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Role of Hematological Parameters in Systemic Sclerosis Patients with Pulmonary System Involvement

Pulmoner Tutulumu Olan Sistemik Skleroz Hastalarında Hematolojik Parametrelerin Rolü

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Abstract

Aim: Systemic sclerosis (SSc) is an autoimmune disease characterized by generalized micro and macroangiopathy. Mortality in SSc is primarily due to pulmonary complications. This study was aimed to reveal the relationship between parenchymal and vascular involvements of the pulmonary system and hematological parameters in patients with SSc.

Material and Method: Participants were divided into three groups: both interstitial lung disease (ILD) and pulmonary hypertension (PH), those with only ILD and those with neither ILD nor PH. Laboratory data were compared between these groups.

Results: ILD was found to be associated with high red cell distribution width (RDW) and erythrocyte sedimentation rate, independent of PH. The platelet (PLT) count was significantly lower, and the RDW to PLT ratio (RPR) level was significantly higher in those with ILD and PH coexistence compared to those with only ILD.

Conclusion: RPR can be used as screening parameters for PH in ILD associated with SSc.

Keywords: Interstitial lung disease, pulmonary hypertension, systemic sclerosis

Öz

Amaç: Sistemik skleroz (SSc), mikro ve makroanjiyopati ile karakterize otoimmun bir hastalıktır. SSc'deki mortalite esas olarak pulmoner komplikasyonlara bağlıdır. Bu çalışma, SSc hastalarının vasküler ve parankimal pulmoner tutulumları ile hematolojik parametreler arasındaki ilişkiyi ortaya koymayı amaçladı.

Gereç ve Yöntem: Katılımcılar, SSc tanılı interstisyel akciğer hastalığı (İAH) ile birlikte pulmoner hipertansiyonu (PH) olanlar, sadece İAH'si olanlar ve İAH ve PH'si olmayan hastalar olmak üzere üç gruba ayrıldı. Laboratuvar verileri bu gruplar arasında karşılaştırıldı

Bulgular: İAH'nIn PH'dan bağımsız olarak yüksek kırmızı hücre dağılım genişliği (RDW) ve eritrosit sedimantasyon hızı ile ilişkili olduğu bulundu. Trombosit (PLT) sayısı anlamlı olarak daha düşüktü ve RDW / PLT oranı (RPO) seviyesi İAH ve PH birlikteliği olanlarda sadece İAH olanlara göre anlamlı olarak daha yüksekti.

Sonuç: RPR, SSc ile ilişkili İAH'da PH için tarama parametreleri olarak kullanılabilir.

Anahtar Kelimeler: interstisyel akciğer hastalığı, pulmoner hipertansiyon, sistemik skleroz

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INTRODUCTION

Systemic sclerosis (SSc) is an autoimmune disease characterized by obliterative vasculopathy, fibrosis of the skin and visceral organs, and has a high mortality rate compared with other rheumatic diseases.[1] The physiopathology of SSc is complex and still poorly understood.[2] Besides, it is well known that the clinical manifestations of SSc based on skin involvement range from limited cutaneous SSc (lcSSc), which indicates better prognosis, to diffuse cutaneous SSc (dcSSc), in which more severe complications develop.[3] Extracutaneous manifestations of SSc include the gastrointestinal tract (90%), musculoskeletal system (45-90%), cardiac (23-32%), and renal involvements (0.5-10%). Interstitial lung disease (ILD) is present in up to 90% of patients with SSc according to high resolution computed tomography (HRCT), and clinically significant ILD is present in approximately 40% of patients with SSc.[3,4] Pulmonary hypertension (PH) is also common in SSc (15%).[5] Three common types of PH in patients with SSc include the following: The World Health Organization (WHO) Group I PH (PAH), WHO Group II PH (PH due to left heart disease), and WHO Group III PH (PH due to ILD).[6,7] Survival in patients with SSc-associated PH and ILD is poor.[7] Compared with isolated SSc-related PH, SSc patients with both PH and ILD have an increased risk of death. Patients with SSc-ILD can also develop PH early on in their SSc disease course.[8-11] It is essential to note that around 50% of patients will never show any signs of progression. Prediction of the course of the disease may cause a difference in treatment choice because early, targeted, and intensive therapy is the key to success in SSc.[12] However, the prognosis is challenging to predict in many cases. Various routinely reported parameters in the complete blood count (CBC) test are considered systemic inflammatory biomarkers in cardiovascular diseases, various cancers, and many rheumatologic diseases.[13] Red cell distribution width (RDW) is considered a complementary measure of multiple pathologic processes that simultaneously occur in SSc, including oxidative stress, thrombosis, inflammation, endothelial dysfunction.[13,14] RDW to platelet ratio (RPR) has been considered a novel, simple, cost-effective biomarker that reflects inflammation severity and combines the prognostic advantages of RDW and PLT.[15] Systemic immune-inflammation index (SII), was an integrated indicator based on peripheral lymphocyte, neutrophil, and platelet counts. SII is an inflammation-based biomarker, which has been shown to be an effective prognostic factor in diseases with an inflammation-related etiology.[16,17] Studies related to the usefulness of globally available and inexpensive CBC tests to assess the severity of SSc are still lacking. This study was aimed to reveal the relationship between parenchymal and vascular involvements of the pulmonary system and CBC parameters, primarily RDW, SII, RPR, in patients with SSc.

