ORIGINAL ARTICLE

Blood Levels of Programmed Cell Death Protein 1 and its ligand (PD-L1) as Predictors of Systemic Sclerosis Severity

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Key words:
Systemic Sclerosis; Programmed Cell Death 1; Programmed Cell Death Ligand 1; B Lymphocytes; T Lymphocytes

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ABSTRACT

Background: *Programmed cell death protein 1 (PD-1) and PD-1 ligand (PD-L1), are key regulators of immune tolerance and are implicated in the pathogenesis of various autoimmune diseases. Systemic sclerosis (SSC) is a complex autoimmune condition characterized by widespread skin fibrosis, involvement of internal organs, and immune dysregulation leading to the production of autoantibodies.* ***Objectives:*** *The aim of this study was to evaluate the expression levels of PD-1 and PD-L1 on CD19+ B lymphocytes and CD3+CD8+ T lymphocytes in patients with SSC. We also aimed to assess the relationship between PD-1/PD-L1 expression and clinical parameters, laboratory findings, and the extent of skin sclerosis in SSC patients.* ***Methodology:*** *45 patients diagnosed with SSC and 45 healthy controls were enrolled in this study. The expression of PD-1 and PD-L1 on CD19+ B cells and CD3+CD8+ T cells was evaluated using flow cytometry on peripheral blood samples.* ***Results:*** *The expression levels of PD-1 and PD-L1 were significantly elevated in both CD19+ B cells and CD3+CD8+ T cells in the SSC group in comparison to the control group (P = 0.001 for all comparisons). Additionally, strong positive correlations were observed between the expression of PD-1 and PD-L1 on both cell types and disease activity in the SCC group.* ***Conclusions:*** *The findings of this study suggest that PD-1 and PD-L1 may contribute to the modulation of disease severity in patients with SSC, highlighting their potential as biomarkers for disease activity.*

INTRODUCTION

Systemic sclerosis (SSC), commonly referred to as scleroderma, is a rare and chronic autoimmune connective tissue disorder characterized by significant skin fibrosis, involvement of internal organs, immune dysregulation resulting in autoantibody production, and abnormalities in small blood vessels. The onset of symptoms is highly variable, leading to considerable differences in clinical manifestations and disease progression[1].

Organs commonly affected by systemic sclerosis include the heart, lungs, gastrointestinal tract, and kidneys. However, the hallmark symptom is scleroderma, characterized by thickened and rigid skin[2]. In the early stages, the disease is marked by abnormal micro-vascular function, inflammation, and autoimmunity. Over time, irreversible structural changes in small blood vessels lead to various forms of organ fibrosis, contributing to the progressive nature of the disease[3].

Specific autoantibodies are among the defining features of SSC[4]. The extent of skin involvement allows the condition to be categorized into two clinical groups: diffuse cutaneous SSC (dcSSC) and limited cutaneous SSC (lcSSC). dcSSC is characterized by skin thickening both proximally and distally to the elbows and knees, often with some involvement of the face. In contrast, lcSSC is marked by skin thickening primarily distal to the elbows and knees[2].

The estimated global prevalence of SSC is 18.87 cases/100,000, with higher incidence and prevalence rates noted among populations in high-income countries[5]. SSC demonstrates a female predominance, and an increasing incidence among females of childbearing age[6].

Extensive skin involvement is associated with increased severity of symptoms in internal organs and a poorer prognosis[7]. The modified Rodnan skin score (mRSS) serves as a validated outcome measure for assessing thickness of skin in clinical studies of SSC[8]. Given that all outcome measures exhibit inherent

measurement variability, it is advisable for patients to be evaluated by the same assessor throughout the trial[9].

T lymphocytes are key components of the adaptive immune system. CD8+ T lymphocytes play a vital role in executing cytotoxic effector functions in the context of autoimmune diseases, infections, and malignancies[10]. In autoimmune conditions, CD8+ T cells bypass several tolerance mechanisms, including thymic selection and the typical requirements for T cell activation. Consequently, they demonstrate aberrant effector activity, leading to damage to the body's own tissues[11]. B lymphocytes also play an important role in the initiation and progression of SSC. Studies utilizing co-culture experiments of B cells and fibroblasts support these findings, showing that B cells directly enhance the production of collagen and extracellular matrix by fibroblasts[12].

