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Research Article

Cell Adhesion Molecules in Systemic Sclerosis – Results from a Portuguese Cohort

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Abstract

Objectives: To determine serum levels of adhesion molecules among patients with Systemic Sclerosis (SSc) spectrum disease and to assess the relationship of these molecules with disease subsets, evolution phase, organ involvement and nail fold capillaroscopic changes.

Methods: E-selectin, P-selectin, Intercellular Adhesion Molecule 1 (ICAM-1) and Vascular Cell Adhesion Molecule 1 (VCAM-1) serum levels were measured by ELISA in a cohort of 48 patients with SSc spectrum disease. The association between these molecules and disease subsets, clinical evolution phase, organ involvement and nail fold capillaroscopic changes was then assessed.

Results: Comparing patients with healthy controls, patients had higher serum levels of E-selectin, P-selectin and VCAM-1 and lower levels of ICAM-1. This pattern was found in all SSc subsets. Increased serum levels of P-selectin and VCAM-1 were found in all disease evolution phases, while E-selectin was significantly higher only in the late SSc. ICAM-1 was significantly lower in intermediate and late disease phases. P-selectin and VCAM-1 were significantly increased, while ICAM-1 was significantly decreased in all nail fold capillaroscopy patterns. E-selectin was elevated only in the late pattern. Serum ICAM-1 levels were higher among patients with lung and heart involvement and VCAM-1 levels were significantly higher in patients with osteoarticular involvement.

Conclusion: We found higher serum levels of P-selectin, E-selectin and VCAM-1 and lower levels of ICAM-1 in SSc spectrum disease patients. P-selectin and VCAM-1 were early and persistent disease markers throughout the course of disease.ICAM-1 might be implicated in the pathogenesis of heart and lung involvement.

INTRODUCTION

Systemic Sclerosis (SSc) is a connective tissue disease with unclear aetiology and pathogenesis, characterized by microvascular changes and immunologic abnormalities leading to fibrosis of the skin and internal organs [1]. Histopathologic hallmarks of early SSc stage are endothelial damage and per vascular inflammatory infiltration with an accumulation of lymphocytes [2].

Molecules known to mediate leucocyte adhesion include selectins and integrins: selectins, namely E-selectin and P-selectin, promote the contact of lymphocyte with endothelial cells; Integrins, help lymphocyte attachment to blood vessels including vascular cell adhesion molecule-1 (VCAM-1) and intercellular cell adhesion molecule-1 (ICAM-1), which facilitate leukocyte endothelial transmigration [3].VCAM-1 (CD106) is expressed on endothelial and epithelial cells, dendritic cells and macrophages [4] and allows the evaluation of the degree of endothelial injury and activity [5]. ICAM-1 (CD54) is constitutively expressed on endothelial and epithelial cells and fibroblasts, and is induced by pro-inflammatory cytokines [6].

Among selectins, E-selectin (CD62E) is specifically expressed by activated endothelial cells, whereas P-selectin (CD62P) is also expressed in platelets [6]. A major difference between them is the time required for their expression: P-selectin is quickly mobilized to the surface of endothelium or platelets, while E-selectin expression is induced by inflammatory cytokines, several hours later [6].

Although there are several studies suggesting that cell adhesion molecules play a major role in SSc pathogenesis [1,2,7,8], and organ involvement [5,7-11] and only a few have studied the association of these molecules with disease subsets, disease phases [3,12] and capillaroscopic changes [13,14].

The aim of this study was to determine the concentrations of circulating VCAM-1, ICAM-1, and E- and P-selectins in patients with SSc and to correlate these values with disease subsets, evolution phase, type of organ involvement and nail fold capillaroscopic changes.

PATIENTS AND METHODS

Study approval

The study was approved by the Ethics Committee and the Board of Directors of the Hospital and all patients signed an informed consent form.

Patients

Between September 2010 and March 2011, sixty-one patients were consecutively selected from a 190-patient-population with SSc, at the Clinical Immunology Unit of Hospital Geral de Santo António, Centro Hospitalar do Porto, Portugal. Four patients were later excluded - three due to overlapping pathologies (mixed connective tissue disease, infection with human immunodeficiency virus, B non-Hodgkin lymphoma) and one for not attending the blood sampling procedure. From the remaining 57 patients, 48 were included in this study (in 9 cases there was no sample available for adhesion molecules measurement).

