Title: Increased serum levels of adhesion molecules ICAM-1 and VCAM-1 in systemic sclerosis are not specific for pulmonary manifestations

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Abstract

Introduction

Studies suggest elevated serum ICAM-1 (intercellular adhesion molecule-1) and VCAM-1 (vascular cell adhesion molecule-1) levels may be markers of pulmonary arterial hypertension in systemic sclerosis (SSc-PAH). We sought to evaluate whether ICAM-1 and VCAM-1 levels are useful screening biomarkers for incident SSc-PAH.

Methods

In this cross-sectional study, four groups were selected from the Australian Scleroderma Cohort Study: Group 1 (*n* =15) had definite PAH; Group 2 (*n* =19) had interstitial lung disease (ILD); Group 3 (*n* =30) were SSc controls; Group 4 (*n* =34) were healthy controls. Serum ICAM-1 and VCAM-1 levels were measured using the Millipore Milliplex MAP Human 2-Plex Panel.

Results

There were no differences in ICAM-1 levels in the PAH versus ILD group (263.0 ± 85.4 vs 380.4 ± 168.3ng/mL, p =0.136), SSc-controls (263.0 ± 85.4 vs 253.1 ± 98.0ng/mL, p =1.00), or healthy controls (263.0 ± 85.4 vs 201.8 ± 57.2ng/mL, p =0.093). Similarly, there were no differences in VCAM-1 level in PAH versus ILD groups (1476.2 ± 434.9 vs 1424.8 ± 527.6ng/mL, p =1.00) and SSc-controls (1476.2 ± 434.9 vs 1409.5 ± 341.1ng/mL, p =1.00). SSc subjects had significantly higher levels of ICAM-1 (297.4 ± 134.0ng/mL vs 201.8 ± 57.2ng/mL, p <0.0001) and VCAM-1 compared to healthy controls (1432.7 ± 427.4ng/mL vs 1125.6 ± 273.4ng/mL, p <0.0001).

Conclusion

Neither ICAM-1 nor VCAM-1 is a specific screening biomarker of SSc-PAH. Instead, increased levels of these adhesion molecules in SSc, irrespective of pulmonary complications, suggest that they may play a role in SSc pathogenesis.

Introduction

Systemic sclerosis (SSc) is a multi-system connective tissue disease characterised by autoimmunity, vasculopathy and fibrosis of the skin and a number of internal organs [1-3]. SSc related pulmonary arterial hypertension (SSc-PAH) remains a leading cause of morbidity and mortality in this population despite improvements in survival associated with screening programs and combination goal-orientated treatment strategies [4,5]. The early detection of SSc-PAH is the cornerstone of optimal disease management; however, current screening programs perform better when the PAH is advanced, and by the time PAH is confirmed by right heart catheterisation (RHC), more than 50% of the pulmonary microcirculation is already occluded [6,7]. This has prompted interest as to whether select biomarkers may better identify those who will develop SSc-PAH, and may even be involved mechanistically in the development of PAH.

Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) have been reported to be elevated in SSc-PAH [8]. ICAM-1 and VCAM-1 are adhesion molecules that are induced by pro-inflammatory cytokines such as IL-1β and TNF-α, and initiate the binding of monocytes to activated and damaged endothelial cells [9,10]. Monocytes can then facilitate the co-stimulation and transmigration of inflammatory cells into the extracellular matrix and contribute to dysregulated angiogenesis [9,10]. When overexpressed, these adhesion molecules can be detected in a circulating soluble form, and are considered markers of underlying endothelial activity and damage. Studies of skin fibroblasts in SSc subjects show an upregulation in ICAM-1 and VCAM-1 expression [11-13]. Rabquer *et al.* demonstrated that ICAM-1 expression by dermal SSc fibroblasts is induced by a number of the cytokines important in SSc pathogenesis (TNF-α, IFN-γ, IL-1β and IL-17) [14]. Similarly, VCAM-1 expression can be induced by TNF-α in a dose-dependent manner [14]. ICAM-1 and VCAM-1 expression have also been shown to correlate with SSc disease activity and severity [15-17].