The receptor known as Programmed Cell Death Protein 1 (PD-1) regulates T cell activity, induces the apoptosis of antigen-specific T cells, and supports the survival of regulatory T cells. This function is crucial for suppressing immune responses and maintaining self-tolerance. This function is regulated by the trans-membrane protein, programmed cell death ligand 1 (PD-L1), which binds to PD-1[13]. The interaction between PD-1 and its ligands plays a key role in the occurrence of autoimmune disorders. *PD-1* gene deficiency can be involved in conditions such as lupus-like glomerulonephritis or autoimmune dilated cardiomyopathy[14]. The aim of current study is to determine the expression levels of PD-1/PD-L1 on CD19+ B cells and CD3+CD8+ T lymphocytes in patients with SSC and to explore the relationship between PD-1/PD-L1 expression, clinical and laboratory findings and the severity of skin sclerosis.

METHODOLOGY

Patients:

This cross-sectional study utilized the 2013 American College of Rheumatology/European League (ACR/EULAR) criteria for diagnosing SSC[15]. Cases were recruited from Outpatient Clinics and Inpatient Departments of Rheumatology and Dermatology at Sohag University Hospitals, while controls were recruited from a group of healthy, age and sex-matched volunteers attending the same hospital for blood donation. Inclusion criteria consisted of individuals diagnosed with SSC according to ACR/EULAR criteria published in 2013, while patients with other collagen diseases were excluded. All participants, both patients and controls, underwent thorough history-taking, clinical examination, and for patients with dcSSC, the severity of skin involvement was assessed using the mRSS[9].

Methods:

Laboratory investigations included several key tests; A complete blood count was performed on an EDTA sample using the XN-1000 (Sysmex, Japan). Erythrocyte sedimentation rate (ESR) was measured using Westergren method. C-reactive protein (CRP) and rheumatoid factor (RF) were assessed using latex agglutination tests provided by Reactivos GPL, Barcelona, Spain. 1:100 dilutions of patient serum were used to test anti-nuclear antibody (ANA) by indirect immunofluorescent microscopy using Hep 20-10/ primate liver (IMMCO Diagnostics, USA). Anti-topoisomerase I antibodies (anti Scl-70) testing was carried out by using a commercially available indirect solid phase enzyme immunoassay kit (Orgentec Diagnostika GmbH /Germany).

PD-1 and PD-L1 expression was measured through flow cytometry. Peripheral blood cells were stained with monoclonal antibodies against CD3, CD19, CD8, PD-1, and PDL-1 (Becton Dickinson, San Jose, California, USA). After staining, the samples were incubated, lysed to remove red blood cells, and centrifuged. The cells were washed with phosphate-buffered saline (PBS) and resuspended for analysis using a FACS Caliber flow cytometer with Cell Quest software (BD Biosciences, USA). A total of 10,000 events per sample were recorded, and the lymphocyte population was identified based on forward and side scatter histograms. The proportions of PD-1+ and PDL-1+ cells within CD8+ and CD19+ lymphocytes were evaluated, with B cells defined as CD19+ according to Kwiecień et al.[16].

Ethical considerations:

The Research Ethics Committee of the Faculty of Medicine at Sohag University approved this study (Approval Number: Soh-Med-24-03-07PD). Informed consents were assigned by participants after explanation of the purpose of the study as a first step to proceed to the study.

Statistical analysis:

Statistical analysis for this study was performed using SPSS V25, incorporating various tests including the mean, standard deviation, Student's t-test, Chi-square test, Pearson's Correlation Coefficient, and Analysis of Variance (ANOVA). The unpaired Student's t-test was applied to compare quantitative data between two groups, while the Chi-square test assessed the independence of categorical variables. Pearson's correlation was used to detect the relationship between two quantitative variables within a group. ANOVA was done to compare quantitative data at different times in the same group. Additionally; test sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy was evaluated using Receiver Operating Characteristic (ROC) curve analysis. Statistical significance was defined as a P-value ≤ 0.05.

