables. The comparison of quantitative variables was performed using the nonparametric Mann–Whitney test. A Chi-square (χ^2) test was performed for comparing categorical data. Linear regression analysis was performed to detect independent predictors of the markers. The receiver operating characteristic (ROC) curve was used to assess the classifier performance of the variables. A p-value of ≤0.05 was considered statistically significant.

Results

Demographic data

In the active group, 14 patients were males (14%), and 86 were females (86%), their ages ranging from 18 to 53 years with a mean value ± SD of 30.38 ± 9.158. In the remission group, four patients were male (5%) and 76 were female (95%), their ages ranging from 18 to 54 years with a mean value ± SD of 33.15 ± 9.28. Both groups were matched in sex and age (p = 0.289 and 0.150, respectively). The disease duration in the active group ranged between 1 and 35 years with a median of 2 years, whereas that in the remission group ranged between 1 and 26 years with a median of 5 years with no statistically significant difference between the two groups (p = 0.109).

Regarding the risk factors of thrombosis; hypertension, diabetes, and smoking, they were matched in both groups as they were found in 26 (26%), 6 (6%), and 2 (2%), respectively, of the active cases and in 24 (30%), 12 (15%), and 0 (0%) independently of remitted patients with p = 0.674, 0.179, and 1, respectively.

Table 1: Clinical manifestations of SLICC diagnostic criteria in both groups

Clinical characteristic	Active group (n = 100), n (%)	Remission group (n = 80), n (%)	p*
Arthritis	90 (90)	72 (90)	1
Nephritis	70 (70)	68 (85)	0.095
Serositis	54 (54)	34 (42.5)	0.278
Neurological	28 (28)	12 (15)	0.140
Pulmonary	4 (4)	6 (7.5)	0.652
Cardiac affection	2 (2)	4 (5)	0.583
Vasculitis	2 (2)	6 (7.5)	0.319
Myositis	2 (2)	6 (7.5)	0.319
Cutaneous manifestation			
Malar rash	68 (68)	56 (70)	0.839
Photosensitivity	64 (64)	58 (72)	0.391
Discoid rash	4 (4)	4 (5)	1
Oral ulcers	40 (40)	40 (50)	0.343
Alopecia	42 (42)	46 (57.5)	0.144

*A P value of ≤ 0.05 is considered statistically significant, SLICC: Systemic lupus international collaborating clinics.

Clinical criteria

The SLICC diagnostic criteria of the clinical and laboratory manifestations at the time of SLE presentation in both groups were matched (p > 0.05) (Tables 1 and 2).

Table 2: Laboratory manifestations of the SLICC diagnostic criteria and treatment lines in both groups

Laboratory characteristic	Active group (n = 100), n (%)	Remission group (n = 80), n (%)	p*
Hematological criteria			
Hemolytic anemia	52 (52)	36 (45)	0.507
Leucopenia	36 (36)	34 (42.5)	0.530
Thrombocytopenia	36 (36)	20 (25)	0.263
Immunological criteria			
Positive ANA	100 (100)	80 (100)	1
Positive anti-dsDNA	45 (45)	44 (55)	0.587
Consumed C3/C4	78 (78)	56 (70)	0.387
APL autoantibodies			
ACL IgM	4 (4)	4 (4)	1
ACL IgG	30 (30)	22 (27.5)	0.795
Anti-b2 GPI IgM	6 (6)	0	0.251
Anti-b2 GPI IgG	34 (34)	28 (35)	0.921
LAC	38 (38)	32 (40)	0.847
Treatment lines in remission phase			
Corticosteroid (mg/day)			
Oral (7.5–30)	64 (64)	58 (72)	0.391
Oral (<7.5)	34 (34)	28 (35)	0.921
Immunosuppressive			
Azathioprine	46 (46)	52 (65)	0.072
Mycophenolate mofetil	22 (22)	24 (30)	0.387
Methotrexate	2 (2)	10 (12.5)	0.085
Leflunomide	10 (10)	6 (7.5)	0.728
Hydroxychloroquine	78 (78)	62 (77.5)	0.955

*A P value of ≤ 0.05 is considered statistically significant. ACL: Anticardiolipin, Anti-B2GPI: Anti-B2-glycoprotein I, APL: Antiphospholipid, LAC: Lupus anticoagulant, ANA: Antinuclear antibody, dsDNA: Double-stranded deoxyribonucleic acid, IgM: Immunoglobulin M, IgG: Immunoglobulin G, SLICC: Systemic lupus international collaborating clinics.