Recent studies have suggested that ICAM-1 and VCAM-1 may be important in SSc-PAH. Pendergrass *et al.* showed significantly higher levels of ICAM-1 gene expression in limited SSc subjects with PAH compared to healthy controls ($p \leq 0.05$); however, no significant differences were seen comparing ICAM-1 gene expression between limited SSc subjects with and without PAH [8]. VCAM-1 gene expression was similar in the SSc and healthy controls; however, higher circulating levels of VCAM-1 were observed in limited SSc subjects with

PAH, compared to limited SSc subjects without PAH and healthy controls [8]. Given the role of IL-1β and TNF-α in inducing ICAM-1 and VCAM-1, Pendergrass *et al.* suggested a role for activated dendritic cells and macrophages in the pathogenesis of SSc-PAH [8,14,18,19].

In this study, we evaluated whether a single measured level of soluble adhesion molecules ICAM-1 and VCAM-1 is useful as a screening biomarker for the detection of incident SSc-PAH. As in our previous studies of biomarkers [20], we also included well-characterised subjects with SSc related interstitial lung disease (SSc-ILD), SSc subjects without cardiopulmonary complications and healthy normal controls to evaluate if any differences observed between groups were specific to SSc-PAH.

Materials and methods

Study design and population

Subjects were selected from the Australian Scleroderma Cohort (ASCS). The ASCS is a multicentre study of risk and prognostic factors for cardiopulmonary outcomes in SSc. The ASCS is approved by human research ethics committees of the participating Australian centres led by St. Vincent's Hospital Melbourne, and subjects provide written consent at recruitment. All patients, except those in the healthy control group, fulfil 2013 EULAR/ACR classification criteria for SSc [21].

In this case-control study, ICAM-1 and VCAM-1 were analysed and compared among four groups of subjects comprising SSc-PAH, SSc-ILD, SSc-controls and healthy controls. The SSc-PAH group comprised 15 consecutive subjects with RHC-confirmed SSc-PAH, based on a mean pulmonary artery pressure (mPAP) ≥25mmHg and pulmonary capillary wedge pressure (PCWP) ≤15mmHg. These subjects had no more than minor changes of ILD on high-resolution CT (HRCT).

The SSc-ILD group (n =19) comprised subjects with significant SSc-ILD, defined as moderate or severe changes of ILD on HRCT, with a forced vital capacity (FVC) <85% predicted, without evidence of significant PAH (either a RHC showing a mPAP <25mmHg and PCWP ≤15mmHg, and/or systolic PAP estimated by Doppler transthoracic echocardiography (TTE) ($sPAP_{TTE}$) <36mmHg).

The SSc-control group (*n* =30) comprised subjects who did not have evidence of cardiopulmonary complications, based on $sPAP_{TTE}$ <30mmHg, normal myocardial function on TTE, diffusing capacity for carbon monoxide corrected for haemoglobin ($DLCO_{corr}$) >70% predicted, forced expiratory volume in 1 second (FEV1, Litres)/ forced vital capacity (FVC, Litres) percentage predicted >0.7, no ILD on HRCT (and in those without an HRCT, FVC ≥80% predicted), and WHO Functional Class I or II.

The healthy control group (*n* =34) comprised normal healthy individuals without evidence of autoimmune disease, or significant known cardiovascular, respiratory or renal disease.

Exclusion criteria for all groups included the presence of abnormal left ventricular systolic or diastolic function for age measured at TTE, abnormal left atrial size, an unrecordable tricuspid regurgitant Doppler signal and estimated glomerular filtration rate (eGFR) <30ml/min. This was to remove the influence of potential confounders such as significant left ventricular and renal dysfunction on ICAM-1 and VCAM-1 levels, Furthermore, all SSc subjects required a recordable $sPAP_{TTE}$, in order to accurately risk stratify those subjects in the non-PAH group who had not undergone RHC.

Cardiac and pulmonary assessments

Left ventricular systolic and diastolic function was determined by two-dimensional TTE. Systolic PAP was estimated by Doppler TTE ($sPAP_{TTE}$) at rest, based on peak velocity of the tricuspid regurgitant jet and estimation of right atrial pressure of 5-10mmHg based on the diameter and respiratory variation of the inferior vena cava. Pulmonary involvement was assessed by a pulmonary function test (PFT) and/or HRCT within three months of serum collection. HRCTs were reported as no, mild, moderate or severe ILD by a radiologist. All $DLCO_{corr}$ (ml/mmHg/min) values are reported as percentage of predicted values, corrected for haemoglobin. All FEV1 (Litres), FVC (Litres) and $FVC/DLCO_{corr}$ values are reported as percentage predicted for sex, race and height.