Thirty-nine patients fulfilled the American College of Rheumatology (ACR) criteria for SSc, while the remaining nine did not present skin involvement and were therefore diagnosed as Pre-Scleroderma, as explained in clinical assessment. The same group of individuals has also met the ACR/EULAR (The European League against Rheumatism) criteria of 2013 [15], as well as 1 of the 9 patients who had no skin involvement due to digital ulcers. However for statistical analysis purposes, this last patient was included in the group of that whodid not present skin involvement. Patients' characteristics are summarized in Tables 1 and 2. Twenty-five healthy individuals were used as controls.

Clinical assessment

Patients were classified as having Pre-Scleroderma (Pre-SSc), limited cutaneous SSc (lcSSc) or diffuse cutaneous SSc (dcSSc) [16].

Numerous definitions for the early stage of disease (in which there is no skin involvement) have been proposed, namely "early scleroderma", "very early scleroderma", "pre-scleroderma" or "limited scleroderma" [17-20]. In this study, we have used the Le Roy and Medsger definition of Pre-Scleroderma, where Raynaud plus scleroderma-type nail fold capillary changes and/or scleroderma-type auto antibodies must be present [17]. Although there is no skin involvement, this stage may evolve to limited or diffuse SSc.

Patients with lcSSc were subdivided in CREST (calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia) and non-CREST groups, according to Lonzetti et al. [21].

Patients that fulfilled the criteria for SSc where divided according to the evolution time of the first disease-related symptom, into "early", "intermediate" and "late SSc" [22]. Among patients with lcSSc, early phase was defined has having less than

Table 1: SSc patients' characteristics (n=48).

Age (years) *Median (range)*	56 (19-80)
Sex *Male: Female*	2:46
Disease subset ***n (%)***	
Pre-SSc	9 (18.8%)
Diffuse cutaneous SSc	12 (25.0%)
Limited cutaneous SSc	27 (56.2%)
CREST	12 (25.0%)
Non-CREST	15 (31.2%)
Disease phase ***n (%)***	
Early	6 (12.5%)
Intermediate	10 (20.8%)
Late	23 (47.9%)
Nailfold capillaroscopic pattern ***n (%)***	
Normal/minor alterations	4 (8.3%)
Early	11 (22.9%)
Active	19 (39.6%)
Late	14 (29.2%)

Abbreviations: SSc, Scleroderma; CREST, Calcinosis; Raynaud Phenomenon; Esophageal Dysmotility; Sclerodactyly; Telangiectasia.

Table 2: Organ involvement according to Medsger Severity Scale*.

ORGAN	SCORE	Pre-SSc (n = 9)	Limited SSc (n = 27)	Diffuse SSc (n = 12)
Skin	0	9 (100)	-	-
	1	-	27 (100)	3 (23.1)
	2	-	-	8 (66.7)
	3	-	-	1 (8.3)
Peripheral	0	4 (44.4)	-	-
Vascular	1	4 (44.4)	15 (55.6)	1 (8.3)
	2	-	2 (7.4)	4 (33.3)
	3	1 (11.1)	10 (37.0)	7 (58.3)
	4	-	-	-
Gastrointestinal	Non-classified	2 (22.2)	1 (3.7)	1 (8.3)
	0	5 (55.6)	16 (59.3)	5 (22.7)
	1	2 (22.2)	10 (37.0)	6 (50.0)
Lung	Non-classified	1 (11.1)	-	-
	0	5 (55.6)	10 (37.0)	2 (16.7)
	1	3 (33.3)	8 (29.6)	6 (50.0)
	2	-	8 (29.6)	1 (8.3)
	3	-	1 (3.7)	1 (8.3)
	4	-	-	2 (16.7)
Heart	Non-classified	3 (33.3)	3 (11.1)	1 (8.3)
	0	5 (55.6)	15 (55.6)	7 (58.3)
	1	1 (11.1)	1 (3.7)	2 (16.7)
	2	-	4 (14.8)	2 (16.7)
	3	-	4 (14.8)	-
Osteoarticular	Non-classified	-	-	-
	0	7 (77.8)	19 (70.4)	11 (91.7)
	1	2 (22.2)	8 (29.6)	1 (8.3)

Results presented as n (%). Abbreviations: SSc, Scleroderma. * From "0" (no documented involvement or without need of treatment, e.g. Raynaud) to "4" (end stage disease)

five years of evolution, while late phase was defined as having ten years or more of evolution. The correspondent cut-off values to define early and late disease phases for dcSSc patients were three and six years, respectively. Intermediate phase of both limited and diffuse SSc ranged between the two numeric values.