RESULTS

Demographic and disease characteristics of patients:

The present study included a cohort of 45 SSC patients and an equal number of healthy controls. The SSC group had a predominance of females (75.56%) and common manifestations such as Raynaud's phenomenon (77.78%), skin ulcers (75.56%), arthritis (55.56%), interstitial lung disease (ILD) (24.44%), and positive anti-Scl-70 antibodies (20.00%) (Table 1).

Table 1: Demographic and disease characteristics of patients with systemic sclerosis (n=45)

Parameter		SSC (n=45)
Age (Years)	**Mean ±SD**	51.711±9.493
Sex	**Female**	34 (75.56%)
	Male	11 (24.44%)
Disease duration (Years)	**Mean ±SD**	5.889 ± 3.518
Raynaud`s phenomenon	**No**	10 (22.22%)
	Yes	35 (77.78%)
Arthritis	**No**	20 (44.44%)
	Yes	25 (55.56%)
Skin ulcer	**No**	11 (24.44%)
	Yes	34 (75.56%)
ILD	**No**	34 (75.56%)
	Yes	11 (24.44%)
mRSS	**Mean ±SD**	16.222 ± 5.838
mRSS	**Mild to Moderate**	34 (75.56%)
	Severe	11 (24.44%)
ESR (mm/1st hour)	**Mean ±SD**	43.022±17.258
CRP	**Negative**	25 (55.56%)
	Positive	20 (44.44%)
ANA	**1/80**	25 (55.56%)
	1/160	11 (24.44%)
	1/320	7 (15.56%)
	1/640	2 (4.44%)
RF	**Negative**	19 (42.22%)
	Positive	26 (57.78%)
mRSS	**Mild to Moderate**	34 (75.56%)
	Severe	11 (24.44%)
Anti-Scl-70	**Negative**	36 (80.00%)
	Positive	9 (20.00%)

SSC: Systemic sclerosis, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, mRSS: Modified Rodnan skin score, ANA: Anti-nuclear antibodies, RF: Rheumatoid factor ILD: Interstitial lung disease, Anti-Scl-70: Anti-topoisomerase I antibodies.

Demographic and laboratory data:

There were no significant differences in age or sex distribution between the groups. Statistically significant differences were observed in PD-1, PD-L1, and CD19. CD8 ($p = 0.071$), CD3 ($p = 0.108$), WBCs ($p = 0.102$), and PLT ($p = 0.397$) showed differences that were non-significant, while hemoglobin (HB) was significantly lower in SSC patients than controls (Table 2).

Table 2: Comparison of demographic and laboratory data of patients with systemic sclerosis and control group.

		Group						T-Test	
		SSC (n=45)			Control (n=45)			t	P-value
Age (Years)	**Mean ±SD**	51.711	±	9.493	53.733	±	9.159	-1.028	0.307
PD1 (%)	**Mean ±SD**	13.783	±	4.537	7.120	±	2.772	8.408	<0.001*
PDL1 (%)	**Mean ±SD**	25.254	±	9.654	6.884	±	3.351	12.058	<0.001*
CD 19 (%)	**Mean ±SD**	17.333	±	7.382	8.900	±	3.614	6.883	<0.001*
CD 8 (%)	**Mean ±SD**	22.627	±	8.917	19.647	±	6.302	1.831	0.071
CD 3 (%)	**Mean ±SD**	71.216	±	9.146	68.444	±	6.874	1.625	0.108
WBCS (Cell/L)	**Mean ±SD**	7.892	±	2.552	7.313	±	1.663	1.275	0.102
PLT (Cell/L)	**Mean ±SD**	278.466	±	92.988	250.133	±	53.186	1.774	0.397
HB (g/dL)	**Mean ±SD**	11.742	±	1.431	13.004	±	0.878	-5.042	<0.001*
Chi-Square		**N**		**%**	**N**		**%**	**χ^2**	**P-value**
Sex	**Female**	34		75.56	32		71.11	0.227	0.634
	Male	11		24.44	13		28.89		

