Journal of Cardiovascular Disease Research

ISSN:0975-3583,0976-2833 VOL12, ISSUE 07, 2021

Anti–Endothelin 1 Receptor Type A Autoantibodies in Pulmonary Arterial Hypertension Associated with Systemic Lupus Erythematosus and Systemic Sclerosis

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Article History: Received: 29.10.2021 **Revised:** 02.11.2021 **Accepted:** 26.11.2021

Abstract

***Objective*:** To detect serum level of autoantibodies against endothelin-1 receptor type-A (anti-ET1RA) in systemic lupus erythematosus (SLE) and systemic sclerosis (SSc) patients and to evaluate its role as a predictive biomarker in disease-associated pulmonary arterial hypertension (PAH).

Methods: 75 patients (25-SLE, 25-SSc, 25-PAH patients due to other entities) and 25 controls were included. Disease activity, functional state of dyspnea, pulmonary function tests, and HRCT chest were performed for SLE and SSc patients—trans-thoracic echocardiography for all patients to detect the signs suggestive of PAH. Serum anti-ET1RA was measured in patients and controls.

***Results**:* PAH was detected in 7 SLE patients (28%) and 6 SSc patients (24%). Serum anti-ET1RA antibodies were positive in 4 (57.14 %) SLE patients with PAH, in 5 (83.33%) SSc patients with PAH, and 18 patients (72%) with other entities of PAH. Serum anti-ET1RA was substantially higher in the patients compared to the control group (p=0.001). It was significantly higher in SLE-PAH and SSc-PAH than those without PAH (p=0.001,p<0.0001). Anti-ET1RA positively correlated with the mean pulmonary artery pressure in SLE and SSc patients (p<0.0001). The best-calculated cut-off value of anti-ET1RA to detect PH obtained by ROC analysis was at 10.39U/ml with 73.7% sensitivity and 97.3% specificity. The risk of PAH was assessed using non-adjusted and fully binary logistic regression models. Anti-ET1RA antibodies were the only independent predictor for PAH in patients with SLE and SSc (95%CI;0.34-10.96) (p=0.037).

Conclusion: Anti-ET1RA antibodies are detected in SLE and SSc patients with PAH serum. They may serve as a predictive biomarker for PAH in patients with connective tissue diseases.

Keywords: Systemic lupus erythematosus, Systemic sclerosis, ET1RA, Pulmonary hypertension.

Introduction:

Pulmonary arterial hypertension (PAH) is a commonly critical clinical condition **(1)**. In clinical trials, connective tissue disease (CTD) patients account for 20-30% of PAH patients. Despite substantial therapeutic improvements, SLE-PAH and SSc-PAH patients have reduced survival compared to patients with idiopathic PAH (IPAH**) (2)**.

The intricate development of PAH involves persistent narrowing of small pulmonary arteries and vascular changes (3). Furthermore, dysregulated immune responses and inflammation are prevalent in CTD-PAH

ISSN:0975-3583,0976-2833 VOL12, ISSUE 07, 2021

and other PAH causes, evidenced by inflammatory infiltration, growth factor expression in remodeled pulmonary vessels, and elevated cytokine and chemokine levels in the bloodstream (4). PAH linked to SLE might exhibit pulmonary vasculitis and immune complex deposition in the affected vessels (2).

Endothelin is a naturally occurring peptide with multiple effects on the vasculature **(5)**. Endothelin type1 is a significant isoform in humans **(6)**. ETA and ETB are separate endothelin receptors that produce differing, occasionally opposing effects **(7)**. They activate their respective receptors located predominantly on vascular smooth muscles (SMCs) and endothelial cells (ECs). ETA and ETB could trigger vasoconstriction, cell proliferation, and local inflammation, inducing obliterative vasculopathy **(8)**.

Riemekasten and co-workers first reported the auto-antibodies in SSc patients**(9),** and were also identified as a marker in SLE that may mediate PAH development**(10)**.

This study aimed to detect serum level of autoantibodies against endothelin-1 receptor type-A (anti-ET1RA) in systemic lupus erythematosus (SLE) and systemic sclerosis (SSc) patients and to evaluate its role as a predictive biomarker in disease-associated pulmonary arterial hypertension (PAH).

Patients and methods:

This cross-sectional, analytical, case-control study was conducted in Minia University Hospital, including 25 SLE patients who met the Systemic Lupus International Collaborating Clinics classification criteria (SLICC 2012) and 25 SSc patients who were diagnosed with systemic sclerosis according to ACR/EULAR 2013 classification criteria **(11, 12)**, compared to 25 patients with different (PH)/PAH entities and 25 healthy age and sex-matched controls. The patients were recruited from Rheumatology and rehabilitation outpatient chest and cardiovascular outpatient clinics from the period ; May 2020 to June 2021. Patients were excluded if they were known to have other connective tissue diseases or overlap syndromes, pulmonary thromboembolism, left-sided heart insufficiency, myocardial infarction, or essential hypertension. Patients who use drugs affecting pulmonary artery pressure were also excluded. The research protocol adhered to the principles outlined in the World Medical Association Declaration of Helsinki. Informed consent was obtained from all patients before their participation. Patients underwent a comprehensive medical history assessment and thorough clinical examination as part of the study.