MATERIAL AND METHOD

Study Population and Design

Patients with SSc were recruited from the Department of Rheumatology between January 2019 and January 2021. Adult individuals who gave written informed consent were enrolled in the study. Demographic characteristics, including age, sex, duration of disease, general medical history, organ involvement, laboratory parameters, imaging tests, and treatment information of the patients were recorded. Data were obtained from the electronic registration database. In addition, echocardiography, and high-resolution CT findings, were obtained from the patient's medical records, while estimated systolic pulmonary artery pressure (sPAP) was measured using the echocardiography method. Exclusion criteria were as follows: acute coronary syndromes, infection, other connective tissue disease, malignancy, heart failure, severe anemia, malnutrition, blood transfusion, hematological disorders, iron deficiency anemia, iron supplementation therapy, thromboembolic disease, cerebrovascular disease, and severe liver or renal insufficiency. This single-center cross-sectional study was designed as a prospective study and was approved by the ethics committee (Decision No: 2021/364). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Classification Criteria

We accepted patients who satisfied the 2013 American College of Rheumatology (ACR) / European League Against Rheumatism (EULAR) classification criteria as SSc.[18] The patients were grouped as lcSSc, and dcSSc based on the classification system proposed by LeRoy et al.[19] Organ involvement was evaluated according to clinical symptoms and the results of various diagnostic tests. The musculoskeletal disease was defined as arthritis, joint contractures, and myositis based on radiographic and laboratory data. Gastrointestinal involvement was defined as the presence of clinical symptoms such as dysphagia, reflux, gastritis, dyspepsia, and diarrhea. The diagnostic criteria for ILD were based on the presence of limited/diffuse-ground-glass or honeycomb opacity on HRCT. PH was diagnosed with pulmonary capillary wedge pressure (PCWP) <15 mmHg, and mean pulmonary artery pressure (PAP) >25 mmHg in right heart catheterization. Estimated sPAP >35 mmHg are used as indicators of probable PH.

Laboratory Measurements

Blood was analyzed in ethylenediaminetetraacetic acid (EDTA) tubes to obtain CBC results, including the platelet (K/μL), lymphocyte (K/μL), neutrophil (K/μL), and monocyte (K/μL) count, RDW (normal range: 11.5%–14.5%), mean platelet volume (MPV) (normal: 7,5–11,5 fl) levels were determined using an automatic blood counting system (Beckman Coulter LH 780, Brea, California, USA) for each participant. RPR was calculated by the formula RDW (%) / platelet count (10^9/L), and SII was calculated by the formula platelet counts

x neutrophil counts/lymphocyte counts. The erythrocyte sedimentation rate (ESR; 0-20 mm/hour) and C-reactive protein (CRP; 0-8 mg/L) of the patient and control groups and also autoantibodies [antinuclear antibody titer (ANA), anti-Scl-70 (ATA), anti-centromere (ACA), anti PM/Scl, anti-RNP, anti-Ro52, and rheumatoid factor (RF)] of the patient group were recorded.

Statistical Analysis

All statistical procedures were conducted using SPSS statistics version 22.0 (IBM, Armonk, NY, USA). Continuous variables were expressed as mean±standard deviation, and categorical variables as numbers (percentages). The distribution of scale variables was evaluated using the Kolmogorov-Smirnov test. Continuous variables were compared using Kruskal-Wallis H and/or Mann-Whitney U tests according to the number of samples. Pearson chi-square and Fisher's exact tests were used in categorical variables, where is appropriate. Receiver operating characteristic (ROC) curve analysis was used to evaluate the predictive value of the determined hemogram parameters. The ROC area under the curve (AUROC) was used to evaluate the discrimination ability of the model. The Youden index ([sensitivity + specificity]-1) was computed to determine the optimal cut-off value with the best combination of sensitivity and specificity. Subsequently, parameters associated with SSc-ILD and SSc-PAH were evaluated by univariate binary logistic regression analysis. Bonferroni correction was applied as post-hoc if significant results were obtained in more than two-sample comparisons. The p-values achieved after post hoc analysis were tabulated in an adjusted manner. A two-sided p-value <0.05 was considered statistically significant.