Based on the SELENA-SLEDAI flare index at sampling; proteinuria, low complement (C3/C4), pyuria, and arthritis were confined to the active group rather than the remission group (p < 0.001). Hematuria, visual

disturbance, thrombocytopenia, and seizure variables were more prevalent in the active SLE cases than in the remitted SLE cases ($p < 0.05$) (Table 3).

Table 3: SELENA-SLEDAI score parameters of the studied groups

SELENA-SLEDAI score parameters	Active group (n = 100), n (%)	Remission group (n = 80), n (%)	p*
Seizure	12 (12)	0	0.032*
Psychosis	6 (6)	0	0.251
Visual disturbance	14 (14)	0	0.016*
Cranial nerve disorder	6 (6)	0	0.251
Lupus headache	10 (10)	0	0.063
Vasculitis	10 (10)	0	0.063
Arthritis	42 (42)	0	<0.001*
Myositis	2 (2)	0	1
Hematuria (> 5 RBCs/HPF)	20 (20)	0	0.002*
Proteinuria (> 0.5 g/24 h)	80 (80)	0	< 0.001*
Pyuria (> 5 WBCs/HPF)	44 (44)	0	< 0.001*
Rash	24 (24)	14 (17.5)	0.453
Alopecia	4 (4)	12 (15)	0.132
Mucosal ulcers	6 (6)	8 (10)	0.695
Pleurisy	36 (36)	16 (20)	0.096
Pericarditis	16 (16)	10 (12.5)	0.639
Low complements			
Low C3	60 (60)	0	<0.001*
Low C4	56 (56)	0	<0.001*
Low C3/C4	60 (60)	0	<0.001*
Fever (> 38°C)	36 (36)	22 (27.5)	0.391
Thrombocytopenia (< 100.000/ml)	40 (40)	16 (20)	0.042*
Leucopenia (< 3000 WBCs/ml)	30 (30)	22 (27.5)	0.795

*A *P* value of ≤ 0.05 is considered statistically significant. HPF: High power field, RBCs: Red blood cells, WBCs: White blood cells.

No significant difference was observed between both groups in relation to the other manifestations: mucosal ulcer, pleurisy, pericarditis, fever, leukopenia, rash, alopecia, myositis, vasculitis, lupus headache, cranial nerve disorder, and psychosis (Table 3).

At sampling, the SELENA-SLEDAI score in the active group ranged between 4 and 42 with a mean ± SD of 16.12 ± 7.36 and that in the remission group ranged from 1 to 3 with a mean ± SD of 2.25 ± 0.67. The degree of activity in the active group was classified as mild-moderate in 36 (36%) patients and severe in 64 (64%) patients.

On follow-up for 6 months, 20 patients in the active group were complicated by deep venous thrombosis (DVT) in the lower limbs, whereas no thrombotic attacks affected the remitted SLE cases. The active cases that were complicated by thrombosis were 18 (90%) males and 2 (10%) females, with a mean ± SD age of 26.6 ± 9.83 years.

Treatment lines

In SLE activity management; corticosteroids were used in the form of pulse therapy of intravenous methylprednisolone (0.5–1 g/day) or oral steroids (prednisolone) with a dose of > 30 mg/day in 86 (86%) and 3 (3%) patients of the active group, respectively. Moreover, cyclophosphamide was administered to 30 (30.0%) cases of the active group to control SLE flare. Our samples were withdrawn before the start of these lines that were not in use in remitted cases (Table 1). The lines of therapy in the remission phase of both groups were matched ($p > 0.05$) (Table 2).