The activity of the disease was assessed employing the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI-2k) **(13)** in SLE patients, the European scleroderma study group score (EscSG score) in SSc patients **(14)**. The functional assessment of dyspnea was performed using the 6-minute walk test **(15)**, the mMRC scale for dyspnea in patients without PH **(16),** and the WHO functional class of dyspnea in patients with PH **(17).**

The cardiologist estimated Pulmonary artery pressure for all the patients, blinded for clinical data by an ultrasound system (Vivid 3, GE) equipped with a GE 3S sector array ultrasound probe. Echocardiography assessed the PH effect on the heart and PAP from continuous wave Doppler measurements (pulmonary artery systolic pressure, mean pulmonary artery pressure). Pulmonary artery systolic pressure was estimated using the

ISSN:0975-3583,0976-2833 VOL12, ISSUE 07, 2021

maximum tricuspid regurgitant jet velocity (TR max) using continuous wave (CW) Doppler and then calculated according to Bernoulli's equation [PASP = 4 $(TR\ max)^2$ **(18).** The mean PAP was evaluated from the RV outflow track (RVOT) acceleration time (AT). The pulse wave of the pulmonic forward flow RVOT signal is estimated at end-expiration at parasternal short axis view. RVOT AT is measured from the beginning of flow to the peak flow velocity; a value > 130 ms is normal, and < 100 ms is suggestive of PH. The mean PAP was calculated using the following equation: [MPAP = 90 - (0.62 × AT_{RVOT}]. Normal mean PAP is <25 mmHg**.**

Pulmonary function assessments were conducted for all patients using the ZAN 300 system (Biomedica, Germany). Various parameters were evaluated, including Forced Vital Capacity (FVC), Forced Expiratory Volume in the First Second (FEV1), the ratio of FEV1 to FVC (FEV1/FVC), Forced Expiratory Flow between 25% and 75% of Vital Capacity (FEF25–75%), and Peak Expiratory Flow Rate (PEF). Additionally, the Diffusing Capacity of the lung for carbon monoxide (DLCO), adjusted for hemoglobin concentration, Total Lung Capacity (TLC), and Transfer Coefficient (KCO) (calculated as DLCO/alveolar volume VA) were measured using the single breath technique. All parameters were expressed as percentages of predicted values based on the patient's age, sex, and height. The pulmonary function tests were defined following the guidelines outlined in the ATS/ERS general considerations for lung function testing document **(19).**

A radiological study was done for SLE and SSc patients using a resolution CT chest on the 16-slice machine (GE Bright Speed, GE Health Care, USA). Chest images were taken considering the following parameters: kV: 120, mA: 300, helical scan, slice thickness: 0.625, interval: 0.625, pitch: 0.562:1, detector configuration: 16 × 0.625, tilt: 0, reconstruction: lung window. Images were reconstructed with a high spatial frequency algorithm and photographed at a window appropriate for viewing the lung parenchyma. The following CT signs were evaluated as defined by the Fleischner Society: (Linear parenchymal opacities, septal thickening, nodular parenchymal opacities, ground glass appearance, airspace consolidation, traction bronchiectasis, cystic changes, honeycombing, pleural involvement, lymph nodal enlargement) **(20).** The mediastinal window was used for viewing vascular, cardiac, and mediastinal abnormalities to indicate the diagnosis of PH (PA diameter ≥29 mm or RV enlargement) **(18).**

Laboratory investigations included the complete blood count, erythrocyte sedimentation rate (ESR: normal level <20 mm/h), semi-quantitative C reactive protein measured by latex agglutination slide test (positive if >6 mg/L), renal function tests including urea (normal range 20-40 mg/dl) and creatinine (normal Range 0.5-1.5 mg/dl), serum anti-nuclear antibody (ANA) (for SLE and SSc patients) (positive if >1.2 U/ml), serum anti-double-stranded DNA (anti dsDNA) (for SLE patients) (positive if >20 U/ml), serum complement (C3, C4) measured by ELISA technique (for SLE and SSc patients) (normal C3: 90–110 ug/ml, C4: 10–35 ug/ml) and serum anti-endothelin-1 receptor type A antibodies level (Anti-ET1RA) (measured for all the patients and controls using the sandwich enzyme immunoassay technique (ELISA); Sino GeneClon Biotech Co., Ltd, China (detection range: 9.20 U/ml – 10.40 U/ml).

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Statistical analysis:

Data were analyzed using a statistical package for social science (SPSS version 19). Quantitative variables were described using mean, standard deviation (SD), and range. Qualitative variables were represented by the number (no.) and percentage (%). Comparisons were made by *Chi-square (χ2) test* to compare qualitative variables. *Student's t-test* was applied to compare the means of different groups in the interval and ordinal variables. One-way ANOVA (Analysis of variance) was used to compare more than two independent groups. Correlation between variables was calculated by Spearman rho and Pearson correlation coefficients (r). Characteristic (ROC) curves were designed to estimate the area under the curve (AUC), sensitivity, and specificity using a calculated distance of 0.1 with Youden's index. Non-adjusted and fully-adjusted binary logistic regression models were used to estimate the risk factors for PAH. Scaled Schoenfeld residuals were employed to assess the proportionality assumption, and 95% confidence intervals (CI) were calculated for the analysis. The p values < 0.05 were considered significant **(21).**

Results:

The mean age of the patients was (39.15±9.97) years (ranging from 22 to 65 years), and the mean duration of the rheumatologic disease (SSc and SLE) was 7.62 years (ranging from 2 to 18 years). The characteristics of the studied SLE and SSc patients are shown in **Table 1.**

Regarding medications, at the time of examination, all the studied lupus patients were on glucocorticoids and antimalarial therapy, as regards DMARDs and immunosuppressive therapy (1 on MTX, 4 patients on cyclophosphamide, 14 on azathioprine therapy and only 2 patients on mycophenolate mofetil). In SSc patients, there were 16 patients on glucocorticoids, 10 on MTX 13 administered cyclophosphamide, 12 on azathioprine, and only 1 patient received mycophenolate mofetil. A comparison of the serum anti-endothelin-1 receptor type A antibodies in the studied patients and controls is shown in **Table 2.**

Table 3 compares patients with and without PH in both SLE and SSc.