RESULTS

Demographics and Clinical Characteristics

There were 202 consecutive patients with SSc, aged between 19 and 80 years, in the patient records with follow-up. However, five patients with critical missing data were excluded. Besides, a total of 24 patients, 12 patients over 70 years of age, and 12 patients with iron deficiency anemia were not included in the study. Of the 24 excluded (11 lcSSc, 13 dcSSc), 8 (33.3%) had PH and 15 (62.5%) had SSc-ILD. 6 (75%) patients with PH were older than 70 years, and only 2 (25%) had lone involvement without SSc-ILD. The median disease duration of the excluded patients was 8 (IQR=10) years, ranging from 1 to 17. Of the remaining 174 patients (101 lcSSc and 73 dcSSc), only 12 were male. The median age was 50.5 (21) and ranged from 19 to 70 years. There was no significant difference between lcSSc and dcSSc in terms of age, gender, disease duration (p=0.778, p=0.217, p=0.114; respectively). A comparison of the patient's demographic characteristics and clinical features with SSc according to skin involvement is presented in **Table 1**. PH was detected in 5 of lcSSc (5.0%) and 13 of dcSSc (17.8%) subjects.

Table 1. Comparison of demographic characteristics and clinical features of patients with scleroderma according to the skin involvement

	Total, n (%)	lcSSc (n: 101)	dcSSc (n: 73)	p value
Sex, n (%)				
Male	12 (6.9%)	9 (8.9%)	3 (4.1%)	0.217
Female	162 (93.1%)	92 (91.1%)	70 (95.9%)	
Age, years		49.0±13.0	49.6 ±13.5	0.778
Age at the diagnosis, years		44.3±12.0	44.2±12.6	0.975
Disease duration, years		4.8±3.1	5.4±2.8	0.114
Clinical manifestations, n (%)				
Anemia of chronic disease †	31 (17.8%)	14 (13.9%)	17 (23.3%)	0.109
SSc-ILD	73 (42.0%)	24 (23.8%)	49 (67.1%)	
Limited NSIP	35 (20.1%)	19 (18.8%)	16 (21.9%)	
Extensive NSIP	23 (13.2%)	3 (3.0%)	20 (27.4%)	<0.001
Limited UIP	10 (5.7%)	2 (2.0%)	8 (11.0%)	
Extensive UIP	5 (2.9%)	0 (0.0%)	5 (6.8%)	
Dyspnea	64 (36.8%)	21 (20.8%)	43 (58.9%)	<0.001
Joint involvement	27 (15.5%)	14 (13.9%)	13 (17.8%)	0.478
Digital ulcers	25 (14.4%)	2 (2.0%)	23 (31.5%)	<0.001
GIT involvement	109 (62.6%)	59 (58.4%)	50 (68.5%)	0.175
Raynaud's phenomenon	160 (92.0%)	90 (89.1%)	70 (95.9%)	0.105
Nail fold capillaroscopy	144 (82.8%)	82 (81.2%)	62 (84.9%)	0.519
Pulmonary hypertension	18 (10.3%)	5 (5.0%)	13 (17.8%)	0.006
Autoantibody positivity, n (%)				
ANA titer				
Negative		8 (7.9%)	1 (1.4%)	
1/100		34 (33.7%)	19 (26.4%)	
1/320		45 (44.6%)	36 (50.0%)	0.026
1/640		2 (2.0%)	2 (2.8%)	
1/1000		12 (11.9%)	14 (19.4%)	
RF		6 (5.9%)	5 (6.8%)	1.000*
Scl-70		19 (18.8%)	34 (46.6%)	<0.001
ACA		46 (45.5%)	10 (13.7%)	<0.001
PMSCL		12 (11.9%)	7 (9.6%)	0.632
RNP3SM		3 (3.0%)	2 (2.7%)	1.000*
Ro-52		8 (7.9%)	9 (12.3%)	0.334

lcSSc: limited cutaneous systemic sclerosis, dcSSc: diffuse cutaneous systemic sclerosis, ILD: interstitial lung disease, NSIP: Non-specific interstitial pneumonia, UIP: usual interstitial pneumonitis, GIT: gastrointestinal tract, † Anemia: <13.5 g/dl in men, <12.0 g/dl in women, Data are expressed as mean±standard deviation or number of patients (percentage). Mann-Whitney U, Pearson chi-square, and Fisher's exact tests were used, where is appropriate. Significant values were shown in bold.

Formation of Comparison Groups

By comparing more than two samples as in **Table 2**, it was feasible to reduce the α (Type 1) error rate and minimize the effect of confounders. In this study, patients with SSc-PH without accompanying SSc-ILD were subsequently excluded due to the scarcity (n=4) of patients with SSc-PH alone in our sample. Our sample was divided into three groups: patients with both ILD and PH (ILD & PH, n=14), those with only ILD (Only ILD, n=59), and those with neither ILD nor PH (No-ILD & No-PH, n=97).