* P<0.05 is significant

SSC: Systemic sclerosis, PD1: Programmed cell death protein 1, PDL1: Programmed death-ligand 1, WBC: White blood cells, Hb: Hemoglobin, PLT: Platelets; CD, Cluster of differentiation

Expression of PD1 and PD-L1 in SSC patients:

Patients with Raynaud's phenomenon, severe skin involvement, and positive anti-Scl-70 antibodies exhibited significantly higher levels of PD-1 expression. In contrast, no significant differences in PD-1 expression were observed based on sex, arthritis, skin ulcers, ILD, CRP, or RF. Additionally, ANA titers showed no association with variations in PD-1 expression (Table 3).

Table 3: Variations of PD1 levels according to clinical and laboratory findings in patients with systemic sclerosis (n=45).

SSC (n=45)		PD1				T-Test	
		N	Mean	±	SD	t	P-value
Sex	**Female**	34	13.581	±	4.505	-0.522	0.604
	Male	11	14.409	±	4.797		
Raynaud`s phenomenon	**No**	10	10.810	±	2.586	-2.484	0.017*
	Yes	35	14.633	±	4.639		
Arthritis	**No**	20	14.235	±	4.814	0.593	0.556
	Yes	25	13.422	±	4.367		
Skin ulcer	**No**	11	14.222	±	4.275	0.365	0.717
	Yes	34	13.641	±	4.671		
ILD	**No**	34	13.810	±	4.543	0.069	0.945
	Yes	11	13.700	±	4.736		
CRP	**Negative**	25	13.366	±	4.471	-0.686	0.496
	Positive	20	14.305	±	4.679		
RF	**Negative**	19	13.453	±	3.967	-0.414	0.681
	Positive	26	14.025	±	4.974		
mRSS	**Mild to Moderate**	34	12.357	±	3.712	-4.420	<0.001*
	Severe	11	18.191	±	4.098		
Anti-Scl-70 positivity	**Negative**	36	12.773	±	4.162	-3.306	0.002*
	Positive	9	17.822	±	3.804		
ANOVA						**F**	**P-value**
ANA	**1/80**	25	13.152	±	5.150	1.036	0.387
	1/160	11	14.255	±	3.733		
	1/320	7	13.849	±	3.291		
	1/640	2	18.850	±	0.919		

SSC: Systemic sclerosis, PD1: Programmed cell death protein 1, CRP: C-reactive protein, mRSS: Modified Rodnan skin score, ANA: Anti-nuclear antibodies, RF: Rheumatoid factor ILD: Interstitial lung disease, Anti-Scl-70: Anti-topoisomerase I antibodies

The analysis of expression of PD-L1 in SSC patients revealed that Raynaud's phenomenon is associated with significantly higher levels of PD-L1. Additionally, patients with severe skin involvement demonstrated higher PD-L1 expression. Moreover, patients with positive Anti-Scl-70 antibodies exhibited significantly elevated PD-L1 levels. However, non-significant differences were found in PD-L1 expression based on sex, arthritis, skin ulcers, ILD, CRP, RF, or ANA titers (Table 4).

Table 4: Variations of PD-L1 levels according to clinical and laboratory findings in patients with systemic sclerosis (n=45).