Serum samples and measurement of ICAM-1 and VCAM-1

All sera were collected from subjects within three months of their annual clinical assessment and cardiopulmonary investigations. All SSc-PAH subjects had serum collected at the time of their RHC and prior to the commencement of advanced pulmonary vasodilator therapy. Blood samples were collected at rest into tubes containing EDTA. Samples were centrifuged and stored at -80°C until used. Levels of ICAM-1 and VCAM-1 were measured in duplicate

using the commercially available Human Multi-Analyte Profiling (MAP) multiplexed immunoassays (Milliplex, Mullipore, Billerica, MA, USA).

Statistical analysis

ICAM-1 and VCAM-1 levels in the SSc-PAH group were compared with the SSc-ILD, SSc-control and healthy control groups using analysis of variance (ANOVA) with Bonferroni multiple test comparison corrections. ICAM-1 and VCAM-1 levels were appropriately transformed to satisfy assumptions of normality and homogeneity of variance. Simple, and multiple linear regression, along with logistic regression was used to determine the correlation of the adhesion molecules with various clinical and laboratory characteristics of the scleroderma subjects. The correlations between transformed ICAM-1 and VCAM-1 levels and RHC measures of cardiopulmonary function were quantified using the Pearson correlation coefficient. All statistical analyses were performed using STATA software (Statacorp, College Station, TX, USA).

Results

Characteristics of the study population

There was no significant difference in the age of the healthy control group (51.2 ± 12.9 years) compared to the SSc-control group (p =0.395). The clinical and investigative characteristics of the subjects with SSc are summarised in Table 1 and Table 2. Compared to the SSc-control group, the SSc-PAH group was significantly older at the time of diagnosis (62.1 ± 10.9 vs 48.7 ± 10.1, p <0.003) and had a longer disease-duration (defined from time of first non-Raynaud's disease manifestation; p =0.002). As expected, the SSc-ILD group had a higher percentage of subjects with diffuse cutaneous SSc (68%) and with anti-scl70 antibodies (53%). There were 14 (74%) subjects in this group that had ever been treated with cyclophosphamide

Comparison of ICAM-1 and VCAM-1 levels between groups

There was no significant difference between ICAM-1 levels in the SSc-PAH group versus SSc-ILD (263.0 ± 85.4 vs 380.4 ± 168.3ng/mL, p =0.136), SSc-PAH group versus SSc-control group (263.0 ± 85.4 vs 253.1 ± 98.0ng/mL, p =1.00), or SSc-PAH group verses the healthy control group (263.0 ± 85.4 vs 201.8 ± 57.2ng/mL, p =0.093; Figure 1). The SSc-ILD group had significantly higher ICAM-1 levels compared to the SSc-control group (380.4 ±

168.3 vs 253.1 ± 98.0ng/mL, p =0.011) and the healthy control group (380.4 ± 168.3ng/mL vs 201.8 ± 57.2, p <0.001; Figure 1).

VCAM-1 was significantly higher in the SSc-PAH group compared to the healthy control group (1476.2 ± 434.9 vs 1125.6 ± 273.4ng/mL, p =0.038; Figure 2). There was no significant difference in VCAM-1 levels between the SSc-PAH and SSc-ILD groups (1476.2 ± 434.9 vs 1424.8 ± 527.6ng/mL, p =1.00) or SSc-PAH and SSc-control groups (1476.2 ± 434.9 vs 1409.5 ± 341.1ng/mL, p =1.00; Figure 2). VCAM-1 levels were higher in the SSc-control group compared to the healthy control group (1409.5 ± 341.1 vs 1125.57 ± 273.4ng/mL, p =0.031; Figure 2). VCAM-1 levels did not differ significantly between the three clinical scleroderma groups (SSc-PAH, SSc-ILD and SSc-control group)

Comparison of ICAM-1 and VCAM-1 levels in SSc subjects to healthy controls

Scleroderma subjects showed significantly higher levels of ICAM-1 (297.4 ± 134.0ng/mL vs 201.8 ± 57.2ng/mL, p <0.001) and VCAM-1 levels compared to the healthy control group (1432.7 ± 427.4ng/mL vs 1125.6 ± 273.4ng/mL, p <0.001). These results remained significant when SSc subjects without cardiopulmonary complications (SSc-control group) and the healthy controls were compared (ICAM-1: 253.1 ± 98.0 vs 201.8 ± 57.2ng/mL, p =0.04; VCAM-1: 1409.5 ± 341.1 vs 1125.6 ± 273.4ng/mL, p =0.001).