Patients with different pulmonary hypertension (PH)/PAH entities other than SLE-PAH and SSc-PAH (n= 25) were found to have a significantly lower serum anti-ET1RA (p< 0.0001) and higher pulmonary artery pressure (p< 0.0001) compared to patients with SLE-PAH and SSc-PAH (n= 13), **Table 4**. In systemic lupus and systemic sclerosis patients, serum anti-ET1RA was significantly positively correlated with RVSP and MPAP (p< 0.0001) and negatively with PAT (p< 0.0001). In patients of other PAH/PH entities, serum ET1RA was negatively correlated with RVSP and MPAP and positively with PAT, but both correlations didn't reach a significant level.

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Table 1: Characteristics of the studied SLE and SSc patients.

Parameter mean ± SD and/or n (%)		SLE patients n=25	SSc patients n=25
Age		34.92 ± 10.30	41.48 ± 11.19
Gender (female)		25 (100%)	22 (88%)
SLEDAI 2k	**Mild**	14 (56%)	
	Moderate	9 (36%)	-------
	Severe	2 (8%)	
EscSG	**Active**	---------	9 (36%)
	Remission		16 (64%)
6 MWT	**Distance (meter)**	284±65.22	227.20±75.51
	O2 saturation-pre	98.56±0.51	98.2±0.78
	O2 saturation-post	96.88±1.26	96.12±1.83
Patients with PH		7 (28%)	6 (24%)
MPAP (mmHg)		17.96 ± 7.11	18.82 ± 7.35
PSPAP (mmHg)		35.88 ± 7.63	35.57 ± 8.06
RVSP (mmHg)		28.88 ± 7.63	28.57 ± 8.06
PAT (mSec)		116.2 ± 11.48	114.8 ± 11.86
IVS (cm)		0.98 ± 0.27	0.90 ± 0.15
PWT (cm)		0.91 ± 0.25	0.89 ± 0.15
LVIDd (cm)		4.59 ± 0.303	4.37 ± 0.37
LVIDs (cm)		2.91 ± 0.32	2.85 ± 0.38
EF (%)		66.04 ± 8.72	63.87 ± 7.22
Aos (cm)		3.12 ± 0.34	3.18 ± 0.32
Aod (cm)		2.94 ± 0.32	2.94 ± 0.33
ASI		9.52 ± 6.09	7.53 ± 6.33
Pericardial effusion		2 (8%)	4 (16%)
FVC %		87.88 ± 13.389	69.20 ± 15.158
DLCO %		77.36 ± 17.308	67.56 ± 19.190
FVC/DLCO %		1.1842 ± 0.281	1.0809 ± 0.332
HRCT chest	**Patients with ILD**	12 (48%)	24 (96%)
	Pleural involvement	7 (28%)	3 (12%)
	Pulmonary artery diameter (cm)	2.48 ± 0.77	2.72 ± 0.84
	Right ventricular diameter (cm)	3.60 ± 0.87	3.80 ± 0.76

SLE: Systemic lupus erythematosus, **SSc:** Systemic sclerosis, **SLEDAI:** systemic lupus erythematosus disease activity index, **EscSG:** European scleroderma study group, **6MWT:** 6-minute walk test, **PH:** pulmonary hypertension, **MPAP:** mean pulmonary artery pressure, **PSPAP:** peak systolic pulmonary artery pressure, **RVSP:** right ventricular systolic pressure, **PAT:** pulmonary acceleration time, **IVS:** inter-ventricular septum, **PWT:** posterior wall thickness, **LVIDd:** left ventricular internal dimension in diastole, **LVIDs:** left ventricular internal dimension in systole, **EF:** ejection fraction, **Aos:** aortic diameter in systole, **Aod:** aortic diameter in diastole, **ASI:** aortic stiffness index, **FVC**: forced vital capacity, **DLCO:** diffusion capacity of carbon monoxide, **HRCT:** high resolution computed tomography, **ILD**: interstitial lung disease.

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Table 2: Comparison of the serum anti-endothelin-1 receptor type A antibodies in the studied patients and controls

Parameter mean ± SD and/or n (%)	**SLE n=25**	**SSc n=25**	**PAH/PH n=25**	**Control n= 25**	**P value**
Anti-ET1RA (positive)	4 (16%)	6 (24%)	18 (72%)	0 (0%)	**<0.0001**
Anti-ET1RA level((U/ml)	10.68 ± 3.27	11.56 ± 4.01	10.56 ± 1.27	8.29 ± 2.31	**0.001**

Table 3: Comparison between patients with and without PH in both SLE and SSc.