SSC		PDL1				T-Test	
		N	Mean	±	SD	t	P-value
Sex	**Female**	34	24.630	±	9.605	-0.759	0.452
	Male	11	27.184	±	10.012		
Raynaud`s phenomenon	**No**	10	17.928	±	6.529	-2.949	0.005*
	Yes	35	27.347	±	9.436		
Arthritis	**No**	20	23.598	±	8.670	-1.030	0.309
	Yes	25	26.579	±	10.357		
Skin ulcer	**No**	11	23.331	±	9.722	-0.756	0.454
	Yes	34	25.876	±	9.695		
ILD	**No**	34	24.769	±	9.880	-0.589	0.559
	Yes	11	26.755	±	9.205		
CRP	**Negative**	25	25.185	±	9.761	-0.053	0.958
	Positive	20	25.341	±	9.771		
RF	**Negative**	19	24.418	±	8.966	-0.492	0.625
	Positive	26	25.865	±	10.259		
mRSS	**Mild to Moderate**	34	23.440	±	8.427	-2.324	0.025*
	Severe	11	30.862	±	11.400		
Anti-Scl-70 positivity	**Negative**	36	23.059	±	8.790	-3.395	0.001*
	Positive	9	34.033	±	8.138		
ANOVA						**F**	**P-value**
ANA	**1/80**	25	24.760	±	9.004	2.028	0.125
	1/160	11	25.391	±	10.050		
	1/320	7	22.434	±	9.781		
	1/640	2	40.550	±	6.435		

SSC: Systemic sclerosis, PDL1: Programmed death-ligand 1, CRP: C-reactive protein, mRSS: Modified Rodnan skin score, ANA: Anti-nuclear antibodies, RF: Rheumatoid factor ILD: Interstitial lung disease, Anti-Scl-70: Anti-topoisomerase I antibodies, ANOVA: Analysis of Variance.

Correlations of PD-1/ PD-L1 in SSC patients:

As shown in table 5, PD-1 expression showed a significant positive correlation with PD-L1 expression ($r = 0.394$, $p = 0.007$) and with the mRSS ($r = 0.630$, $p < 0.001$). However, non-significant correlations were found between PD-1 expression and age, CD19+, CD8+, or CD3+ T cell counts, other laboratory parameters, or disease duration. Similarly, PD-L1 expression showed significant positive correlations with CD19+ B cell counts ($r = 0.302$, $p = 0.044$), and CD8+ T cell counts ($r = 0.371$, $p = 0.012$). Additionally, PD-L1 expression was correlated significantly with total white blood cell (WBC) counts ($r = 0.296$, $p = 0.049$). However, no significant associations were observed between PD-L1 expression and age, platelet count, hemoglobin levels, ESR, mRSS, or disease duration.

Table 5: Correlation between the Expression of PD-1 and PD-L1 and Various Laboratory and Clinical Parameters

Correlations				
SSC	**PD-1**		**PD-L1**	
	r	**P-value**	**r**	**P-value**
PD-L1	0.394	0.007*		
Age	0.311	0.438	0.025	0.872
CD 19	0.259	0.086	0.302	0.044*
CD 8	0.111	0.470	0.371	0.012*
CD3	0.055	0.720	-0.090	0.556
WBCS	0.253	0.093	0.296	0.049*
PLT	0.214	0.158	-0.118	0.442
HB	-0.033	0.829	-0.020	0.897
ESR	-0.089	0.561	0.042	0.786
mRSS	0.630	<0.001*	0.345	0.120
Duration (Years)	-0.071	0.643	0.268	0.075

SSC: Systemic sclerosis, PD1: Programmed cell death protein 1, PDL1: Programmed death-ligand 1, WBC: White blood cells, Hb: Hemoglobin, PLT: Platelets, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, mRSS: Modified rodnan skin score, ANA: Anti-nuclear antibodies, RF: Rheumatoid factor

Predictors of PD-1/ PD-L1 in SSC Patients:

Using multiple linear regression model, the only significant predictor of PD-1 expression was the mRSS, which shows a positive association with PD-1 levels. Other factors, such as Raynaud's phenomenon, Anti-Scl-70 positivity, PD-L1 expression and age, did not significantly predict PD-1 expression (Table 6)