Clinical correlates of ICAM-1 and VCAM-1 levels in SSc subjects

Clinical and laboratory characteristics of the SSc subjects were evaluated to see if any specific parameters correlated with ICAM-1 and VCAM-1 levels. There were no significant correlations between ICAM-1 levels and age (p =0.163), disease duration (p =0.960), modified Rodnan skin score (MRSS) (p =0.508), disease subtype (limited or diffuse; p =0.689), erythrocyte sedimentation rate (ESR; p =0.628) or C-reactive protein (CRP; p =0.697). In univariate logistic regression analysis, anti-centromere antibody was associated with lower ICAM-1 levels (OR 0.07, 95% CI: 0.012 - 0.464), with trends towards higher ICAM-1 levels in subjects with the diffuse cutaneous subtype (OR 3.87, 95% CI: 0.88 - 16.88, p =0.072). There were no significant associations between ICAM-1 levels and the presence of Raynaud's phenomenon, digital ulcers or calcium channel blocker use, renal dysfunction, or the presence of anti-scl70 antibodies (all p >0.10).

There were no correlations between VCAM-1 levels and age (p =0.516), disease duration (p =0.082), MRSS (p =0.212), disease subtype, ESR (p =0.535) or CRP (p =0.837). Similarly,

univariable logistic regression models showed no relationship between VCAM-1 levels and the presence of Raynaud's phenomenon, digital ulcers, calcium channel blocker use, renal dysfunction, autoantibodies (anti-centromere and anti-scl-70) and disease subtype (all p >0.10).

Correlation of ICAM-1 and VCAM-1 with markers of SSc PAH severity

The correlation of ICAM-1 and VCAM-1 with markers of PAH severity was evaluated. ICAM-1 levels significantly correlated with mean pulmonary artery pressure (mPAP) at RHC (r =0.54, p =0.038), but not with mean right atrial pressure (mRAP) (r =0.21, p =0.462) or pulmonary vascular resistance (PVR) (r =0.49, p =0.109). Further, treating mPAP as a dichotomous variable, higher ICAM-1 levels were noted in those subjects with a mPAP ≥40mmHg (315.2 ± 95.8 vs 228.2 ± 60.3ng/mL, p =0.049). Of the non-invasive measures, ICAM-1 correlated best with $sPAP_{TTE}$ (r =0.64, p =0.010), but not DLCO (r =-0.07, p =0.801) or 6MWD (r =-0.36, p =0.205).

VCAM-1 levels showed a non-statistically significant correlation with mPAP (r =0.45, p =0.091), but not with mRAP (r =0.02, p =0.934) or PVR (r =0.40, p =0.198). However, on dichotomising mPAP to cut points ≥40 and <40mmHg, VCAM-1 levels were not significantly higher (1666.0 ± 445.8 vs 1349.7 ± 402.1ng/mL, p =0.176) in those with a higher mPAP.

Correlation of ICAM-1 and VCAM-1 with markers of SSc-ILD

In the SSc-ILD group, there was a trend towards the correlation of ICAM-1 levels and DLCO levels (r =-0.45, p =0.080). This relationship was not significant in a logistic regression model that included a current or past history of smoking (OR 1.46, 95% CI: 0.75 - 2.81, p =0.357). Further, ICAM-1 levels did not correlate with FVC, as either a continuous (r =0.04, p =0.884) or categorical variable (FVC ≥75 vs <75, p =0.760). ICAM-1 levels did not differ between subjects who had or had not previously received cyclophosphamide therapy (p =0.365).

There were no significant correlations between VCAM-1 levels and FVC (r =0.03, p =0.910), DLCO (r =0.04, p =0.896), or $sPAP_{TTE}$ (r =0.18, p =0.532). There were significantly lower levels of VCAM-1 in subjects who had been treated with cyclophosphamide (1137.2 ± 335.1 vs 2000.2 ± 321.6ng/mL, p <0.001).

Discussion

Higher circulating levels of ICAM-1 and VCAM-1 were observed in SSc subjects compared to healthy controls, but were not specific for the pulmonary complications of SSc-PAH; a significant positive correlation between ICAM-1 level and mPAP at RHC was also demonstrated.