Parameter mean ± SD and/or n(%)	SLE with PH n=7	SLEwithout PH n=18	P value	SSc with PH n=6	SSc without PH n=19	P value
Disease duration (year)	8.28 ± 5.93	6.61 ± 3.32	0.376	11 ± 4.09	7.26 ± 3.63	**0.043**
Age (year)	31.71 ± 10.78	36.17 ± 8.71	0.294	45.33 ± 12.42	40.26 ± 10.85	0.344
Gender Female	7 (100%)	18 (100%)	---	6 (100%)	16 (84.21%)	0.299
Acute cutaneous lupus	1 (14.29%)	10 (55.56%)	0.062		--------	
Chronic cutaneous lupus	5 (71.43%)	5 (27.78%)	**0.045**			
Oral ulcers	6 (85.71%)	13 (72.22%)	0.478			
Skin tightness limited		--------		2 (33.33%)	7 (36.84%)	0.876
MRSS				22.16 ± 5.98	19.05±6.99	0.337
Digital pitting scars				6 (100%)	16 (84.21%)	0.299
Digital ulcers				5 (83.33%)	6 (31.58%)	**0.026**
Raynaud's	6 (85.71%)	4 (22.22%)	**0.004**	6 (100%)	18 (94.74%)	0.566
Vasculitic lesions	2 (28.57%)	3 (16.67%)	0.504		--------	
Telangiectasia	0 (0%)	0 (0%)	---	6 (100%)	3 (15.79%)	**<0.0001**
Arthritis	3 (42.86%)	8 (44.44%)	0.943	3 (50%)	8 (42.11%)	0.734
Myalgia	2 (28.57%)	8 (44.44%)	0.467	3 (50%)	10 (52.63%)	0.910
Palpitation	3 (42.86%)	1 (5.56%)	**0.022**	0 (0%)	5 (26.32%)	0.160
Dyspnea	6 (85.71%)	9 (50%)	0.102	5 (83.33%)	17 (89.47%)	0.687
Pre-syncope	1 (14.29%)	0 (0%)	0.102	1 (16.67%)	0 (0%)	0.069
Cough	4 (57.14%)	8 (44.44%)	0.568	6 (100%)	18 (94.74%)	0.566
Expectorations	4 (57.14%)	5 (27.78%)	0.170	2 (33.33%)	4 (21.05%)	0.539
Pleuritic chest pain	3 (42.86%)	3 (16.67%)	0.169	1 (16.67%)	2 (10.53%)	0.687
Dry mouth	5 (71.43%)	2 (11.11%)	**0.003**	3(50%)	4 (21.05%)	0.169
Dry eye	3 (42.86%)	2 (11.11%)	0.075	4 (66.67%)	5 (26.32%)	0.073
SLEDAI 2k score	11±4.65	10.33±6.71	0.812		-------	
EscSG score		--------		2.58 ± 1.68	2.76 ± 1.64	0.818
6MWT distance	233.57 ± 86.68	303.61± 43.58	**0.012** 0.091	133.33± 34.73	256.84 ± 58.33	**0.0001** **0.004**
O2 saturation-pre	98.28 ± 0.48	98.66 ± 0.48	**<0.0001**	97.5 ± 0.54	98.47 ± 0.69	**0.001**
O2 saturation-post	95.14 ± .89	97.55 ±		94.16 ± 0.75	96.73 ± 1.62	

		0.51				
MPAP (mmHg)	28.35 ± 2.07	13.92 ± 2.86	**<0.0001**	29.43 ± 2.52	15.47 ± 4.58	**<0.0001**
RVSP (mmHg)	39.39 ± 2.51	24.80 ± 4.10	**<0.0001**	40.07 ± 2.28	24.93 ± 5.22	**<0.0001**
PAT (mSec)	99.42 ± 3.35	122.72 ± 4.62	**<0.0001**	97.66 ± 4.03	120.21 ± 7.38	**<0.0001**
Pericardial effusion	2 (28.57%)	0 (0%)	**0.018**	2 (33.33%)	2 (10.53%)	0.184
FVC %	85.57 ± 10.39	88.77 ± 14.55	0.6015	72.16 ± 5.19	68.26 ± 17.17	0.593
DLCO %	57.85 ± 18.21	84.94 ± 9.39	**0.0001**	46 ± 8.22	74.36 ± 16.38	**0.0005**
FVC/DLCO %	1.54 ± 0.25	1.04 ± 0.12	**<0.0001**	1.60 ± 0.24	0.91 ± 0.11	**<0.0001**
Pleural Involvement in CT	4 (57.14%)	3 (16.67%)	**0.043**	1 (16.67%)	2 (10.53%)	0.687
Anti-ET1RA (positive)	4 (57.14 %)	0 (0%)	**<0.0001**	5 (83.33%)	1 (5.26%)	**<0.0001**
Anti-ET1RA level (U/ml)	13.87 ± 4.46	9.44 ± 1.51	**0.001**	17.52 ± 3.93	9.68 ± 1.24	**<0.0001**

Table 4: Comparison between patients with different pulmonary hypertension (PH)/PAH entities and patients with SLE-PAH & SSc-PAH.

Parameters mean ± SD and/or n (%)	**PAH/PH entities (n=25)**		**SLE-PAH & SSc-PAH (n=13)**		**P value**
Clinical diagnosis	**COPD**	15 (60%)	**SLE**	**7 (53.8%)**	--
	IPAH	7 (28)	**SSc**	**6 (46.2%)**	
	CTEPH	3 (12%)			
Age (year)	43.12 ± 7.29		38 ± 13.12		0.129
Gender Female	20 (80%)		13 (100%)		0.084
Anti-ET1RA (positive)	18 (72%)		9 (69.3%)		
	COPD	12 (48%)	**SLE**	4 (30.8%)	0.858
	IPAH	6 (24%)	**SSc**	5 (38.5%)	
	CTEPH	0 (0%)			
Anti-ET1RA titer (U/ml)	10.56 ± 1.27		15.56 ± 4.47		**<0.0001**
MPAP (mmHg)	31.63 ± 3.57		28.85 ± 2.26		**0.015**
RVSP (mmHg)	51.08 ± 7.70		39.71 ± 2.34		**<0.0001**
PAT (mSec)	94.12 ± 5.77		98.61 ± 3.64		**0.015**

discrimination between CTD-PAH (SSc-PAH and SLE-PAH) and non–CTD-PAH, the sensitivity was 62% with a specificity of 100% (AUC= 0.720) **(Figure 2)**. All differences of these groups were highly significant (p< 0.0001).