Table 6: Multiple Linear Regression Model for Predicting PD-1 Expression

SSC	Unstandardized Coefficients		Standardized Coefficients	t	P-value
	B	Std. Error	Beta		
Raynaud`s phenomenon	1.109	1.384	0.103	0.801	0.428
Anti-Scl-70 positivity	1.869	1.486	0.167	1.258	0.216
PDL1	0.055	0.066	0.118	0.842	0.405
Age	-0.082	0.057	-0.173	-1.438	0.158
mRSS	0.365	0.098	0.470	3.743	0.001*
a. Dependent Variable: PD1					

SSC: Systemic sclerosis, PD1: Programmed cell death protein 1, PDL1: Programmed death-ligand 1, mRSS: Modified rodnan skin score, Anti-Scl-70: Anti-topoisomerase I antibodies

For PD-L1 expression, significant positive predictors include CD8 expression, while Raynaud's phenomenon and Anti-Scl-70 positivity serve as marginally significant predictors. However, PD-1 expression, CD19 expression, WBCs and mRSS do not significantly predict PD-L1 expression (Table 7).

Table 7: Multiple Linear Regression Model for Predicting PD-L1 Expression

SSC	Unstandardized Coefficients		Standardized Coefficients	t	P-value
	B	Std. Error	Beta		
Raynaud`s phenomenon	5.694	3.084	0.248	1.846	0.073
Anti-Scl-70 positivity	6.393	3.331	0.268	1.919	0.063
PD1	0.442	0.372	0.208	1.190	0.242
CD 19	0.243	0.202	0.186	1.199	0.238
CD 8	0.339	0.145	0.313	2.334	0.025*
WBCS	0.305	0.225	0.181	1.357	0.183
mRSS	-0.395	0.346	-0.239	-1.141	0.261
a. Dependent Variable: PDL1					

SSC: Systemic sclerosis, PD1: Programmed cell death protein 1, PDL1: Programmed death-ligand 1, WBC: White blood cells, mRSS: Modified rodnan skin score, Anti-Scl-70: Anti-topoisomerase I antibodies

Diagnostic Value of PD1/ PDL1 in Patients with SSC:

The ROC curve analysis for PD-1 demonstrates a favorable balance between sensitivity and specificity, with a sensitivity of 82.22% and a specificity of 91.11%, indicating that the PD-1 test (cut off value >10.1) is effective in accurately identifying SSC. The ROC curve for PD-L1 (cut off value >10) exhibits exceptional sensitivity at 100.00%, successfully identifying all individuals with SSC, while also maintaining a reasonably high specificity of 88.89%. The overall accuracy of PD-L1 test is 98.3%, indicating strong performance in distinguishing between the SSC and control groups (Table 8, Figure 1).

Table 8: Validity of PD1 and PDL-1 levels for discrimination between systemic sclerosis and control groups.

ROC curve between SSC and Control						
	Cutoff	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**
PD1	>10.1	82.22	91.11	90.2	83.7	89.5%
PDL1	>10	100.00	88.89	90.0	100.0	98.3%

SSC: Systemic sclerosis, PD1: Programmed cell death protein 1, PDL1: Programmed death-ligand 1, PPV: Positive predictive value, NPV: Negative predictive value.

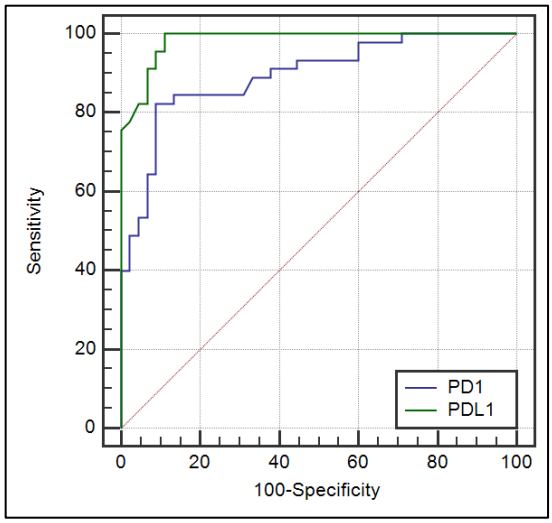

Fig. 1: ROC curve for PD-1 and PD-L1 for differentiation between systemic sclerosis group and the control group