Levels of PS activity, C4BP, and total TFPI

The active SLE cases showed a PS activity level ranging from 27 to 100 with a median of 77.15%, a C4BP level ranging from 22 to 150 with a median of 37.3 ng/ml, and a TFPI level ranging from 4 to 9 with a median of 4.9 ng/ml. The remission group had a PS activity level ranging from 60 to 99 with a median of 78.7%, a C4BP level ranging from 23 to 280 with a median of 69.8 ng/ml, and a TFPI level ranging from 4 to 9 with a median of 4.8 ng/ml. A statistically significant difference was observed between both groups in relation to the PS activity levels ($p = 0.038$, OR = 1.027, and 95% CI = 1.001–1.053) and C4BP levels ($p = 0.001$, OR = 1.030, and 95% CI = 1.012–1.047). While no statistically significant difference was observed in the total TFPI levels between the two groups ($p = 0.836$, OR = 1.044, and 95% CI = 0.700–1.574).

In the active SLE patients, no correlation was found between the PS activity, C4BP, and total TFPI levels, whereas in the remission group, the C4BP levels were negatively correlated with the PS activity levels and positively correlated with the total TFPI levels (Table 4).

Furthermore, no statistically significant difference was observed between the PS activity, C4BP, and total TFPI levels along with age and sex in both groups ($P > 0.05$) (Table 4).

Table 4: Correlation between levels of PS activity, C4BP, and total TFPI levels in both groups and their correlations to the demographic characteristics

Characteristic	PS activity		C4BP		TFPI	
	Active group (n = 100)	Remission group (n = 80)	Active group (n = 100)	Remission group (n = 80)	Active group (n = 100)	Remission group (n = 80)
PS activity						
r*	1.000	1.000	−0.112	−0.519	−0.227	−0.080
p*	-	-	0.439	0.001*	0.113	0.625
C4BP						
r*	−0.112-	−0.519-	1.000	1.000	0.026	0.490
p*	0.439	0.001*	-	-	0.858	0.001*
Total TFPI						
r*	−0.227-	−0.080-	0.026	0.490	1.000	1.000
p*	0.113	0.625	0.858	0.001*	-	-
Demographic characteristic	PS activity		C4BP		TFPI	
	Active group (n = 100)	Remission group (n = 80)	Active group (n = 100)	Remission group (n = 80)	Active group (n = 100)	Remission group (n = 80)
Age						
r*	0.099	−0.095-	−0.061-	−0.008-	−0.065-	0.025
p	0.496	0.559	0.674	0.959	0.654	0.876
Sex						
Median in male/female	73.4/77.3	67.8/80.5	39/36.8	127.75/67.6	5.1/4.9	6.7/4.8
p*	0.476	0.153	0.791	0.082	0.120	0.066

*A *P* value of ≤ 0.05 is considered statistically significant. C4BP: C4 binding protein, PS: Protein S, r: Correlation coefficient, TFPI: Tissue factor pathway inhibitor.

Table 5: Levels of PS activity, C4BP, and total TFPI to SLICC diagnostic criteria and SELENA-SLEDAI score parameters in active SLE cases