We used Medsger´s severity scale where the involvement of each one of the nine organs (general, peripheral vascular, skin, joints/tendons, muscles, gastrointestinal (GI) tract, lungs, heart and kidneys) may range from 0 (with no documented involvement or with no need for treatment, e.g. Raynaud) to 4 (end-stage disease).This tool presents some limitations as a measure of activity due to its lack of sensitivity in the presence of SSc severity improvement [23]. Nevertheless, this is a quite useful instrument as a prognostic measure [24].

At the time of study enrolment, a complete clinical profile was established for each patient and the degree of organ involvement was assessed with medical history, physical examination and complementary tests. Organ involvement was evaluated using the Medsger's scale cut-off point, except for osteoarticular evaluation, since it does not value joint involvement but only the distance between thumb and thinner eminence, which was not systematically evaluated in our patients. The Modified Rodnan Skin Score was the tool chosen for evaluating skin involvement [25]. Joint evaluation was performed through physical exam and complemented by image exams (radiography and/ or joint echography) whenever justified. DAS 28 (*Disease Activity Score Calculator for Rheumatoid Arthritis*) was used to classify the activity of this involvement [26]. Muscular involvement was evaluated by physical examination and complemented by biochemical study (creatine phosphokinase, aldolase and glutamate oxaloacetate transaminase). Gastrointestinal tract involvement was assessed during upper gastrointestinal endoscopy, esophageal manometry. Among symptomatic patients, the assessment was made using gastric scintigraphy. Involvement of the lower GI tract was evaluated using barium tests, glucose hydrogen breath test, ultrasound, and colonoscopy and anorectal manometry, whenever justified.

Chest teleradiography, high resolution computed tomography, spirometry, diffusing capacity of the lungs for carbon monoxide (DLCO) corrected for hemoglobin and alveolar volume, and two-dimensional echocardiogram with evaluation of pulmonary artery systolic pressure (PASP) were used to evaluate pulmonary involvement. PASP was considered abnormal when greater than 35 mmHg. Right heart catheterization was performed in patients with PASP above or equal to 40 mmHg in order to confirm pulmonary hypertension. Forced vital capacity (FVC) and DLCO were considered abnormal when lower than 80%. Patients with lung fibrosis and/or alveolitis affecting at least the lung bases were considered to have major pulmonary alterations; criteria for minor alterations included septal hypertrophy and limited zones of fibrosis and/or ground glass opacities. Cardiac conduction disturbances and/or arrhythmias were detected by electrocardiogram and/or Holter, and systolic dysfunction was evaluated with two-dimensional echocardiogram, namely through the calculation of the left ventricular ejection fraction. Finally, a score introduced by Cutolo et al. [27], was used to classify nail fold microvascular changes as observed by capillaroscopy (early, active and late capillaroscopic patterns of microvascular damage).

Laboratory analysis

Blood samples were collected in plain Vacutainer tubes and serum a liquots were frozen at -40ºC before analysis. Serum concentrations of soluble VCAM-1 (IBL International; Ref BE59051), soluble ICAM-1 (IBL International; Ref BE59011), soluble P-selectin (IBL International; Ref BE59081) and soluble E-selectin (IBL International; Ref BE59061) were measured using ELISA assays (IBL International, Hamburg, Germany), in accordance with manufacturer instructions.

Statistical analysis

Statistical analysis was performed using the IBM Statistical Package for Social Sciences (SPSS 20.0) software (SPSS Inc, IL, and U.S.A.). Data are presented as mean and standard deviation. Kolmogorov–Smirnov test was used to evaluate sample normality distribution. All studied variables presented a non-normal distribution. Non-parametric tests were used for single comparisons of continuous variables between two (Mann Whitney U test) or three (Kruskal-Wallis test) groups. Spearman's correlation coefficient (r_s) was used to evaluate the association between two continuous variables.

Regarding the relationship between Medsger's severity scale and adhesion molecules serum levels, despite this scale having a sufficient number of points in order to be used to compute correlation coefficients, patients exhibited a very diverse number of different scores (from 2 to 4); therefore, for each organ we compared patients at the lowest score (0 or 1) with those presenting higher scores. The null hypothesis was rejected when $p<0.05$.