Table 2. Comparison of sex, age, clinical manifestations, and laboratory parameters of patients with SSc according to pulmonary involvement such as ILD and PH

	Patients with SSc				Adjusted p-values		
	ILD & PAH A, n=14	Only ILD B, n=59	No-ILD & No-PAH C, n=97	p-value	p1- value A vs. B	p2-value A vs. C	p3-value B vs.C
Sex, n (%) †							
Male	0 (0.0%)	5 (8.5%)	7 (7.2%)	N/A	N/A	N/A	N/A
Female	14 (100.0%)	54 (91.5%)	90 (92.8%)				
Age, years ‡	56.3±9.1	54.1±11.8	44.9±13.1	<0.001	1.000	<0.001	<0.001
Age at the diagnosis, years ‡	50.9±9.7	48.1±11.5	40.6±12.0	<0.001	1.000	0.013	0.001
Disease duration, years ‡	5.4±2.3	6.0±2.8	4.3±3.1	<0.001	1.000	0.216	<0.001
Clinical manifestations, n (%)							
Cutaneous subset†							
Limited cutaneous SSc	3 (21.4%)	21 (35.6%)	75 (77.3%)	<0.001	Not Sig	Sig	Sig
Diffuse cutaneous SSc	11 (78.6%)	38 (64.4%)	22 (22.7%)				
ILD subset §							
NSIP	8 (57.1%)	50 (84.7%)	-	0.032	-	-	-
UIP	6 (42.9%)	9 (15.3%)					
Raynaud's phenomenon †	13 (92.9%)	55 (93.2%)	89 (91.8%)	0.943	-	-	-
NF capillaroscopy finding †	9 (64.3%)	51 (86.4%)	81 (83.5%)	0.137	-	-	-
Autoantibody positivity, n (%)							
ANA titer ‡							
Negative	0 (0.0%)	3 (5.1%)	6 (6.2%)				
1/100	2 (14.3%)	20 (33.9%)	30 (30.9%)				
1/320	7 (50.0%)	27 (45.7%)	45 (46.4%)	0.117	-	-	-
1/640	2 (14.3%)	0 (0.0%)	2 (2.1%)				
1/1000	3 (21.4%)	9 (15.3%)	14 (14.4%)				
RF †	1 (7.1%)	5 (8.5%)	5 (5.2%)	N/A	N/A	N/A	N/A
Scl-70 †	7 (50.0%)	21 (35.6%)	25 (25.8%)	0.124	-	-	-
ACA †	2 (14.3%)	13 (22.0%)	39 (40.2%)	0.021	Not Sig	Sig	Sig
PMSCL†	2 (14.3%)	4 (6.8%)	12 (12.4%)	0.489	-	-	-
RNP3SM †	2 (14.3%)	1 (1.7%)	2 (2.1%)	N/A	N/A	N/A	N/A
Ro-52 †	1 (7.1%)	8 (13.6%)	8 (8.2%)	0.525	-	-	-
Hemogram parameters ‡							
Hemoglobin (g/dl)	12.9±1.3	12.9±1.2	13.3±1.2	0.124	-	-	-
Neutrophil (109/l)	4.4±1.6	4.2±1.6	4.3±1.6	0.840	-	-	-
Lymphocyte (109/l)	1.6±0.7	1.9±0.7	2.1±0.7	0.009	0.116	0.009	0.481
Monocytes (109/l)	0.54±0.27	0.57±0.19	0.53±0.15	0.174	-	-	-
Platelet (109/l)	235±67	291±73	268±69	0.017	0.034	0.473	0.103
Plateletcrit (%)	0.21±0.05	0.24±0.5	0.23±0.05	0.061	-	-	-
MPV (fl)	8.9±1.6	8.3±0.9	8.6±0.9	0.104	-	-	-
RDW (%)	15.9±1.6	15.2±1.7	14.5±1.6	<0.001	1.000	0.004	0.002
NLR	3.14±1.71	2.44±1.23	2.27±1.09	0.112	-	-	-
MLR	0.37±0.18	0.33±0.17	0.27±0.10	0.033	1.000	0.203	0.079
PLR	160±57	171±71	141±62	0.025	1.000	0.545	0.028
RPR (10-7 x mm3)	7,32±2.42	5.61±1.49	5.71±1.49	0.046	0.045	0.062	1.000
SII (103/mm3)	713±414	714±393	613±342	0.228	-	-	-
ESR (mm/hour)	31.9±21.7	25.3±17.6	18.8±18.1	0.001	1.000	0.024	0.009
CRP (mg/l)	8.1±6.6	7.5±7.4	5.9±7.5	0.009	1.000	0.159	0.017

SSc: systemic sclerosis, PAH: pulmonary hypertension, ILD: interstitial lung disease, NSIP: Nonspecific interstitial pneumonia, UIP: usual interstitial pneumonitis, NF: nail fold, MPV: mean platelet volume; RDW: red cell distribution width; PLR: platelet/lymphocyte ratio; NLR: neutrophil/lymphocyte ratio; MLR: monocytes/lymphocyte ratio; RPR: RDW/platelet ratio, SII: systemic immune-inflammation index (calculated by multiplying NLR by platelet), ESR: erythrocyte sedimentation rate; CRP: C-reactive protein, N/A: not applicable; Sig: significant Data are expressed as mean±standard deviation or number of patients (percentage). Kruskal-Wallis H ‡, Mann-Whitney U, Pearson chi-square † and Fisher's exact § tests were used, where is appropriate. Bonferroni correction was applied as post-hoc if significant results were obtained in more than two sample comparisons Significant values were shown in bold. Adjusted p1 for the difference between A and B groups; adjusted p2 for the difference between A and C groups; adjusted p3 for the difference between B and C groups