SLICC diagnostic criteria	PS activity, median (range)	p*	C4BP, median (range)	p*	Total TFPI, median (range)	p*
ACL IgM (+)	79 (77.3–80.7)	0.790	41.85 (36.5–47.2)	0.552	5.6 (5.4–5.8)	0.198
ACL IgM (-)	76.7 (27.1–100)		37.05 (22.1–150)		4.9 (4–9.2)	
ACL IgG (+)	70.3 (27.1–87.3)	0.019*	42.6 (25.6–111.2)	0.415	5.1 (4.4–6.2)	0.324
ACL IgG (-)	80 (29.2–100)		37.3 (22.1–150)		4.9 (4–9.2)	
AntiB2GPI IgM (+)	80.7 (39.5–90.3)	0.848	31.6 (26–36.50)	0.159	5.8 (4.7–8.5)	0.249
AntiB2GPI IgM (-)	77 (27.1–100)		37.75 (22.1–150)		4.9 (4–9.2)	
AntiB2GPI IgG (+)	77.3 (27.1–93.3)	0.362	37.9 (22.2–150)	0.407	5.1 (4.6–9.2)	0.096
AntiB2GPI IgG (-)	77 (29.2–100)		37.3 (22.1–107.3)		4.9 (4–8.5)	
LAC (+)	76.4 (27.1–94.4)	0.234	36.8 (22.2–56.9)	0.653	5.1 (4.2–9.2)	0.193
LAC (negative)	78.2 (29.2–100)		37.3 (22.1–150)		4.9 (4–8.5)	
Nephritis (+)	77.3 (29.4–100)	0.295	37.3 (22.1–150)	0.882	4.9 (4–7.6)	0.840
Nephritis (-)	75.8 (27.1–90.3)		37.3 (22.7–107.3)		4.8 (4.5–9.2)	
SELENA-SLEDAI score parameters	PS activity, median (range)	p*	C4BP, median (range)	p*	Total TFPI, median (range)	p*
Seizure (+)	78.05 (29.4–84.2)	0.387	46.55 (38.4–59.4)	0.032*	4.7 (4–5.9)	0.147
Seizure (-)	77.15 (27.1–100)		36.25 (22.1–150)		4.9 (4.1–9.2)	
Pleurisy (+)	79 (33–98.4)	0.808	37.75 (22.1–107.3)	0.984	5.1 (4.1–8.5)	0.043*
Pleurisy (-)	76.35 (27.1–100)		37.05 (22.2–150)		4.8 (4–9.2)	
Lupus headache (+)	80.1 (30.2–98.4)	0.594	46.8 (44.1–73.9)	0.025*	4.8 (4.6–5.9)	0.758
Lupus headache (-)	76.4 (27.1–100)		36.5 (22.1–150)		4.9 (4–9.2)	
Low complement (+)	76.7 (27.1–100)	0.384	37.75 (22.1–150)	0.642	5.1 (4.2–8.5)	0.042*
Low complement (-)	81.1 (29.2–98.4)		37.05 (22.7–69.9)		4.8 (4–9.2)	
Pyuria (+)	74 (27.1–96.5)	0.007*	38.6 (22.7–150)	0.211	4.9 (4.5–7.6)	0.604
Pyuria (-)	82 (29.4–100)		36.65 (22.1–107.3)		4.9 (4–9.2)	
Vasculitis (+)	80.1 (30–98.4)	0.528	46.5 (42.6–69.9)	0.034*	4.8 (4.1–5)	0.237
Vasculitis (-)	76.4 (27.1–100)		36.5 (22.1–150)		4.9 (4–9.2)	

*A *P* value of ≤ 0.05 is considered statistically significant. C4BP: C4 binding protein, IgM: Immunoglobulin M, IgG: Immunoglobulin G, LAC: Lupus anticoagulant, Anti-B2GPI: Anti-B2-glycoprotein I, PS: Protein S, TFPI: Tissue factor pathway inhibitor, SLICC: Systemic lupus international collaborating clinics, (+): positive, (-): negative.

Correlation to active SLE patients' criteria

For the SLICC diagnostic criteria, no statistical significance was found for the levels of PS activity, C4BP, and total TFPI regarding the presence and absence of either nephritis or APL Abs, except for ACL IgG Abs. The active cases with positive ACL IgG Abs showed lower PS activity levels than those with negative ACL IgG (Table 5).

Regarding the SELENA-SLEDAI score parameters, a statistically significant difference in PS activity levels was observed between the cases expressing pyuria and those who did not. The patients who suffered from seizures, lupus headache, and vasculitis showed higher C4BP levels compared with those who did not. Moreover, the patients who presented with pleurisy and low complement levels showed higher total TFPI levels than those who did not (Table 5).

The other SELENA-SLEDAI score parameters showed no statistically significant difference in their presence or absence regarding the PS activity, C4BP, and total TFPI levels (p > 0.05).