RESULTS

Serum levels of cell adhesion molecules were highly variable among patients and controls (Table 3). Considering groups combined (patients and controls), positive associations were found between P-selectin and E-selectin and between P-selectin and VCAM, while a negative association was observed for P-selectin and ICAM-1. However, when considering controls and patients separately, the association between P-selectin and E-selectin was significant among controls but not among patients. In contrast, the association between P-selectin and VCAM-1 has reached statistical significance among patients but not among controls. The association between P-selectin and ICAM-1 was non-significant in both groups (Figure 1). No statistically significant associations were observed between the serum levels of the three referred molecules and age ($p>0.050$ for all correlations; data not shown).

Comparing study sample individuals with healthy controls, highly significant differences between both groups were found for serum levels of P-selectin, E-selectin, VCAM-1 and ICAM-1 (Table 3). For the first three molecules, patients had higher levels comparing with controls, while ICAM-1 serum levels were lower inpatients (Table 3).

P-selectin and VCAM-1 serum levels were significantly higher in Pre-SSc, lcSSc and dcSSc patients in comparison with

Table 3: VCAM-1, ICAM-1, E-selectin and P-selectin levels in study population and healthy controls, and according to Systemic Sclerosis subsets.

	VCAM-1 (ng/ml)	p*	ICAM-1 (ng/ml)	p*	E-selectin (pg/ml)	p*	P-selectin (pg/ml)	p*
Healthy controls (n=40)	833 (112-3143)		337 (158-536)		43 (19-72)		100 (50-228)	
Study population (n=48)	1469 (167-11000)	**<0.001**	205 (130-697)	**<0.001**	55 (21-90)	**0.012**	181 (123-610)	**<0.001**
Pre-SSc (n=9)	962 (595-2856)	**0.035**	205 (144-344)	**0.004**	55 (36-68)	**0.050**	172 (143-216)	**<0.001**
SSc (n=39)	1472 (167-11000)	**<0.001**	218 (130-697)	**<0.001**	54 (21-90)	**0.028**	188 (123-610)	**<0.001**
lcSSc (n=27)	1684 (237-11000)	**<0.001**	**193 (130-697)**	**<0.001**	53 (21-90)	**0.074**	190 (123-610)	**<0.001**
dcSSc (n=12)	1123 (167-4147)	**0.019**	238 (143-381)	**0.028**	57 (23-75)	**0.065**	175 (126-262)	**<0.001**
lcSSc / non-CREST (n=15)	1823 (237-11000)	**0.002**	177 (130-526)	**0.001**	48 (27-84)	0.205	190 (130-610)	**<0.001**
lcSSc / CREST (n=12)	1469 (363-5565)	**0.003**	234 (155-697)	0.056	54 (21-90)	0.113	186 (123-242)	**<0.001**

Results presented as median (range) values. p* values: Patients vs. Controls (Mann-Whitney U test).
Abbreviations: SSc, Scleroderma; dcSSc, diffuse cutaneous Scleroderma; lcSSC, limited cutaneous Scleroderma; CREST, Calcinosis, Raynaud phenomenon, Esophageal dysmotility, Sclerodactyly, and Telangiectasia; VCAM-1, Vascular Cell Adhesion Molecule-1; ICAM-1, Intercellular Cell Adhesion Molecule-1. One patient missing for ICAM-1.

