The relationship between vascular biomarkers and disease characteristics in systemic sclerosis: elevated MCP-1 is predominantly associated with fibrotic manifestations

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Received on May 26, 2016; accepted in revised form on September 10, 2016.

Clin Exp Rheumatol 2016; 34 (Suppl. 100): S110-S114.

Key words: systemic sclerosis, fibrosis, biological markers

Competing interests: none declared.

ABSTRACT

Objective. *To determine the relationship between vascular biomarkers reflecting the vascular injury and organ involvement in systemic sclerosis (SSc).*

Methods. *Seventy-two SSc patients (66 female) fulfilling 2013 ACR/EULAR Criteria were evaluated. Serum samples of patients were collected for flow-cytometric analysis of sCD40L, tPA, MCP-1, sE-selectin, IL-8, IL-6, VEGF, sP-selectin, TGF-β1 and VCAM levels (Bender MedSystems) in SSc patients and 20 healthy controls. Results were compared with Pearson chi-square / Fisher's and Mann Whitney tests.*

Results. *Levels of MCP-1 were found to be elevated in patients with diffuse cutaneous SSc, flexion contractures, FVC<80%, DLCO<80%, pulmonary fibrosis and high acute phase response (p=0.002, p=0.005, p=0.045, p=0.005, p=0.036, p=0.006, respectively), TGF-β1 in patients receiving immunosupressives (p=0.001), sE-selectin in patients with high acute phase response (p=0.028), sCD40L in patients with lcSSc (p=0.011) and smoking habitus (p=0.032). MCP-1 and sE-selectin levels were correlated with disease activity score (r=0.243, p=0.040 and r=0.303, p=0.010), MCP-1 and TGF-β1 were correlated with severity of pulmonary involvement (r=0.323, p=0.006 and r=0.312, p=0.008).*

Conclusion. *MCP-1 was the prominent biomarker correlated with the manifestations related to fibrosis, disease activity score and severity of pulmonary involvement. Treatment and smoking may have an effect on cytokine profile. Vascular biomarkers can be used to predict the characteristics and severity of SSc warranting prospective studies.*

Introduction

Systemic sclerosis (SSc) is a heterogeneous connective tissue disease which can effect multiple target organs, characterised by vascular damage and autoimmune inflammation preceding vascular and interstitial fibrosis. Initial microvascular abnormalities are possibly functional and might be reversible. Progressive obliterative vasculopathy and tissue hypoxia, activating innate and adaptive immune systems cause inflammation and fibroblast activation at later stages resulting in irreversible process of structural damage in multiple organs (1-3).

The skin and lung are the commonly effected organs that should be carefully evaluated. Evaluation of skin fibrosis by modified Rodnan skin score (MRSS), a measure of the extent of the skin involvement, was shown to reflect the severity and mortality of SSc (4, 5) and should be monitored. High-resolution computed tomography (HRCT) of the lungs and pulmonary function tests are recommended tools to reveal the restrictive lung disease due to fibrosis. But all these measurements are able to estimate these manifestations merely after the fibrotic process established.

All studies including nail-fold capillaroscopy and different biomarkers target to distinguish severe patients and clarify the stages of SSc to allow earlier therapeutical approach. Capillaroscopic abnormalities and some pro-inflammatory or pro-fibrotic cytokines were shown to be related to different clinical manifestations and/or histopathological findings in SSc (1, 6-13). In this prospective study, we planned to evaluate the relationship between vascular biomarkers that may reflect the vascular injury and organ involvement in SSc.

Patients and methods

A total of 72 SSc patients at age of <18 and fulfilling ACR / EULAR classification criteria (14) were included in this prospective cross-sectional study.

Demographics, onset of symptoms, organ involvements, treatment details, laboratory and imaging including acute phase response (ESR>20 mm/hour and/or CRP>5mg/L were accepted as high acute phase response), serology, echocardiography, respiratory function tests, HRCT of chest were noted into a pre-defined protocol. MRSS, Valentini activity score (different scores of 10 parameters, the total possible activity score was 10) (15) and Medsger severity score (Score of 0 to 4 for 9 involvements, the total possible severity score was 36) (16) were evaluated. After permission was obtained from local ethics committee, all patients signed informed consent to be a participiant.