Linear regression and ROC curve

For the 180 SLE patients, the levels of PS activity and C4BP were negatively independent predictors of the SELENA-SLEDAI score. As the SELENA-SLEDAI score increased by 0.383 and 0.312, every decrease one unit (one standard deviation) of the level of PS activity and C4BP, respectively. No association between TFPI levels and SELENA-SLEDAI score was found (Table 6).

On follow-up, thrombotic complications were found during the SLE flare of 20 patients (20%) of the active group in the form of DVT. The active cases with thrombosis had lower median and range values in the PS activity and C4BP levels [36.05 (27–48) and 33.8 (22–46), respectively] compared to those who did not have [80.4 (30–100) and 38.7 (22–150), independently], p <0.001 and 0.031, respectively. The median and range values of total TFPI in the active cases with and without thrombosis were [5.00 (4–9) and 4.90 (4–9), respectively] with no statistically significant difference (p = 0.574).

Table 6: Linear regression of levels of PS activity, C4BP, and total TFPI to SELENA-SLEDAI score

Dependent variable	Independent variables	SD	p*	Standardized beta	95% CI
SELENA-SLEDAI score	PS activity	19.196	<0.001*	−0.383−	(−0.265–) - (–0.088–)
	C4BP	47.65	0.002*	−0.312−	(−0.095–) − (–0.021)
	Total TFPI	1.071	0.525	0.064	(–1.121–)−(2.182)

*p ≤ 0.05 is statistically significant. C4BP: C4 binding protein, PS: Protein S, r: Correlation coefficient, TFPI: Tissue factor pathway inhibitor, CI: Confidence interval, SD: Standard deviation.

ROC curve was constructed for thrombosis incident risk in SLE active group. As PS activity was found to be as an excellent classifier for the active SLE cases to thrombotic event risk (AUC = 0.948, p < 0.001, and 95% CI = 0.851–1) with a cut-off value of 46.45% (sensitivity = 92% and specificity of 90%). Whereas C4BP level act as a fair classifier (AUC = 0.722, p = 0.031, and 95% CI = 0.555–0.890) with a cut-off value of 35.25 ng/ml (sensitivity = 70% and specificity = 80%). However, total TFPI levels failed to be such a classifier (AUC = 0.441, p = 0.569, and 95% CI = 0.222–0.661) (Figure 1).

Discussion

Thrombosis, either venous or arterial, is considered one of the most common causes of worldwide mortality [22], [23], including SLE deaths [6]. Inflammatory diseases contribute to thrombotic risk

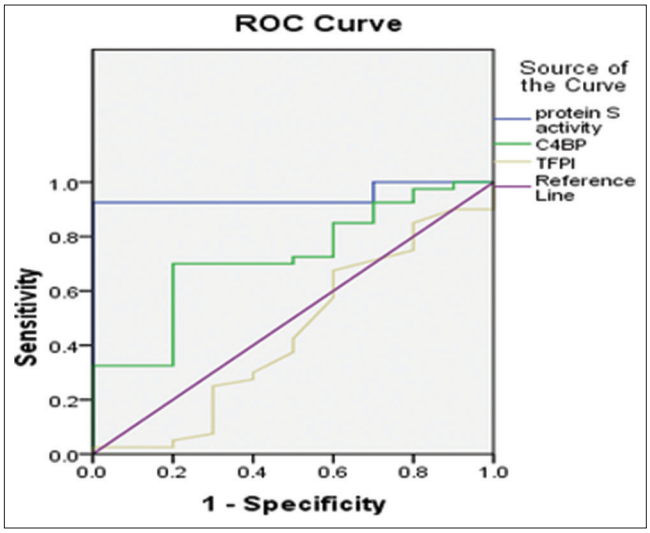

Figure 1: ROC curve for thrombosis incident risk in SLE activity

through hypercoagulability by decreasing the natural inhibitors of hemostasis, recumbence with venous stasis, and overexpression of inflammatory markers [24]. The complement pathway and its interactions with coagulation parameters play an important role in SLE flare [25]. One of the complement components, C4BP, is an essential inhibitor for the activation of the complement pathway and PS. The anticoagulant function of PS is represented in the enhancement of the antithrombotic actions of APC and TFPI [26].