These results differ from those of Pendergrass *et al.*, in which higher circulating levels of ICAM-1 and VCAM-1, and increased ICAM-1 gene expression, were found in subjects with limited SSc with PAH versus controls [8]. There are a number of potential reasons for these differences. Firstly, all the SSc-PAH cases included in our study were 'incident' and treatment naïve. In contrast, the study by Pendergrass *et al.* included a number of the study participants already commenced on advanced pulmonary vasodilator therapies, potentially introducing the effect therapies and clinical response may have on serum ICAM-1 and VCAM-1 levels. Iannone *et al.* previously reported that bosentan reduced levels of the adhesion molecules in 10 subjects with SSc-PAH treated for 12 months [22]. However, Sfikakis *et al.* found no alterations in ICAM-1 level with lower dose bosentan [23]. Studies on the effect of intravenous iloprost on soluble adhesion molecules for the therapy of Raynaud's phenomenon in SSc subjects showed decreased ICAM-1 and/or VCAM-1 levels with iloprost therapy [24,25]. A study that measures ICAM-1 and VCAM-1 levels pre- and post-initiation of therapy is required to elucidate the impacts of therapy on the levels of these adhesion markers. This was not possible in the current study as data was not available for drug usage within three months of serum draw. Secondly, all subjects in our study were diagnosed in the context of a screening program that may have selected for subjects with a milder form of the disease; unfortunately, data relating to functional class and pulmonary haemodynamics were not provided in the study by Pendergrass *et al.* Thirdly, Pendergrass *et al.* included subjects with extensive ILD in the 'PAH' group which may have influenced ICAM-1 levels given that the highest absolute ICAM-1 levels were observed in the ILD group. Lastly, Pendergrass *et al.* included some subjects in the PAH group with a PCWP ≥15mmHg, albeit it after careful adjudication; current RHC definitions of PAH require a PCWP <15mmHg, and a raised PCWP potentially includes subjects with WHO Group 2 pulmonary hypertension due to left heart disease [26].

A significant positive correlation was observed between ICAM-1 and mPAP, with the highest ICAM-1 levels noted in those with an mPAP ≥40mmHg. These findings have not previously been reported in SSc-PAH, although Sungprem *et al.* found a significant linear correlation between ICAM-1 levels and mPAP in children with PAH complicating congenital heart disease [27]. ICAM-1 and VCAM-1 are thought to mediate vascular inflammation by initiating the binding of monocytes to the endothelium, leading to the adhesion and extravasation of inflammatory cells [9,10]. Gene expression studies in limited SSc-PAH subjects have suggested a stable increase in expression of the ICAM-1 gene over the course of approximately one year [8]. This may be due to increases in the flow pulsatility of distal pulmonary arteries, as can be seen in PAH [28]. While the exact role of SSc specific autoantibodies in SSc-PAH remains uncertain, an *in vitro* study that included SSc subjects demonstrated that autoantibodies against U1-RNP and dsDNA could lead to an upregulation of ICAM-1 in pulmonary artery endothelial cells, together with other adhesion molecules and class II MHC molecules. Taken together, these results raise the possibility that ICAM-1 may be involved in the pathogenesis of SSc-PAH, and thereby act as a biomarker of PAH disease progression or severity.

Higher levels of circulating ICAM-1 and VCAM-1 were observed in SSc subjects, irrespective of cardiopulmonary complications. This is consistent with previous studies showing the over-expression of ICAM-1 and VCAM-1 in SSc skin and serum [11-13,29-31]. In other studies, ICAM-1 expression has been shown to be inducible by TNFα, IFN-Y, IL-β, and IL-17, whilst VCAM-1 expression is highly inducible by TNF-α [14,18,19]. These adhesion molecules also functionally mediate myeloid cell adhesion in SSc skin [14]. In bleomycin-induced SSc mouse models, ICAM-1 has also been shown to contribute to skin and lung fibrosis [32]. The highest overall levels of adhesion molecules have been observed in the early inflammatory stage of SSc, and in subjects with rapid disease progression [29]. In this study, the lowest ICAM-1 levels were seen in those subjects with the anti-centromere antibody, with trends towards higher levels in subjects with diffuse cutaneous skin involvement. Denton *et al.* showed the correlation of VCAM-1, but not ICAM-1, with clinical progression [15]. Overall, it appears likely that adhesion molecules are involved in the processes of endothelial cell injury and extracellular matrix deposition that characterises the pathogenesis of SSc.