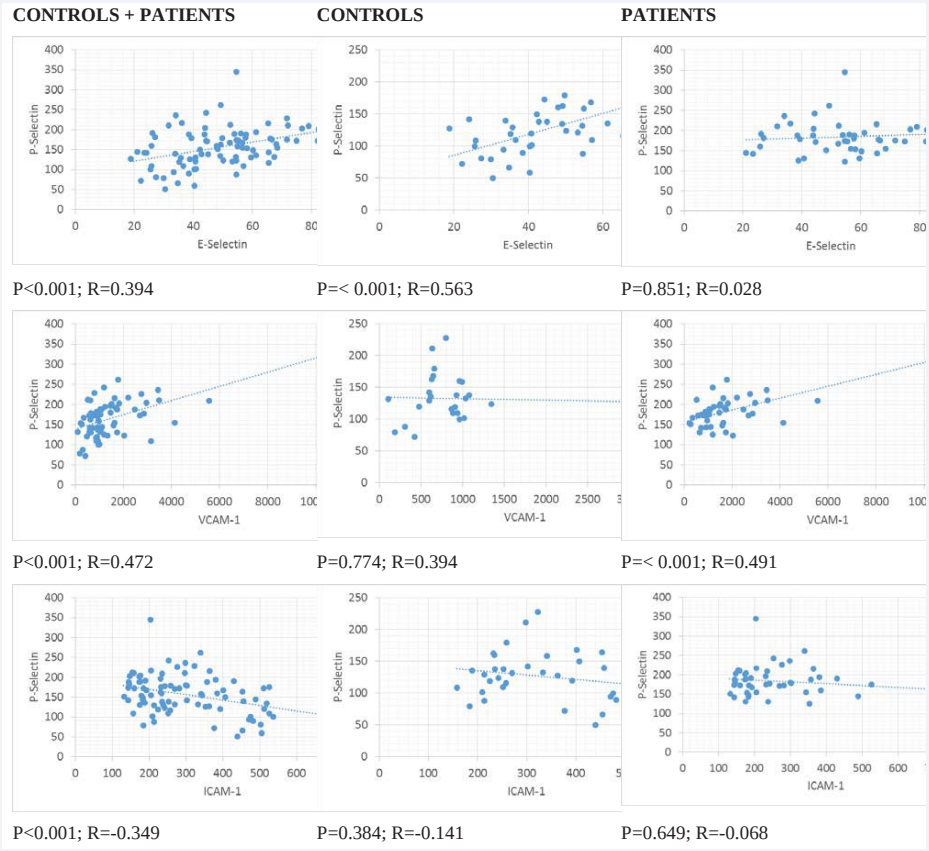

Figure 1 Controls/Patients, Controls e Patients - the association between E-selectin, VCAM-1 and ICAM-1 for both groups.

controls. In contrast, ICAM-1 concentrations were significantly lower in Pre-SSc, lcSSc and dcSSc patients. For E-selectin, there was a tendency regarding Pre-SSc and a statistically significant difference in SSc with E-selectin concentrations being higher among patients. However, when we analyzed disease subsets (lcSSc and dcSSC), only central tendencies were found (Table 3).

Concerning patients with lcSSc, non-CREST and CREST, statistically significant differences were observed between for P-selectin and VCAM-1 (Table 3). These differences were not observed for E-selectin. ICAM-1 concentrations were lower in non-CREST and in CREST patients, while differences have only reached statistical significance in the former case.

When comparing all subgroups, we verified that for all adhesion molecules no statically significant differences were found, between Pre-SSc and SSc, lcSSc and dcSSc, CREST and non-CREST patients (p>0.050 in all cases).

Analyses according to disease evolution phases revealed that P-selectin and VCAM-1 serum levels were significantly higher in all phases as compared to controls (Table 4). Regarding E-selectin, there were no differences in early SSc phase. However, as the disease evolved, increased E-selectin values were observed, reaching statistical significance in late disease ICAM-1 concentrations were significantly lower in intermediate and late disease stages, as compared to controls (Table 4).

Comparisons between the three disease evolution phases (performed using Kruskal-Wallis test) showed no significant differences for VCAM-1 (p=0.808), E-selectin (p=0.529) and P-selectin. For ICAM-1 differences were found between the three phases (p=0.013); post-hoc analysis (Mann-Whitney test) revealed significant differences between early and late phases (p=0.004) but not between early and intermediate (p=0.065) nor intermediate and late (p=0.255).

Due to the small sample size of some groups, an additional analysis was made by combining adjacent disease phases. Significant differences between early + intermediate phase and controls were found for VCAM-1 (p=0.001) and P-selectin (p<0.001), but not for ICAM-1 (p=0.198) nor for E-selectin (p=0.115). When intermediate and late phase were combined, significant differences (vs. controls) were found for all adhesion molecules (VCAM-1: p=0.001; ICAM-1: p<0.001; E-selectin: p=0.018; P-selectin: p<0.001).

Regarding the patter n of nail fold capillaroscopy, P-selectin levels were significantly higher in all patterns as compared to controls (Table 5). For E-selectin, statistical significance was observed only for the late pattern, with a tendency in the active pattern, and both capillaroscopic patterns presenting higher values. VCAM-1 serum levels were significantly higher in all capillaroscopic patterns; whereasICAM-1 concentrations were significantly lower in all capillaroscopic patterns (Table 5).

Comparisons between the three capillaroscopic patterns (Kruskal-Wallis test) showed no statistically significant differences (VCAM-1: p=0.893; ICAM-1: p=0.717; E-selectin: p=0.182; P-selectin: p=0.288).