Our study confirmed the relationship between PS activity and C4BP levels with SLE activity and its thrombotic risk complications that were not found in relation to total TFPI levels. In our study, PS activity was measured using the Siemens coagulation analyzer, while both C4BP and TFPI were analyzed by ELISA. To our knowledge, this is the first research to demonstrate the link between PS activity, C4BP, and TFPI in active and remitted SLE cases.

Bertolaccini *et al*. [27] measured the free PS plasma and activity levels in 184 SLE patients and 99 normal people. They reported that the free PS plasma levels were concomitant to its activity in the SLE patients and controls.

Moreover, Song *et al*. [28] examined the free PS plasma and activity levels in 27 SLE patients. They reported that the free PS antigen and activity levels were in good correlation ($r = 0.851$, $p < 0.001$).

The results of the present study revealed that the PS activity and C4BP levels were lower in the active cases than in the remission group. The lowered PS activity had a 1.027 increased risk of SLE flare ($p = 0.038$), whereas the lowered C4BP levels had a 1.030 increased risk of SLE activity ($p = 0.001$). Moreover, the PS activity and C4BP levels were found to be independent predictors of the SELENA-SLEDAI scores in SLE patients ($p < 0.001$ and 0.002, respectively).

The present study disclosed the complement consumption level as part of the SELENA-SLEDAI score during SLE activity. C3, C4, and C3/C4 consumption were found in 30 (60%), 28 (56%), and 31 (62%) active SLE cases, respectively. Moreover, no complement consumption was observed in the remission group, indicating a highly statistically significant difference between both groups ($p < 0.001$). This is consistent with the evidence of low complement levels during SLE activity that was routinely analyzed for diagnosis and disease monitoring [29], [30].

According to our results, Jung *et al*. (2019) [31] investigated 111 SLE patients regarding the relationship between the plasma levels of free PS and the disease activity. The cases were classified as those with low levels of PS (<50%) and those with a normal PS level (>50%). They documented that PS was correlated with SLE disease activity manifestations. Furthermore, the low PS group was associated with reduced levels of C3 and C4 compared with the group with normal PS levels.

Suh *et al*. [32] also reported that PS is a useful marker of disease activity as decreased free PS levels were found to be related to signs of SLE activity, which is correlated with the complement consumption of either C3 or C4 ($p < 0.0001$). The free plasma level of PS was correlated with the active British Isles Lupus Assessment Group (BILAG) score.

In the present study, the lower C4BP levels in our active cases can be explained by the C4BP consumption that is found to occur during the acute and probably chronic activation of the classic complement pathway [33]. The complement activation in SLE activity has many roles, such as the removal of cell debris, mediation of immune function (e.g., opsonization), cell activation, and target cell lysis [31].

Bergamaschini *et al*. measured the complement components and their degradation products in classical complement activation conditions rather than inflammatory states. In severe post-transfusion complement-mediated anaphylaxis, the reduction in the C4 levels was correlated with the C4BP levels. Furthermore, the C4BP-C4b complex was consumed and cannot be detected in hereditary angioedema with acute complement activation [33].

In the present study, higher C4BP levels were observed in the active cases suffering from seizures, vasculitis, and lupus headache. This could be because C4BP is an acute-phase reactant; its level may be elevated in some inflammatory conditions, which could overcome its consumption [34].

Our results indicated that the SLE active cases that were complicated by thrombosis during an SLE flare showed lower PS activity and C4BP levels than those who did not have ($p < 0.001$ and 0.031, respectively). The PS activity and C4BP levels were considered excellent and fair classifiers for thrombosis risk in active SLE patients as their AUC were 0.948 and 0.722, respectively.