Regarding sensitivity and specificity tests, the results of ROC analysis for anti-ET1RA antibodies are shown in **Table 5**. The sensitivity of anti-ET1RA antibodies to discriminate between SSc-PAH and SLE-PAH was 83% with a specificity of 71% (AUC= 0.7143) **(Figure 1)**. As regards the

ISSN:0975-3583,0976-2833 VOL12, ISSUE 07, 2021

Table 5: ROC analysis for anti-ET1RA antibodies to discriminate between different PAH/PH entities

PAH/PH entities	Sensitivity	Specificity	AUC
SSc-PAH vs. SLE-PAH	83%	71%	0.7143
CTD-PAH vs. non–CTD-PAH	62%	100%	0.720

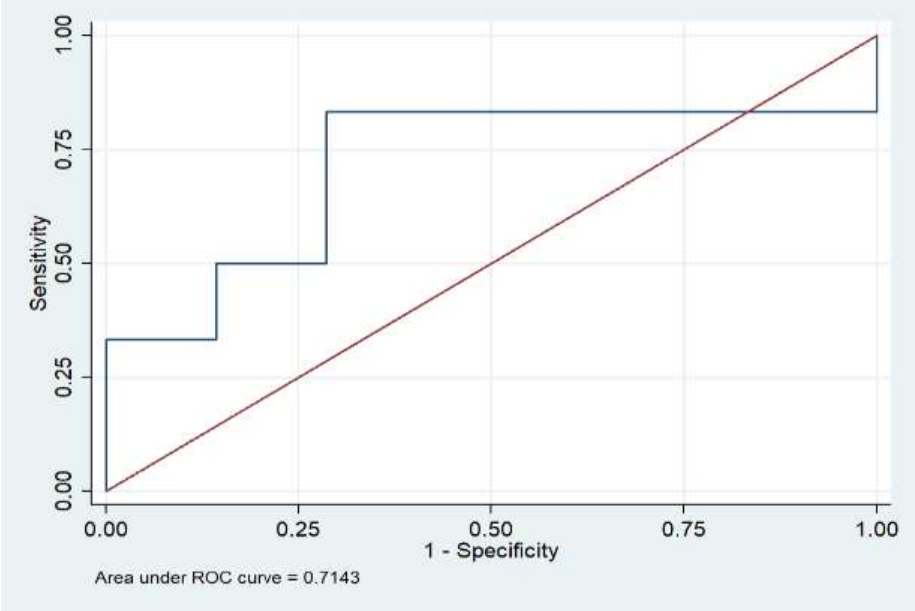

Figure 1: ROC curve analysis for anti-ET1RA antibodies to discriminate between SSc-PAH vs. SLE-PAH

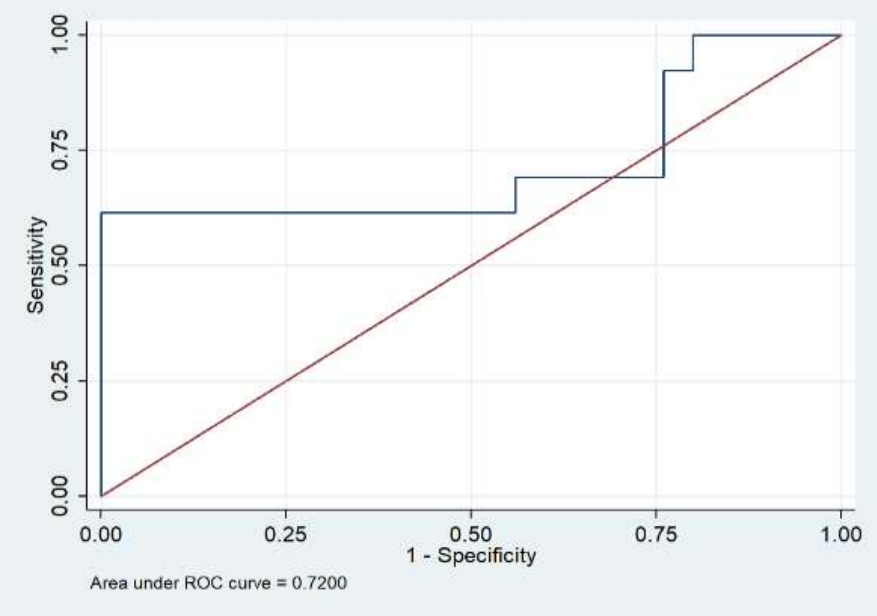

Figure 2: ROC curve analysis for anti-ET1RA antibodies to discriminate between CTD-PAH vs. non–CTD-PAH

The best-calculated cut-off for anti-endothelin-1 receptor type A antibodies titer to predict pulmonary hypertension was also obtained by ROC analysis (**Figure 3)**. The area under the curve for this measure was 0.863 (p< 0.0001). ROC curve analysis set an optimal cut-off at 10.39 U/ml for the detection of anti-ET1RA antibodies that had a sensitivity of 73.7% and a specificity of 97.3% for the development of PAH in patients of SLE, SSc, and other PAH/PH entities. The positive predictive value was 96.43%, with a negative predictive value of 76.60% **(Table 6).**

Regarding ROC analysis in SLE and SSc patients, a cut-off at 10.36 U/ml for anti-ET1RA antibodies had a sensitivity of 87 % and a specificity of 85 % (AUC 0.9150) to detect PAH compared to controls. A slightly higher cut-off (10.44 U/ml) was needed for the detection of PH in other PAH/PH entities (100% sensitivity and 72% specificity) (AUC 0.8752).

Table 6: Sensitivity and specificity tests for anti-ET1RA titer for PAH prediction in SLE patients, SSc patients, and other PAH/PH entities.