Analysis comparing patients presenting organ involvement (score ≥ 1) with patients without organ involvement (score=0), showed that patients with articular disease had significantly lower levels of VCAM-1 and a tendency to lower P-selectin serum levels [median = 852 (237 to 1721) vs. 1647 (167 to 11000) p=0.007 and median = 164 (141 to 211) vs. 188 (123 to 610) p=0.066, respectively]. No differences in the serum levels of P-selectin, E-selectin, VCAM-1 and ICAM-1 were observed for the remaining analyzed organs (p>0.1 in all cases). However, patients having lung and/or heart involvement showed a tendency to have higher ICAM-1 levels, as compared to patients with no involvement of these organs [median = 238 (133 to 526) vs. 185 (130 to 697) p=0.099 and median = 273 (174 to 697) vs. 185 (133 to 526) p=0.089, respectively]. A zero Medsger's skin score corresponds indeed to the Pre-SSc subset.

In relation to organ involvement severity, when comparing patients having a score=1 with patients having ascore ≥ 2, we observed a trend for higher E-selectin serum levels in patients presenting more serious lung involvement (score ≥ 2) [median = 58 (34 to 84) vs. 44 (21 to 90) p=0.066] and a trend for higher ICAM-1 levels in patients with more serious heart involvement [median = 290 (176 to 697) vs. 191 (174 to 269) p=0.066]. No significant differences were found for other organ analysis regarding the remaining molecules.

Table 4: VCAM-1, ICAM-1, E-selectin and P-selectin levels in study population and healthy controls and according to Systemic Sclerosisevolution phase.

	VCAM-1 (ng/ml)	p*	ICAM-1 (ng/ml)	p*	E-selectin (pg/ml)	p*	P-selectin (pg/ml)	p*
Healthy controls (n=40)	833 (112-3143)		337 (158-536)		43 (19-72)		100 (50-228)	
Early SSc (n=6)	1419 (742-4147)	**0.004**	394 (205-526)	0.535	50 (21-67)	0.696	181 (145-235)	**0.002**
Intermediate SSc (n=10)	1645 (237-11000)	**0.015**	195 (146-697)	**0.029**	56 (26-82)	**0.069**	177 (123-345)	**<0.001**
Late SSc (n=23)	1472 (167-11000)	**0.002**	191 (130-365)	**<0.001**	52 (23-90)	**0.050**	193 (126-610)	**<0.001**

Results presented as median (range) values; * p values: Patients vs. Controls (Mann-Whitney U test)
Abbreviations: SSc, Scleroderma; VCAM-1, Vascular Cell Adhesion Molecule-1; ICAM-1, Intercellular Cell Adhesion Molecule-1.

Sub-analysis for lung involvement revealed no differences (p>0.1; data not shown) for TC changes, pulmonary function tests and PASP considered separately.

Sub-analysis for vascular involvement displayed no differences (p>0.1; data not shown) between Raynaud disease with or without digital ulcers.

DISCUSSION

Adhesion molecules play an important role in Scleroderma pathogenesis. Selectins mediate tethering and rolling of leucocytes on the endothelium [10], whereasVCAM-1 and ICAM-1promote leucocyte transmigration over the endothelium [3].

Several studies have compared adhesion molecules levels between SSc patients and healthy controls in serum [3,5,10-12,28-30] and in tissues [28,31,32].

Our results are line with those previously reported, showing higher serum levels of P and E-selectins and VCAM-1 in SSc spectrum disease patients (Pre-SSc and SSc). In contrast, we observed lower levels of ICAM-1 in SSc spectrum disease patients, with the exception of higher levels found in the early phase of the SSc disease. Despite not having a definite answer for this last result, according to Wolf et al. [33], one possible explanation could be the presence of agonist anti-ICAM-1 antibodies in serum from patients with SSc that activate reactive oxygen species production and VCAM-1 expression. However, the authors do not address the evolution of ICAM-1 serum levels in the presence of these antibodies.

Regarding disease subsets, results reported in the literature are conflicting. Valentini et al., described higher levels of E-selectin and ICAM-1, but not of VCAM-1, in patients with Pre-SSc, as compared to healthy controls [14]. On the other hand, Kumanovics et al., found no differences in E-selectin levels between patients with Pre-SSc and healthy controls [30]. Concerning limited vs. diffuse SSc, some authors have described higher levels of E-selectin and VCAM-1 in both subsets [5,34], while others have reported higher levels of VCAM-1 in diffuse [13,34] or limited [35] SSc.