Even though the highest ICAM-1 levels were found in the SSc-ILD group, there were no significant differences in the adhesion molecules in SSc-ILD versus SSc groups without ILD.

Furthermore, there was no relationship between ICAM-1 and FVC as a marker of ILD severity. Similarly, there were no relationships between VCAM-1 and FVC, consistent with the findings of Alzawawy *et al.* who reported no correlation in VCAM-1 levels in subjects with mild versus moderate-severe ILD on CT imaging [33]. Therefore, unlike in idiopathic pulmonary fibrosis wherein raised ICAM-1 and ICAM-2 have been demonstrated, raised ICAM-1 levels did not appear to be specific for SSc-ILD [34].

Interestingly, we demonstrated lower levels of VCAM-1 in subjects who had previously received cyclophosphamide for SSc-ILD. Unfortunately the cross-sectional design of this study did not enable an assessment of pre- and post-treatment VCAM-1 levels, ILD severity, or clinical response, which limits the inferences we can draw from this finding. Whilst relatively little is published in the scleroderma literature, in literature concerning idiopathic pulmonary fibrosis, elevated VCAM-1 levels have been associated with increased mortality that is thought to be mediated through TGF-β fibroblast proliferation [35,36]. Studies using cyclophosphamide have suggested that treatment might boost angiogenesis through the normalisation of the endothelial cell-matrix interactions, reduction of endothelial cell apoptosis and rebalance of dysregulated angiostatic factors [37]. VCAM-1 is typically expressed by activated endothelial cells in response to inflammatory cytokines, and cyclophosphamide is known to both modulate lymphocyte function and suppress inflammation. In this way, it can be postulated that CYC assists in the healing of vascular endothelial cells, and thereby reduces VCAM-1 levels [38].

The strength of this study was that all SSc-PAH cases were newly diagnosed, treatment naïve, and diagnosed according to internationally accepted RHC criteria. Furthermore, the study design built on our previous studies by having well characterised non-PAH SSc and non-SSc groups, and via the comprehensive collection of clinical parameters we were also able to adjust for a large number of the relevant clinical variables and confounders in SSc.

However, the main limitation of this study was the small size of the study-group and the cross sectional study design. In order to better understand the role of these molecules in SSc subjects with cardiopulmonary complications, the serial measurement of ICAM-1 and VCAM-1 levels in subjects at risk of, or evolving PAH or ILD may provide utility. Alternately, the measurement of adhesion molecules across the pulmonary circulation, by simultaneously assaying pulmonary-arterial and systemic arterial blood concentrations at RHC, looking for a ‘step-up’ of adhesion molecule levels across the pulmonary circulation may provide useful information. In SSc-ILD, comparing serum levels of adhesion molecules

with those obtained from broncho-alveolar lavage, and correlating with ILD severity on PFT and HRCT may have a role.

Other limitations of this study included lack of measurement of ICAM-1 and VCAM-1 gene expression levels, and lack testing to see whether there is a correlation between these adhesion molecules and pro-fibrotic and pro-inflammatory cytokines such as TGF-β, IL1, IL6 and TNF. Future *in vitro* experiments are also necessary to confirm findings at a cellular level in skin or lung fibroblasts.

The findings from this study indicate that neither ICAM-1 nor VCAM-1 can be regarded as a specific screening peripheral blood biomarker of SSc-PAH. Instead, increased levels of these adhesion molecules in SSc, irrespective of pulmonary complications, suggest that they may play a role in the pathogenesis of SSc.

Figure captions

Fig. 1 Comparison of ICAM-1 levels between SSc. clinical groups. Boxplot with whiskers extending to 1.5 IQR below the lower quartile and 1.5 IQR above the upper quartile. Outliers represented by circles. ICAM-1: soluble intercellular adhesion molecule-1, SSc: systemic sclerosis; PAH: pulmonary arterial hypertension; ILD: interstitial lung disease

Fig. 2 Comparison of VCAM-1 levels between SSc. clinical groups. Boxplot with whiskers extending to 1.5 IQR below the lower quartile and 1.5 IQR above the upper quartile. VCAM-1: soluble vascular cell adhesion molecule-1, SSc: systemic sclerosis; PAH: pulmonary arterial hypertension; ILD: interstitial lung disease