Sensitivity	73.7%
Specificity	97.3%
Positive predictive value (95%CI)	96.43% (92.23% - 100.63%)
Negative predictive value (95%CI)	76.60% (67.01% - 86.18%)

CI: Confidence interval

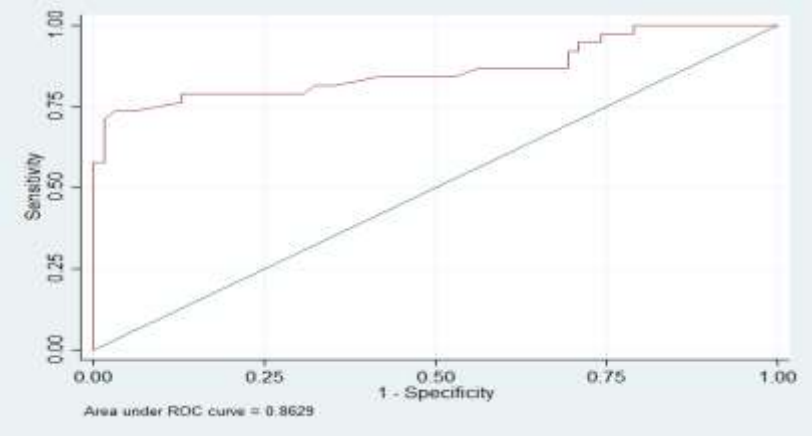

Figure 3: ROC curve for anti-ET1RA titer to predict PAH SLE, SSc patients, and other PAH/PH entities

no more variables could be removed without significantly altering the model. Among all the risk factors included that showed significance in univariate analysis (6MWT distance, O2 saturation post-6MWT, DLCO%, RV diameter), ET1RA antibodies were found to be the only independent predictor for PAH in patients with SLE and SSc (95% CI; 0.34-10.96) (p= 0.037).

The risk of PAH was estimated using non-adjusted and fully binary logistic regression models. All risk factors considered significant based on the univariable regression analyses were entered into initial multivariable logistic regression models (**Table 7)**. The models were streamlined by eliminating each non-significant variable and/or those causing the least impact on significance. This process was repeated until

Table 7: Logistic regression analysis for risk of PAH

Variable	β ± SE	95% CI	P value¥	β ± SE	95% CI	P value*
Age	0.03±0.03	-0.03-0.10	0.295	-0.11±0.10	-0.31-0.09	0.288
6MWT distance (m)	-0.02±0.01	-0.03 -0.01	**0.001**	0.02 ±0.02	-0.01- 0.05	0.251
O2 saturation post-6MWT	1.20±0.32	-1.83-0.57	**<0.0001**	-1.92±1.18	-4.23-0.39	0.104
DLCO%	-0.12±0.04	-0.19 -0.05	**0.001**	0.11± 0.09	-0.07-0.28	0.231
RV diameter (cm)	1.80±0.55	0.73-2.87	**0.001**	4.39 ±2.24	-0.01-8.79	0.051
ET1RA positivity	4.39±1.18	2.08-6.70	**<0.0001**	5.65±2.71	0.34-10.96	**0.037**

(β) Beta coefficient, (SE) Standard error, (CI) Confidence interval, significant P value< 0.05
¥ Unadjusted, *Fully adjusted, **6MWT:** 6-minute walk test, **DLCO:** diffusion capacity of carbon monoxide, **RV:** Right ventricular, **ET1RA:** endothelin-1 receptor type A antibodies.

Discussion:

Pulmonary arterial hypertension was more frequent in systemic lupus patients (28%) than in systemic sclerosis patients (24%). This finding concorded with other registries **(22-24),** which found that CTD-PAH patients were more likely to have SLE as the underlying CTD than SSc. On the other hand, other registers (mainly Western cohorts) **(25, 26)** found that SSc-PAH comprised most of the CTD-PAH population**.** This difference may result from the different prevalence of connective tissue disease between countries, study populations, nature of the study, sample size, and diagnostic methods used.

Regarding the demographics, patients with SLE-PAH were younger (31.71±10.78 years) than those with SSc-PAH (45.33 ± 12.42 years). This finding agreed with other studies **(24, 27***)* that found younger age in patients with SLE-PAH compared to SSc-PAH. In our study, both SLE and SSc patients with PAH had a longer disease duration (8.28 ± 2.24 years and 11 ± 4.09 years) than those without PAH (6.61 ± 0.78 years and 7.26 ± 3.63 years), but no significant difference was found regarding gender and the mean age. In concordance, other studies had reported significantly longer disease duration in SLE patients with PAH than those without PAH **(28, 29).**

Comparing the clinical findings in SLE patients with and without PAH in our study, a significant difference was detected regarding the presence of Raynaud's phenomenon in patients with SLE-PAH compared to SLE patients without PAH (P= 0.004). This finding was in concordance with the study by Lian et al. and Kasparian et al., who found a significant increase in the presence of Raynaud's phenomenon in SLE-PAH group and considered it as one of the predictors for PAH development in SLE **(30, 31**). The link

between pulmonary hypertension (PH) and Raynaud's phenomenon can be explained by the vasospasm characteristic of Raynaud's phenomenon. This phenomenon represents cutaneous vasospasm, which might manifest as a systemic vascular disorder. This systemic disorder can result in pulmonary arterial vasoconstriction, raising pulmonary vascular resistance and ultimately causing pulmonary hypertension (32).