In our study, when patients were compared to controls, both selectins and VCAM-1 exhibited higher levels in all disease subsets (although no statistical significance was found for E-selectin) whereas lower levels were observed for ICAM-1.

Several studies have observed the association between the evolution phases of SSc andadhesion molecules levels. Valim et al., found higher levels of E-selectin in the first four years of disease [13]. Hasegawa et al., evaluated ICAM-1 and E- and P-selectins in early SSc (<3 years) and concluded that serum levels of these adhesion molecules were higher in patients as compared to controls [12]. In our study, we found higher levels of these 3 molecules, as well as higher levels ofVCAM-1 in early phase, although statistical significance was achieved only for P-selectin and VCAM-1. For patients in intermediate and late phases, ICAM-1 was significantly lowered (compared to controls). As for the other molecules, patients' values were also higher, with statistically significant differences observed for P-selectin and VCAM-1 in both intermediate and late phases and for E-selectin in late phase. These results can be partially explained by the small number of patients included in the early and intermediate phases.

Concerning the capillaroscopic patterns, Valim et al., found a relationship of E-selectin levels with capillary deletion, but not with capillary ectasia [13]. Similarly, we found significantly higher E-selectin levels in the late capillaroscopic pattern. On the other hand, we found significantly higher P-selectin and VCAM-1 levels and significantly lower levels of ICAM-1 in all capillaroscopic patterns. To the best of our knowledge, these results have not been reported so far.

Some studies have evaluated the relationship between adhesion molecules and organ involvement (Table 6), disease severity and activity [4,36].

Some of the results we highlighted and discussed didn't reach statistical significance, being only marginally significant. This option was driven by the potential relevance of some of these results for future research, considering the influence of using our small sample size.

In accordance with previously reported data, our results on peripheral vascular involvement revealed no significant differences between the levels of adhesion molecules and the presence of digital ulcers [5,11,34,37]. In opposition, S fikaki et

Table 5: Relation between capillaroscopic patterns and VCAM-1, ICAM-1, E-selectin and P-selectin serum levels in Pre-SScand SSc patients (n=47).

	VCAM-1 (ng/ml)	p*	ICAM-1 (ng/ml)	p*	E-selectin (pg/ml)	p*	P-selectin (pg/ml)	p*
Healthy controls (n=40)	833 (112-3143)		337 (158-536)		43 (19-72)		100 (50-228)	
Early (n=11)	1117 (268-3496)	**0.014**	205 (136-489)	**0.007**	55 (21-75)	0.208	173 (143-216)	**<0.001**
Active (n=19)	1647 (237-11000)	**0.003**	190 (144-697)	**0.002**	54 (23-79)	0.083	189 (123-345)	**<0.001**
Late (n=14)	1488 (363-4147)	**<0.001**	232 (146-430)	**0.006**	58 (27-90)	**0.005**	178 (126-262)	**<0.001**

Results presented as median (range) values; * p values: Patients vs. Controls (Mann-Whitney U test). Abbreviations: SSc, Scleroderma; VCAM-1, Vascular Cell Adhesion Molecule-1; ICAM-1, Intercellular Cell Adhesion Molecule-1.

Table 6: Adhesion molecules and organ involvement: results of previous studies.

Previous studies	ORGAN/ ADHESION MOLECULES
Nomura et al. [29]; Hasegawa et al. [12]; Ihn et al. [34,40].	**P-selectin** and **ICAM-1** had statiscally higher levels in ILD patients than in patients without ILD [29]; Higher levels of **ICAM-1** in ILD patients with opposite correlation between **ICAM-1** and FVC in Scl70 patients [12]; Higher **ICAM-1, VCAM-1** and **E-selectin** levels in patients with lung involvement evaluated by spirometry and DLCO [34,40]
Iverson et al. [11], Hasegawa et al. [12] Kumánovics G et al. [30].	Positive relationship between **E-selectin** level and fibrosis on chest x-ray [11], diffuse fibrosis on CT scan [30] and DLCO and FVC [11,12,30]
Yanaba et al. [10], Iverson et al. [11].	Raised levels of **PSGL-1** are associated with a lower frequency and severity of pulmonar involvement which seems to show a role of **P-selectin** in pulmonar fibrosis [10]; correlation between **P-selectin** levels and DLCO but not FVC [11]
Pendergrass et al. [9]; Iannone et al [37]	Correlation between **ICAM-1** and **VCAM-1** molecules and PAH in limited SSc [9]; Higher **VCAM-1** levels in patients without PAH versus PAH patients
Sfikaki et al. [38].	Higher **ICAM-1** levels in patients with digital ulcers
Veale et al [39]	Opposite correlation between **ICAM-1** and skin score
Alzawawy et al [5], Iverson et al [11], Ihn et al. [34], Iannone F [37]	No significant differences between the levels of adhesion molecules and the presence of digital ulcers
Alzawawy et al. [5], Iverson et al. [11], Kumánovics G et al. [30] Iannone F [37].	No significant differences between the levels of adhesion molecules and skin score
Ihn et al. [34,40].	Positive relationships between **VCAM-1** [34] and **ICAM-1** [40] levels and osteoarticular involvement