Association of Hemogram Parameters and SSc-ILD in SSc Patients

Comparison of gender, age, clinical findings, and laboratory parameters of patients with SSc according to pulmonary system involvement is presented in **Table 2**. Post-hoc analysis results and adjusted (adj.) p-values for significant parameters were on the table's right side. ILD was found to be associated with advancing age, late onset of disease, diffuse cutaneous SSc, negative ACA, and high RDW and ESR, independent of the presence of concomitant PH (both, adj. p2<0.05 and adj. p3<0.05).

Association of Hemogram Parameters and SSc-PAH in SSc-ILD Patients

When the relationship between the presence of PAH and hemogram parameters in patients with SSc-ILD was evaluated, the groups were identical in terms of age (adj. p1=1.000). There was no significant difference in parameters such as gender, age, disease duration, age at diagnosis, cutaneous subset, and autoantibodies in patients with and without SSc-PH, provided that ILD was present (adj. p1>0.05). It was determined that UIP was significantly more common than NSIP in the coexistence of SSc-PH and SSc-ILD (p=0.032). It was noted that the platelet count was significantly lower, and the RPR level was significantly higher in those with ILD and PH coexistence compared to those with only ILD (adj. p1=0.034, adj. p1= 0.045; respectively).

The relationship of Medication in SSc Treatment with Hemogram Parameters

As seen in **Table 3**, neither azathioprine nor cyclophosphamide was administered in any patients withoutpulmonary system involvement (both p<0.001). These two drugs, frequently used in SSc will affect hemogram parameters due to their bone marrow suppression effect. It is challenging to predict whether the hemogram parameters associated with pulmonary involvement are related to the nature of the disease or the drugs used. However, we analyzed the relationship between azathioprine and cyclophosphamide and hemogram parameters (**Table 4**). It was noteworthy that the RDW and ESR associated with ILD were significantly higher, and the lymphocyte count was significantly lower in patients using both azathioprine and cyclophosphamide. However, neither platelet count nor RPR level was associated with either drug use.

Table 3. Medications administered to patients with SSc according to the presence of SSc-ILD

Medications, n (%)	Patients with SSc (n=170)				Adjusted p-values		
	ILD & PH A, n=14	Only ILD B, n=59	No-ILD &No-PH C, n=97	p-value	p1- value A vs. B	p2-value A vs. C	p3-value B vs.C
Hydroxychloroquine	14 (100.0%)	59 (100.0%)	97 100.0%)	N/A	-	-	-
Azathioprine	13 (92.9%)	39 (66.1%)	0 (0.0%)	<0.001	Not sig	Sig	Sig
Methotrexate	0 (0.0%)	0 (0.0%)	2 (2.1%)	N/A	-	-	-
Mycophenolate mofetil	0 (0.0%)	1 (1.7%)	1 (1.0%)	N/A	-	-	-
Cyclophosphamide	6 (42.9%)	17 (28.8%)	0 (0.0%)	<0.001	Not sig	Sig	Sig
Pentoxifylline	0 (0.0%)	6 (10.2%)	17 (17.5%)	0.130	-	-	-
Prostaglandin analogue	2 (14.3%)	5 (8.5%)	3 (3.1%)	N/A	-	-	-
PDE inhibitor	1 (7.1%)	0 (0.0%)	0 (0.0%)	N/A	-	-	-
Bosentan	1 (7.1%)	0 (0.0%)	0 (0.0%)	N/A	-	-	-
Nifedipine	0 (0.0%)	6 (10.2%)	14 (14.4%)	0.262	-	-	-
Ritixumab	1 (7.1%)	6 (10.2%)	0 (0.0%)	N/A	-	-	-
Tocilizumab	0 (0.0%)	0 (0.0%)	1 (1.0%)	N/A	-	-	-

SSc: systemic sclerosis, P-H: pulmonary hypertension, ILD: interstitial lung disease, N/A: not applicable; Sig: significant, Data are expressed as the number of patients (percentage). Pearson chi-square test was used. Bonferroni correction was applied as post-hoc if significant results were obtained in more than two-sample comparisons. Significant values were shown in bold. Adjusted p1 for the difference between A and B groups; adjusted p2 for the difference between A and C group; adjusted p3 for the difference between B and C groups

Table 4. The relationship of azathioprine and cyclophosphamide with hemogram parameters, ESR and CRP levels