This study found another significant difference regarding sicca manifestation (dry mouth) between SLE patients with and without PAH (P=0.003). This was also in agreement with other studies that detected an increased prevalence of pulmonary diseases, including pulmonary artery hypertension, in lupus patients with sicca manifestations compared to those without associated sicca manifestations. They suggested that the pathogenesis was almost related to associated anti-Ro/SS-A antibodies as a mediator of endothelial injury resulting in PH **(33, 34).**

As regards the clinical findings in SSc patients with and without PAH, a significant difference was found regarding the presence of digital ulcers and telangiectasia in the PAH group, but patients of both groups had increased prevalence of Raynaud's phenomenon (RP) with no significant difference detected. These findings have also been reported by Huang et al., who found a significantly increased prevalence of digital ulcers and telangiectasias in patients with SSc-PAH than those without. They also reported the absence of significant differences in the presence of Raynaud's phenomenon between both groups **(29)**. Telangiectasias and digital ulcers are considered microvascular lesions, and their pathogenesis (a non-inflammatory vasculopathy) is similar to that of PAH in SSc. Hence, the association between these symptoms and the presence of PAH is biologically plausible. Regarding Raynaud's phenomenon, the absence of significant difference between the PAH and non-PAH groups was perhaps due to the high prevalence of Raynaud's phenomenon in both groups. Previous studies in SSc patients have suggested that the severity of Raynaud's phenomenon is positively associated with the likelihood of PAH **(35)**.

No significant difference was found in comparing the clinical manifestations suggestive of pulmonary arterial hypertension (dyspnea, chest pain, syncope) between patients with and without PAH in both SLE and SSc. This was reported previously by many reviews, which confirmed that reliance on the development of symptoms suggestive of PH in CTD is insensitive for the early diagnosis of PAH and is often confounded by other manifestations of CTD. More severe symptoms such as syncope/pre-syncope and oedema only occur after extensive pulmonary vasculopathy has developed, so echocardiographic screening for early detection of PH in SLE and SSc patients is a must **(36-38).**

Regarding the disease activity score in SLE patients, no significant difference was found in the mean SLEDAI-2k score between SLE patients with and without PH (p= 0.812). A negative correlation was reported between disease activity scores (SLEDAI 2k) in SLE patients with MPAP; this correlation didn't reach a significant level. This reflects that PH is not related to disease activity in SLE and

ISSN:0975-3583,0976-2833 VOL12, ISSUE 07, 2021

that patients with high pulmonary artery pressure may have low disease activity and confirms the importance of echocardiographic screening of PH even in those with low disease activity.

Other studies also reported that the SLEDAI score did not differ significantly between the PH and non-PH groups. They confirmed that pulmonary hypertension in patients with SLE may occur even when non-pulmonary disease activity was quiescent **(39-2).**

In contrast to our results, the studies by Troncoso et al. and Mirfeizi et al. detected that PH in SLE was significantly associated with higher SLEDAI scores and higher SLEDAI scores in patients who had elevated pulmonary artery pressure rates **(43, 44).** These discrepancies are attributed to genetic factors, the age distribution, sample size, the nature of the study, a diagnostic measure used for PH detection, variable disease duration, and associated organ involvement in SLE patients enrolled in each study. Consequently, further research is necessary to investigate the mechanisms involved in this issue.

Regarding systemic sclerosis patients in our study, no significant difference was found in the mean EscSG score between those with and without PAH. A positive correlation was found in SSc patients between disease activity score (EscSG) and MPAP, but this correlation didn't reach a significant level (p= 0.762).

In agreement with our results, other studies found no significant correlation between EScSG scoring and the presence of pulmonary hypertension. They concluded that the EScSG activity index might not reflect sufficiently the pulmonary vascular involvement and the lung-related disease activity in systemic sclerosis **(45, 46).**

In this study, comparing functional assessment of dyspnea in SLE and SSc patients between those with and without PH revealed a significant difference in the mean distance at which the patient stopped the six-minute walk test and the mean oxygen saturation assessed after the test. In concordance with our results, the studies by Gadre et al. and Mirfeizi et al. also reported a statistically significant decrease in distance walked in a 6-minute test and saturation of oxygen in those with PH. They concluded that 6MWT is a sensitive tool for predicting SSc-PH and should be used to predict its clinical outcomes **(47, 48).**

In contrast to our results, other studies found that the 6MWT lacks specificity as an outcome measure in SSc-PH and SLE-PAH **(49, 50).** They concluded that the 6MWD relates to broad factors that raise doubts about the specificity of the 6MWD for assessing specific organ damage and cannot solely be used to evaluate PAH. The differences between these studies and our results were almost due to variations in the disease activity in the studied population and the variability in associated organ affection. Most of our study population with PH had a low disease activity, so the decrease in exercise capacity is not related to those confounding factors. Many reviews concluded that the 6MWT should be considered a standardized part of the CTD PAH evaluation because of its ease, non-invasive nature, and low cost. However, 6MWT interpretation should consider vascular and musculoskeletal exercise limitations **(51, 52).**

Regarding the echocardiographic assessment measures in SLE patients with and without PH, a significant difference in pericardial effusion was found in SLE patients with and without PH (p= 0.018). This finding agreed with other studies that found a significant difference in the prevalence of pericarditis in SLE patients with PH than those without PH **(39, 53).**

As regards echocardiographic assessment measures in patients of other PH/PAH entities [15 (COPD), 7 (IPAH) and 3 (CTEPH)], their pulmonary artery pressure was found to be significantly higher than that in patients of SLE-PAH & SSc-PAH (p< 0.0001). In concordance with our results, the study by Becker et al. reported that mPAP was significantly lower in CTD-PAH than IPAH and CTEPH (p= 0.029) **(8).**

In this study, lupus and systemic sclerosis patients with PH had a significantly lower diffusion capacity of carbon monoxide (DLCO) and higher mean FVC/DLCO ratio than those without PH. The FVC% was low in both SSc patients with and without PH; no significant difference was found between both groups. This decrease in FVC% reflects lung parenchymal affection (which is prevalent in SSc with and without PH in our study) rather than reflecting pulmonary vascular affection. So, our results agree that the decrease in DLCO% is the most specific pulmonary function for early detection of PAH, especially in the absence of marked parenchymal affection (high FVC/DLCO ratio).