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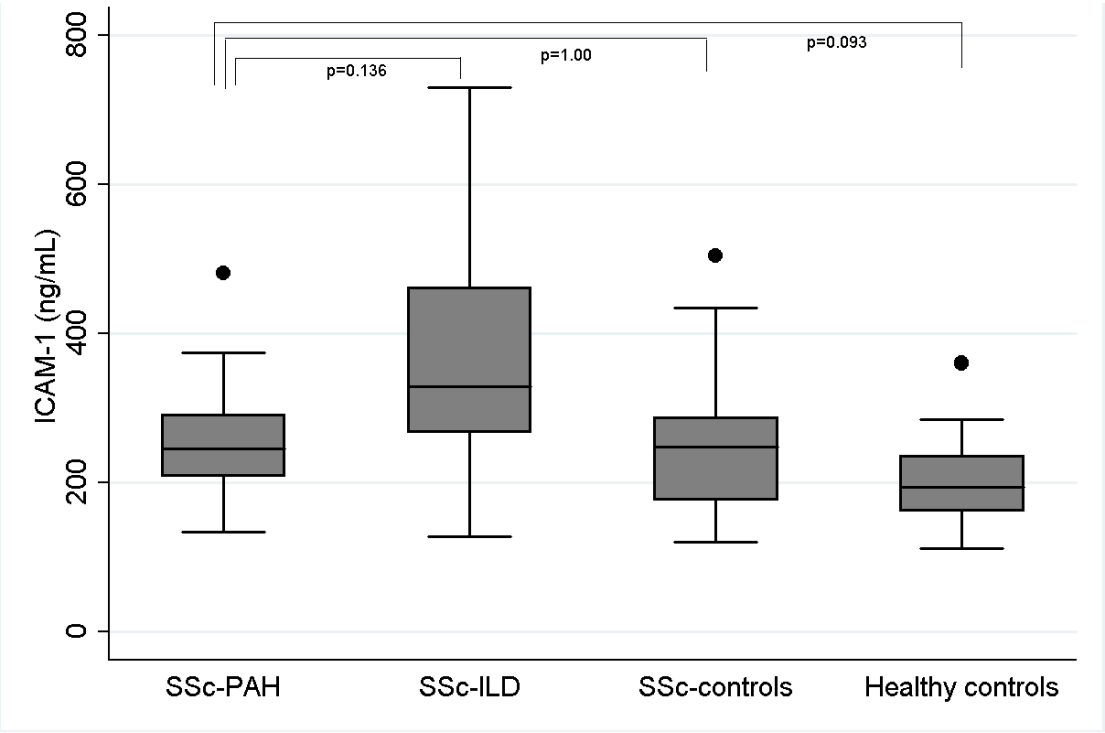
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200
400
600
800
SSc-PAH
SSc-ILD
SSc-controls
Healthy controls

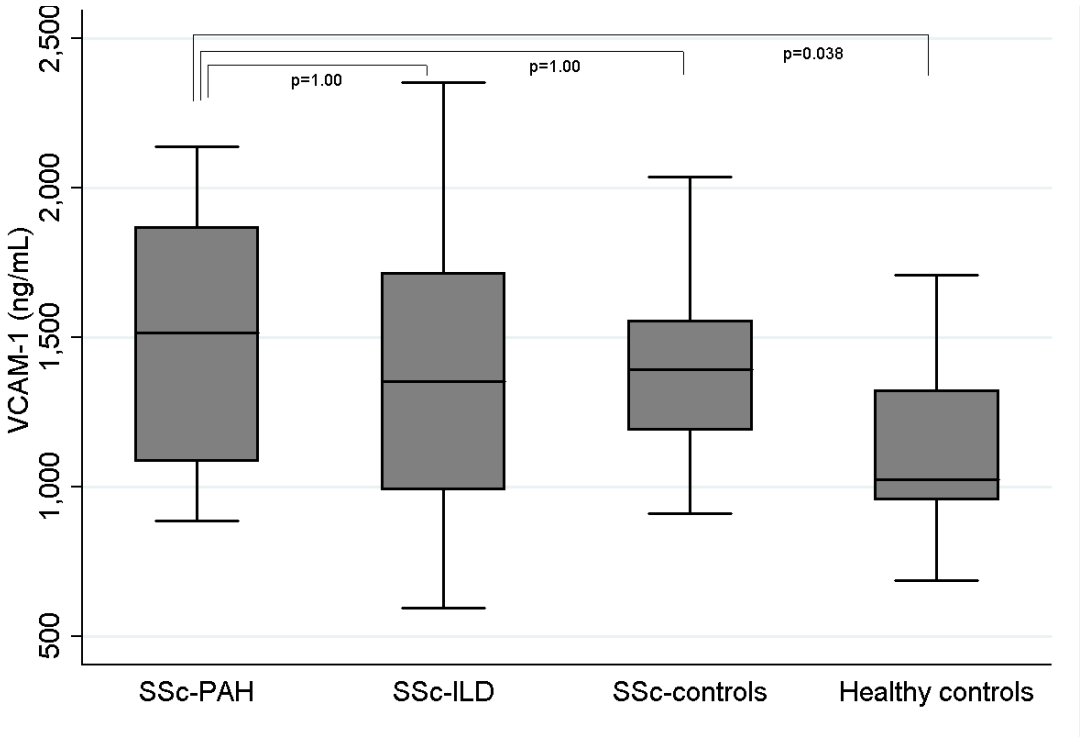
VCAM-1 (ng/mL)
500
1,000
1,500
2,000
2,500
p=1.00
p=1.00
p=0.038
SSc-PAH
SSc-ILD
SSc-controls
Healthy controls