Hemogram parameters (n=170)	Azathioprine		p-value	Cyclophosphamide		p-value
	Yes (n=52)	No (n=118)		Yes (n=23)	No (n=147)	
Hemoglobin (g/dl)	12.8±1.2	13.3±1.2	0.015	12.9±1.1	13.2±1.2	0.321
Neutrophil (109/l)	4.25±1.67	4.31±1.59	0.692	4.45 ±1.73	4.27±1.59	0.726
Lymphocyte (109/l)	1.78±0.68	2.08±0.68	0.005	1.68±0.58	2.04±0.69	0.019
Monocytes (109/l)	0.55±0.22	0.54±0.15	0.681	0.53±0.21	0.55±0.17	0.518
Platelet (109/l)	273 ±62	273±76	0.638	278±66	272±73	0.341
Plateletcrit (%)	0.23±0.05	0.23±0.05	0.908	0.22 ±0.06	0.23±0.05	0.760
MPV (fl)	8.4±1.1	8.6±0.9	0.196	8.1±1.1	8.6±0.9	0.009
RDW (%)	15.9±1.7	14.5±1.6	<0.001	15.6±1.9	14.7±1.7	0.045
NLR	2.67±1.36	2.29±1.13	0.082	2.89±1.34	2.33±1.18	0.039
MLR	0.34 ±0.16	0.29±0.12	0.031	0.33±0.14	0.30±0.14	0.162
PLR	173±67	145±64	0.006	180±61	149±66	0.011
RPR (10-7 x mm3)	6.08 ±1.71	5.71±1.59	0.112	5.98±1.90	5.78±1.60	0.707
SII (103/mm3)	724±392	627±354	0.107	812±452	632±348	0.046
ESR (mm/hour)	28.1±20.3	19.6±17.3	0.003	28.9±20.4	20.1±18.2	0.039
CRP (mg/l)	7.5±6.2	6.2±7.9	0.010	6.9 ±6.0	6.5±7.6	0.317

MPV: mean platelet volume; RDW: red cell distribution width; PLR: platelet/lymphocyte ratio; NLR: neutrophil/lymphocyte ratio; MLR: monocytes/lymphocyte ratio; RPR: RDW/platelet ratio, SII: systemic immune-inflammation index (calculated by multiplying NLR by platelet), ESR: erythrocyte sedimentation rate; CRP: C-reactive protein. Data are expressed as mean±standard deviation. Mann-Whitney U test was used. Significant values were shown in bold.

ROC Analysis in the Prediction of Pulmonary System involvement in SSc

The cut-off values, sensitivity, and specificity of the relevant hemogram parameters were presented in **Figure 1**. The RDW and ESR values in the ROC curve with the best balance of sensitivity and specificity to determine SSc-ILD were >14.0% (78.1% sensitivity, 56.4% specificity) and >15 mm/h (68.5% sensitivity, 61.4% specificity) according to the results from the Youden index. The optimal RPR cut-off point was ≥5.39 (10-7×mm³) with a sensitivity of 71.4% and specificity of 57.6% in predicting concomitant SSc-PAH in patients with SSc-ILD (p=0.015). Platelet count [(cut-off value<206.5 (10^9/l)] was useful in predicting SSc-PH (50.5% sensitivity, 88.1% specificity, p=0.013) (**Figure 1B**).

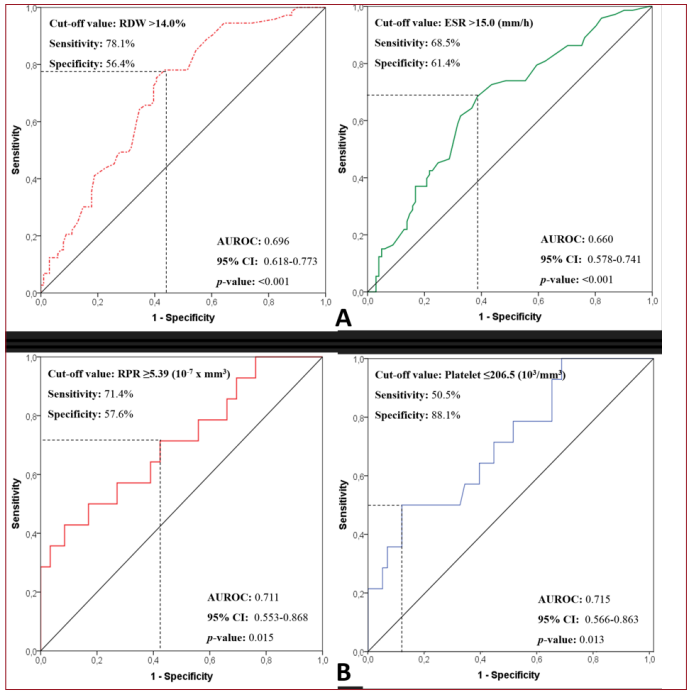

Figure 1. Receiver operating characteristic curve (ROC) analysis for assessing the performance of relevant hemogram parameters (RDW, ESR, RPR, platelet) in SSc-ILD in SSc patients and PH in patients with SSc-ILD. The cut-off values, sensitivity, and specificity of the relevant hemogram parameters were demonstrated.