Abbreviations: SSc, Scleroderma; VCAM-1, Vascular Cell Adhesion Molecule-1; ICAM-1, Intercellular Cell Adhesion Molecule-1; ILD, interstitial lung disease; DLCO, diffusing capacity of the lungs for carbon monoxide ; FVC, Forced vital capacity; PAH, pulmonary arterial hypertension; CT scan, computed tomography.

al., found higher ICAM-1 levels among those patients [38]. Also in line with our results, some studies have analyzed the relationship between skin score and adhesion molecules and the majority has failed to reach statistically significant associations [5,11,30,37]. The only exception was Veale et al., who described an opposite correlation between ICAM-1 and the skin score [39]. Concerning lung involvement, Nomura et al., found that only P-selectin and ICAM-1had statistically significant higher levels in interstitial lung disease (ILD) patients [29]. Similarly, Hasegawa et al., observed higher levels of ICAM-1 in ILD patients and a negative correlation between ICAM-1 and FVC in anti-Scl70 patients [12]. Additionally, Ihn et al., showed higher E-selectin [34], VCAM [34] and ICAM [40] levels in patients with lung involvement evaluated by spirometry and DLCO [34]. Moreover, Pendergrass et al. [9], found a correlation between ICAM-1 and VCAM-1 and pulmonary arterial hypertension (PAH) in limited SSc, while Iannone et al. [37], found higher VCAM-1 levels in patients with no PAH. In the present study, we found no association between higher VCAM-1 levels and lung involvement or severity (CT scan, PASP, DLCO and FVC) but we found a marginal relationship between higher ICAM levels and lung involvement among patients.

Some studies established a positive relationship between E-selectin levels and fibrosis on chest x-ray [11], diffuse fibrosis on CT scan [30], and DLCO and FVC alterations [11,12,30]. These results suggest E-selectin as a potential pulmonary fibrosis evolution and severity biomarker [30]. Our results are in accordance with these studies, positively relating E-selectin levels with the severity of lung involvement. Moreover, Yanaba et al., reported that increased serum levels of P-selectin glycoprotein ligand 1 (PSGL-1) were associated with a lower frequency and severity of pulmonary involvement, which, in turn, suggests the role of P-selectin in pulmonary fibrosis [10]. Furthermore, Iversen et al., found a correlation between P-selectin levels and DLCO but not with FVC [11]. In our study, P-selectin levels were not associated with pulmonary involvement or severity.

To the best of our knowledge, our results concerning the relationship between ICAM-1 levels and heart involvement and severity are the first reported in the literature. This makes it hard to discuss these results; however, it also highlights their relevance and, therefore, of our study.

Inh et al., described positive relationships between VCAM-1 [34] and ICAM-1 [40] levels and osteoarticular involvement. However, our results showed a negative relationship between VCAM-1 levels and osteoarticular involvement.

Ihn et al. [34], demonstrated that, in patients with SSc, E-selectin levels showed no correlation with either ICAM-1 or VCAM-1 levels, whileICAM-1 and VCAM-1 serum levels were significantly correlated In our study we found a positive correlation between P-selectin and E-selectin in controls but not in SSc spectrum disease patients. On the other hand, a positive correlation between P-selectin and VCAM-1 levels was observed among patients but not among the control group. These results may contribute to clarify the pathogenesis of SSc.

In summary, P-selectin and VCAM-1 were consistently increased in all disease phases, subsets and capillaroscopic patterns, making these molecules early and persistent markers throughout the course of the disease.

The systematic observation of elevated E-selectin levels in advanced stages of disease with late capillaroscopic changes, or in a broader sense, among patients with a more severe organ involvement, suggests that E-selectin has a wide impact in disease progression and is related to lung pathogenesis and severity.

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