DISCUSSION

Multiple organ systems are affected in SSC, necessitating a coordinated healthcare team that includes many specialties including Rheumatology, Dermatology, Gastroenterology, Nephrology, Cardiology, and Pneumology to effectively manage the condition. Early diagnosis and monitoring of progression of SSC are vital for successful treatment[17]. Our findings indicate that the expression levels of PD-1 and PD-L1 are significantly elevated in patients with SSC. Moreover, there were strong positive correlations between these expression levels and disease activity within this patient group.

Immunological checkpoints are regulatory pathways that are crucial for maintaining the balance and tolerance of the immune system. Among these, the PD-1/PD-L1 pathway has garnered significant attention in the context of autoimmune diseases. This pathway becomes activated following activation of immune cells and is involved in their proliferation and differentiation[18, 19]. Our findings show that PD-L1 expression was significantly positively correlated with CD19+ B cell counts. Presence of PD-1 and PD-L1 may increase CD19+ B cells in SSC. This rise in B cells could contribute to the production of the autoantibodies characteristic of SSC and can result in tissue damage[20]. Yoshizaki et al.[21] observed similar findings and proposed that these abnormalities may be involved in the excessive activation of B lymphocytes seen in SSC.

Our findings indicate that increased levels of CD8+ T lymphocytes may contribute to tissue damage and fibrosis in patients with SSC. Fuschiotti[22] noted that CD8+ T lymphocytes in SSC can interact with other immune cells, including B lymphocytes and myeloid cells, to promote and sustain inflammatory and fibrotic processes. Notably, increased CD8 expression serves as a significant positive predictor of PD-L1 expression, highlighting the activity of these cells in SSC. Furthermore, the mRSS exhibited a positive association with PD-1 levels, underscoring the role these proteins play in disease pathogenesis and progression.

These findings are consistent with results of Yanaba et al.[23], who reported higher serum levels of soluble PD-1 in patients with dcSSC than patients with lcSSC and healthy controls. They also observed a positive correlation between serum levels of PD-1 and severity of skin sclerosis[23]. Similarly, Fukasawa et al.[24] found that levels of soluble PD-1 and soluble PD-L2 were elevated in the sera of patients with SSC, and these levels correlated with immunological abnormalities and the extent of fibrosis.

Elahee et al.[25] found that individuals with SSC and ILD, particularly those with more severe lung involvement, exhibited an increased presence of a specific population of helper T lymphocytes with high levels of PD-1, absence of CXCR5 and ICOS, and expression of HLA-DR, indicating a cytotoxic phenotype. This potential cytotoxicity within the CD4+

T lymphocytes could serve as a prognostic biomarker for disease severity in patients with SSC.

Zanatta et al.[26] evaluated the *PD-1 C>T rs2227981* single nucleotide polymorphism in relation to SSC. While their study found no association between the *PD-1 rs2227981* polymorphism and SSC, this does not rule out the potential association of other PD-1 polymorphisms with the disease. Additionally, their findings cannot be generalized as the study was conducted at a single center.

CONCLUSIONS AND RECOMMENDATIONS

This study shows that the expressions of PD-1 and PD-L1 on CD19 B cells and CD3+ CD8 T cells were significantly elevated in patients with SSC compared to normal controls, and these expressions were strongly correlated with disease activity. These results reinforce the notion that PD-1 and its ligand PD-L1 may serve as regulators of immune activation in SSC patients, potentially providing valuable insights for future prognostication and development of treatment strategies. Further longitudinal studies to monitor changes in PD-1 and PD-L1 expression levels over time in SSC patients are recommended to correlate these changes with disease progression and treatment responses, and to investigate the underlying mechanisms by which PD-1 and PD-L1 influence immune activation in SSC.

Conflict of Interest: The authors declare that they have no competing interests.
Financial Disclosures: Nothing to declare.

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