Results of the Binary Logistic Regression Analysis

Factors associated with pulmonary system involvement with univariate binary logistic regression analysis were evaluated in **Table 5**. In addition to well-known risk factors associated with SSc-ILD such as age, general skin involvement, and ACA negativity; RDW (odds ratio 4.62 [%95CI 2.34–9.11], p<0.001) and ESR (3.46 [1.83–6.53], p<0.001) elevation were also independent risk factors indicating SSc-ILD. In patients with SSc-ILD, the involvement pattern compatible with UIP increased the risk of SSc-PH 4.17 [1.17-14.90] times (p=0.028). There were significant increases of 3.4 [1.08-12.11] and 7.43 [2.00-27.58] times in the risk of SSc-PAH in high RPR [≥5.39 (10-7 x mm³)] and low platelet [<206.5 (109/l)] levels, respectively (p=0.047, p=0.003; respectively).

DISCUSSION

SSc is characterized by generalized micro and macroangiopathy. Mortality in SSc is primarily due to pulmonary complications: in the largest observational study conducted to date, the leading cause of death was ILD; 17% and PH 15%.[4] In this study, we investigated the relationship between parenchymal and vascular involvements of the pulmonary system and CBC parameters, primarily RDW, SII, RPR, in patients with SSc. It was found that IAH was associated with RDW and ESR independent of PH. RPR was significantly higher in patients with IAH and PH coexistence compared to IAH alone. Previous studies reported risk factors for poor survival in SSc such as male sex, diffuse cutaneous subtype, age at disease onset, African origin, presence of anti-Scl-70 antibody, and specific organ involvement. Identifying autoantibodies is clinically valuable for helping diagnosis and in predicting the development of certain clinical manifestations and prognoses.[3] ACA is associated with lcSSc and PH, whereas anti-Scl70 antibodies are associated with dcSSc and pulmonary fibrosis.[6] The present study confirms this data. ILD is an early complication in SSc, and in some patients (~4%), the first clinical symptom of SSc is directly related to ILD.[6] PH is a progressive and potentially

Table 5. Evaluation of the factors associated with pulmonary system involvement

Univariate logistic regression analysis	βi	Odds ratio	95% CI		Wald value	p value	Nagelkerke R Square
			Lower	Upper			
SSc-ILD (n=73/174)							
Age	0.59	1.06	1.03	1.09	17.98	<0.001	0.154
Generalized skin involvement	1.88	6.55	3.35	12.80	30.26	<0.001	0.235
ACA negativity	0.92	2.64	1.32	5.28	7.55	0.006	0.061
RDW >14.0%	1.53	4.62	2.34	9.11	19.44	<0.001	0.157
ESR >15 mm/hour	1.24	3.46	1.83	6.53	14.61	<0.001	0.114
SSc-PAH in patients with SSc-ILD (n=14/73)							
SSC-ILD subset: UIP	1.43	4.17	1.17	14.90	4.82	0.028	0.099
RPR ≥5.39 (10-7 x mm3)	1.29	3.40	1.08	12.11	3.57	0.047	0.094
Platelet <206.5 (103/mm3)	2.01	7.43	2.00	27.58	8.98	0.003	0.186

βi – Regression coefficient; CI – confidence interval; SSc-ILD – Systemic sclerosis-associated interstitial lung disease; SSc-PAH – Systemic sclerosis-associated pulmonary hypertension, UIP – usual interstitial pneumonitis, RDW – red cell distribution width; ESR – erythrocyte sedimentation rate; RPR – RDW/platelet ratio