Table 1 Comparison of clinical characteristics of subjects in SSc. clinical groups

Characteristics	SSc-PAH	SSc-ILD	SSc-control	p-value
Number, *n*	15	19	30	-
Age at onset, years	44.5 ± 12.9	40.3 ± 15.6	40.6 ± 13.2	0.620
Age at study, years	62.1 ± 10.9	51.1 ± 12.7	48.7 ± 10.1	<0.001
Disease duration, years	19.2 ± 12.4	10.8 ± 7.9	7.8 ± 7.2	0.002
Female, *n* (%)	14 (93)	14 (74)	30 (100)	0.015
Disease subtype				
Limited, *n*	13	6	23	0.001
Diffuse, *n*	2	13	7	
ANA, *n*	14	17	30	0.217
Anti-Scl70, *n*	1	10	5	0.002
Anti-cent, *n*	7	0	16	<0.001
MRSS	10.4 ± 12.1	12.4 ± 8.5	7.6 ± 7.5	0.214

SSc: systemic sclerosis; PAH: pulmonary arterial hypertension; ILD: interstitial lung disease; ANA: anti-nuclear antibody; Anti-Scl70: anti-topoisomerase-1 antibody; Anti-cent: anti-centromere antibody; MRSS: modified Rodnan skin score

Table 2 Comparison of investigation parameters among subjects in SSc. clinical groups

Investigations	SSc-PAH	SSc-ILD	SSc-control	p-value
TTE parameters				
TRV (m/s)	3.8 ± 0.7	2.5 ± 0.4	2.20 ± 0.2	<0.001
sPAP (mmHg)	65.8 ± 27.3	32.1 ± 5.3	26.3 ± 2.6	<0.001
RHC results				
mPAP (mmHg)	40.2 ± 12.5	-	-	-
mRAP (mmHg)	10.1 ± 3.1	-	-	-
PVR (wood units)	6.2 ± 3.4	-	-	-
PFT				
FVC (% pred)	75.5 ± 24.3	67.4 ± 14.3	102.8 ± 13.4	<0.001
$DLCO_{corr}$ (% pred)	45.6 ± 11.7	49.9 ± 11.7	86.8 ± 13.0	<0.001
$FVC/DLCO_{corr}$	1.76 ± 0.38	1.46 ± 0.31	1.20 ± 0.20	<0.001
6MWD (m)	337 ± 100	467.7 ± 84.4	520 ± 48.6	<0.001
C-reactive protein (mg/L)	8.6 ± 9.7	8.4 ± 13.8	5.9 ± 10.0	0.642
ESR (mm/hr)	24.7 ± 13.3	22.5 ± 14.0	12.4 ± 14.5	0.010^

SSc: systemic sclerosis; PAH: pulmonary arterial hypertension; ILD: interstitial lung disease; TTE: transthoracic echocardiography; TRV: tricuspid regurgitant velocity; sPAP: systolic pulmonary artery pressure; RHC: right heart catherisation; mPAP: mean pulmonary artery pressure; mRAP: mean right atrial pressure; PVR: pulmonary vascular resistance; PFT: pulmonary function test; FVC: forced vital capacity (% predicted); DLCO: diffusion capacity of lung for carbon monoxide (% predicted); 6MWD: six minute walk distance; ESR: erythrocyte sedimentation rate

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