Moreover, the active SLE cases showed lower levels of PS activity in cases with positive ACL IgG that could be attributed to their lowered PS activity levels (p = 0.019). Conversely, the PS activity, C4BP, and TFPI levels showed no statistically significant difference between the presence and absence of anti-B2GPI Abs, LAC, and lupus nephritis (p > 0.05) that excludes their affection on the measured parameters.

In accordance with our findings, Bertolaccini *et al*. [27] revealed that anti-PS antibodies are more frequent in SLE patients with thrombosis than in those with thrombosis in the normal controls (29% vs. 4%) (p < 0.0001, OR 9.5, 95% CI 3.07–29.3).

Furthermore, Suh *et al*. [32] measured the free PS levels in 107 SLE patients and 45 matched healthy controls. They found lower concentrations of free PS in positive ACL Abs in SLE patients and no changes in the levels of LAC or anti-B2GPI Abs. Moreover, Seriolo *et al*. [35] examined the ACL and free plasma PS levels in 184 rheumatoid arthritis patients. They confirmed the association between low PS levels and positive ACL that mediates the thrombotic events in secondary APL rheumatic arthritis patients. They also stated that the patients with low free PS levels showed a higher percentage of thrombosis (54%) and increased positivity of ACL Abs (50%) compared with those with normal PS levels, showing 11% thrombosis and 15% positive ACL Abs with the same p value (p = 0.01).

Several researchers found reduced levels of PS in SLE patients with subsequent thrombotic susceptibility in the form of venous and arterial thrombosis [32]. This is explained by the essential role of PS anticoagulants as a cofactor for APC and TFPI [31].

In APS with secondary SLE, the autoantibodies formed against PS, especially ACL lead to thrombosis with a reversible and temporary reduction of PS levels [32]. Researchers have reported that these antibodies are associated with decreased PS activity as they bind to the free portion of PS, neutralizing its function as well as increasing its clearance by immune complexes [27].

In the present study, regarding the SELENA-SELEDI score, lower levels of PS activity were observed in the cases who suffered from pyuria than in those who did not. Moreover, PS has been proven to react with Tyro3, Axl, and Mer (TAM) receptors on apoptotic cells and stimulate their clearance without subsequent inflammations. Hence, PS deficiency leads to an accumulation of debris that induces an autoimmune response and widespread inflammation [36].

The present study demonstrated that the TFPI antigen levels were not different between the SLE cases during activity and remission. They were also not considered as a classifier for thrombosis risk during activity (p > 0.05). However, higher levels of TFPI were observed in the active SLE cases expressing pleurisy or low complement levels compared with those who were free from these manifestations (p = 0.043 and 0.042, respectively).

In contrast to the findings of the present study, Qin *et al*. (2019) [30] investigated the urine samples of biopsy-proven lupus nephritis (LN) patients. Active LN (ALN) patients showed higher urinary levels of TFPI (p < 0.001) compared with the inactive LN patients, which also acted as a good classifier (AUC = 0.74, p < 0.0001). They also found that urinary levels of TFPI correlated positively with renal SLEDAI (rSLEDAI) (r = 0.40, p < 0.0001).

Despite the association between lower TFPI levels and the increased risk of venous thrombosis due to its anticoagulant function [12], both high and low levels of TFPI have been detected in APS and LA-positive patients [37]. Some research revealed lower levels of plasma TFPI in SLE patients compared with normal controls, whereas others showed higher levels of free TFPI that were correlated with endothelial disruption and SLE activity [30].

The heterogeneity in research findings may be related to different ethnicities, associated comorbidities, and method variability. This encourages further studies to confirm the relations between the investigated parameters in immune disorders.

Due to a lack of funding, our study's limitations were presented in the absence of a confirming method for PS activity. However, we based our assessment on its confirmation in previous studies of different ethnicities with the avoidance of confounding factors.

Conclusions

Our research revealed for the first time the relationships between PS activity, C4BP, and TFPI in the active and remission phases of SLE. PS activity and C4BP levels can be implanted as independent predictors of SLE activity and the risk of thrombosis during an SLE flare. There was no association found between total TFPI levels and SLE activity or its thrombotic consequences.

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