In concordance with our results, many studies reported this significantly lower DLCO% and higher FVC/DLCO ratio in the PAH group compared to the non-PH group in their studies **(54-56).**

In this study, anti-ET1RA was positive in 4 (57.14 %) of SLE-PAH (7 patients) with no positive results in the non-SLE-PAH group (p< 0.0001). There was a significant difference in the mean value of its level between both groups (p= 0.001). The serum level of these antibodies was significantly positively correlated with RVSP and MPAP (p< 0.0001) and negatively with PAT (p< 0.0001).

These results align with the research conducted by Guo et al., demonstrating a higher occurrence of ET1RA autoantibodies in SLE-associated PAH compared to SLE patients without PAH. Their study revealed a notable correlation between ET1RA autoantibody levels and pulmonary artery systolic pressure measured through echocardiography. Guo and colleagues identified ET1RA autoantibodies as a relevant mechanistic biomarker in SLE, potentially contributing to the development of PAH in SLE patients (10).

Regarding anti-ET1RA in systemic sclerosis patients of our study, it was more prevalent than in patients with SLE-PAH (5 (83.33%)). The mean anti-ET1RA level was significantly higher in the SSc-PAH group than in the SSc non-PAH group. In concordance with our results, the study by Riemekasten et al. also reported that patients with SSc had higher levels of anti-ET1RA autoantibodies compared with other studied groups (RA, Primary Sjögren syndrome, PRP, Morphea, Control). Patients with high anti-ET1RA autoantibody levels were more likely

to develop disease complications such as pulmonary hypertension **(9).**

In this study, serum anti-endothelin receptor antibodies in patients with pulmonary hypertension of other entities were positive in 18 (72%) [12 (48%) COPD patients, 6 (24%) IPAH patients, no positive results were found in CTEPH patients]. When we compared the mean anti-ET1RA titer between this group and SLE-PAH & SSc-PAH, it was significantly higher in SLE and SSc PAH patients (P< 0.0001). This difference may reflect the pathogenic role of these autoantibodies as a mediator of vascular endothelial reactivity and pulmonary vasculopathy in connective tissue disease associated with PAH, which had been reported in previous studies **(8,57).**

The best-calculated cut-off for ET1RA antibodies titer to detect pulmonary hypertension obtained by ROC analysis in our study in patients of SLE, SSc, and PAH/PH entities was at 10.39 U/ml. This cut-off value matches the reference range used in our study (9.20- 10.40 U/ml). Regarding ROC analysis in SLE and SSc patients, a cut-off at 10.36 U/ml for anti-ET1RA antibodies had a sensitivity of 87 % and a specificity of 85 % (AUC 0.9150) to detect PAH. A slightly higher cut-off (10.44 U/ml) was needed for the detection of PH in patients with other PAH/PH entities compared to controls (100% sensitivity and 72% specificity) (AUC 0.8752).

The study by Riemekasten et al. found that a cut-off value of 10.4 U/ml was optimal for the detection of anti-ET1RA antibodies in SSc patients compared to controls (83.7% sensitivity and 77% specificity); in disagreement with our results, they found that a higher cut off at 15.74 U/ml was optimal for the diagnosis of PAH in SSc patients (79% sensitivity and 73% specificity) **(9).** This difference may be due to variability in the number of included SSc patients (298 patients) and their race, the method used to detect pulmonary artery pressure (right heart catheterization). Further research on this issue will be required, including more patients.

In this study, anti-ET1RA antibodies were also found to differentiate between different PAH/PH entities. These findings were also reported before in the study by Becker et al., who found that anti-ET1RA antibodies had a sensitivity of (70.0%) and specificity of (82.4%) (AUC; 0.754) to discriminate between non–SSc-PAH vs. SSc-PAH. The sensitivity and specificity of these antibodies to differentiate between CTD-PAH vs. non–CTD-PAH were (72.5% and 78.1%) respectively (AUC= 0.786) **(8).**

As regards the predictive role of anti-ET1RA antibodies, it was estimated by calculating the predictive values using the ROC curve analysis (PPV= 96.43%, NPV= 76.60%) and by assessing the risk of PAH using non-adjusted and fully binary logistic regression models. Anti-ET1RA antibodies were found to be the only independent predictor for PAH in patients with SLE and SSc (95% CI; 0.34-10.96) (p= 0.037). This predictive role agreed with the prospective analysis by Becker et al., who reported that anti-ET1RA Abs predict the development of SSc-PAH, and high levels of these antibodies can predict SSc-PAH–related mortality. They concluded that anti-ET1RA antibodies are predictive and prognostic biomarkers in SSc-

PAH **(8).** In disagreement with our results, the study by Guo et al. found that anti-ET1RA autoantibodies performed moderately in terms of PAH predictive value in SLE patients (PPV= 71%, NPV= 59.5%) **(10).** The difference between these studies could probably be due to study populations, sample size, underlying CTD, and method used to detect pulmonary artery pressure in each study. Therefore, further research is required to determine the cause of variation regarding this issue.

We concluded that anti-endothelin-1 receptor type A antibodies are detected in SLE and SSc patients with pulmonary hypertension. They may serve as a biomarker for pulmonary hypertension prediction and could be included in laboratory assessment of lupus and systemic sclerosis patients who were suspected to have PH.

Consent for publication: Not applicable.

Competing interests: Not applicable.

Source of funding: Not applicable

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