mortal disease that often presents non-specific symptoms leading to delayed diagnosis.[5,7] Patients with SSc and PH and ILD (SSc-PH-ILD) generally have a worse prognosis than those without SSc and SSc-PAH without ILD.[8-11] SSc-related PH-ILD has a 3-year survival rate of 21%.[11] Despite several clinical and hemodynamic parameters such as right heart catheterization for evaluating PH, the invasive, subjective, and unstable nature and very high costs limit their use. Blood-based biomarkers that reliably identify SSc-ILD patients at risk of PH would significantly improve screening, potentially leading to improved survival, and provide novel mechanistic insights into early disease.[12] In recent years, accumulating evidence has indicated the potentially great diagnostic and prognostic value of complementary components of CBC.[13-15] Along with other hematological inflammatory indices, SII seems to be a simple and inexpensive tool to predict the progression of various diseases.[16,17] SII has been widely used in oncology since 2014 with promising results. However, SII was not significantly changed in our study. Previous studies have reported the value of RDW in predicting adverse outcomes in malignant tumors, autoimmune diseases, cardiovascular and thrombotic disorder.[20-22] Furthermore, RDW can be a prognostic marker of adverse outcomes in patients with the PH of different etiologies.[20] The importance of RDW has been recently recognized in patients with connective tissue disease-associated ILD.[23] Farkas et al. reported that RDW was higher in patients with SSc, particularly those with dcSSc and those with anti-Scl.[14] Wang et al. found increased RDW to have a diagnostic value in chronic thromboembolic PH in SSc patients.[24] Zhao et al. demonstrated an independent association between RDW and PH in lcSSc and dcSSc.[25] The SSc-PH group had significantly higher RDW values compared to the SSc group without pulmonary disease. Thus, RDW in SSc may represent an integrative measure of multiple pathological processes, including extensive vasculopathy, fibrosis, or ongoing inflammation.[26,27] We demonstrated that the RDW value is significantly higher in patients with SSc-ILD than those without ILD, independent of concomitant PH. Besides age, general skin involvement, and ACA negativity, RDW and ESR elevation were independent risk factors indicating SSc-ILD. Isolated PH patients were not included in our study because of their rarity. Therefore, this study does not predict PH without ILD. Although we found ansignificant relationship between ILD and RDW and ESR, this result should be treated with caution as it is likely that patient medication affects these parameters significantly. Besides RDW, increasing number of reports emphasize the inflammatory and prognostic significance of the RPR.[28-32] Although the pathophysiological role of the inflammation marker RPR remains unclear, its elevation augments the probability of an increased RDW and a decreased platelet count. RPR is considered a strong predictor of the severity of fibrosis and cirrhosis in patients with chronic hepatitis and a valuable prognostic marker of inflammation in acute pancreatitis, myocardial infarction, and some malignancies.[28-32] Positive associations between increased RPR and the incidence of cardiovascular events in hemodialysis patients were identified.[31] Liu et al. stated that RPR had the highest accuracy in predicting advanced liver fibrosis compared to other non-invasive tests.[33] Wang et al. found that RPR can predict significant fibrosis and liver cirrhosis with relatively high accuracy.[34] Xie S. et al. SLE patients had significantly higher RPR than healthy individuals, and RPR level was correlated with clinical disease activity in SLE.[35] RPR has been proven to predict the prognosis of patients with severe burns and severe acute pancreatitis.[36,37] These results showed that RPR is regarded as an indicator of systemic inflammatory response. Our study also further supports the evidence that RPR is elevated in patients with SSc-ILD- PH compared to SSc-ILD patients without PH. The pathophysiology of RPR elevation in PH is likely multifactorial since the pathogenesis of PH is related to inflammation, oxidative stress, and endothelial dysfunction. PH could lead to systemic hemodynamic disorders and tissue hypoxia. Tissue hypoxia provokes an inflammatory response and oxidative stress, both of which may disrupt erythrocyte turnover and may lead to anisocytosis and increased RDW levels. Another possible explanation for increased RDW is that chronic inflammation may shorten the half-life of erythrocytes, change the membrane characteristics, and cause to increase in RDW values.[20,21] PLT plays acrucial role in the process of hemostasis in the body. Decreased PLT count is a common pathological phenomenon in acute and critically ill patients. Decreased PLT in children with sepsis is an important sign of severe inflammation in the body.[38,39] Guo Feng et al. found that the PLT of severe burn patients decreases considerably in 1–2 weeks post-injury. Distinctly low PLT also strongly predicted the poor outcome of operations.[39] RPR has recently been considered a novel index marker that reflects inflammation severity by combining the prognostic advantages of RDW and PLT. However, the reason why the imbalances between RDW and platelet count could be a significant prognostic factor remains uncertain. It is noteworthy that the RDW and ESR associated with ILD were significantly higher in patients using both azathioprine and cyclophosphamide. However, neither platelet count nor RPR level was associated with either drug use, which is an important finding of our study. We consider that the RPR may profit to detect SSc-PH at earlier stages, and thus better outcomes can be achieved. Although the precise mechanism remains unclear, the present study indicates for the first time a potential prognostic value of the inflammatory marker RPR in SSc-PH-ILD patients. Taken together, the present results suggest that these routinely available parameters, which may be obtained non-invasively and economically, may be repurposed as novel diagnostic parameters for PH in SSc-ILD patients.

Limitations

Our study has limitations that must be acknowledged. First, due to its retrospective nature, it was impossible to standardize at what point testing was performed in the natural history of the disease. Another limitation was that isolated PH patients were not included in the study because of their rarity. Therefore, this study does not predict PH without ILD. Another limitation is that all of our included patients in this study were SSc and SSc patients were compared within themselves. The healthy control group was not included in the study. This RPR based screening strategy should also be studied in other at-risk populations with larger samples sizes.

CONCLUSION

RPR can be used as one of the parameters for screening PH in SSc-ILD. To the best of our knowledge, the present study is the first research that evaluated the RPR value and explored its clinical significance in SSc patients. Therefore, a large prospective study should now validate using RPR in a screening strategy to diagnose SSc-PH earlier.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was designed as a prospective study and was approved by the ethics committee (Decision No: 2021/364)

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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