Detection of biomarkers by multiplex bead array kits

Serum samples of SSc patients and healthy controls were centrifugated after phlebotomy at 3000 rpm 10 minutes and separated serum was stored at -20ºC till detection of the markers. Human Cardiovascular 6plex FlowCytomix [including soluble soluble CD40 ligand (sCD40L), interleukin-6 (IL-6), interleukin-8 (IL-8), monocyte chemoattractant protein-1 (MCP-1), sP-Selectin, tissue plasminogen activator (t-PA)], human E-selectin, human vascular endothelial growth factor (VEGF), human soluble vascular cell adhesion molecule-1 (sVCAM-1), human transforming growth factor beta-1 (TGF-beta1-) (total form) FlowCytomix Simplex non-magnetic beads kits (all from e-bioscience formerly Bender MedSystems GmbH, Vienna, Austria) were determined by flow-cytometer and was performed according to manifacturer's instructions. Beadfluorescence measurement was performed by a flow cytometry apparatus (Becton Dickinson Immunocytometry Systems GmbH). The mean levels were compared with healthy subjects and between groups according to organ involvements.

Statistical analysis

For statistical analysis of the data 'SPSS v. 16 for Windows' programme was used. Groups were compared with student's *t* and Mann Whitney U tests for continuous variables or Pearson chi-square/Fisher's for categorical variables. Results were presented as mean ± standard deviation (± SD). *p*-values <0.05 were considered significant. Mean levels of biomarkers were presented after the exclusion of outliers. The distribution and median levels of biomarkers among groups according to organ involvement were summarised by box-plot graphs.

Table I. Demographics and disease characteristics of SSc patients.

Age (mean ± SD)		44.9 ± 12.7
Gender (F/M) (n) (%)		66/6 (%92/8)
Duration of Raynaud's (year) (mean ± SD)		5.8 ± 5.9
Duration of non-Raynaud symptom (year) (mean ± SD)		3.2 ± 2.3
Skin(n) (%) Involvement	Diffuse Cutaneous SSc (dcSSc)	23 (%32)
	Limited Cutaneous SSc (lcSSc)	49 (%68)
Serology	ANA(+)	66 (%92)
	Anti-centromere (+)	15 (%21)
	Anti-Scl70 (+)	34 (%47)
Organ (n) (%) Involvement	Raynaud's	70 (97%)
	Digital Ulcer History	33 (46%)
	Telangiectasia	40 (50%)
	Arthritis	37 (51%)
	Renal Crisis	4 (5%)
	Contracture-t.friction rubs	12-6 (20-8%)
	Disphagy-Reflux-Diarrhoea	54 (75%)
	FVC<%80-DLCO<%80	19-28 (26-39%)
	Pulmonary fibrosis	18 (25%)
	PAP>40 mmHg	5 (7%)
Treatment(n) (%)	No treatment	16 (22%)
	Calcium Channel Blockers-PPI	55 (76%)
	Immunosupressives	41 (57%)
	Steroids (<7.5 mg/day)	36 (50%)

Results

Demographics and disease characteristics of SSc patients were summarised in Table I.

The mean±SD scores of MRSS, activity and severity were 9.1±7.9, 1.5±2.0 and 4.9±2.6, respectively in SSc patients. Subgroup analysis of the distribution of severity scores showed that peripheral vascular involvement score was higher in patients with smoking habitus (n=32, 44%) (1.7±0.7 *vs*. 1.3±0.6, *p*=0.039).

When compared to healthy controls, levels of tPA (4036±6961 *vs*. 2415±1279, *p*=0.02), MCP-1 (*p*=0.001), sE-selectin (*p*=0.008), TGF-β1 (*p*=0.001) (Table II) were higher, sP-selectin (287±86 *vs*. 364±137, *p*=0.011) and IL-8 (22±80 *vs*. 49±73, *p*=0.001) were lower, VEGF (776±591 *vs*. 704±363), VCAM (3945±1754 *vs*. 3231±1435), sCD40L (27847±33315 *vs*. 24620±13051), IL-6 (0.6±2.8 *vs*. 0) were similar in SSc patients.

MCP-1 levels were significantly higher in patients with dcSSc, flexion contractures, FVC<80%, DLCO<80%, pulmonary fibrosis and high acute phase response (*p*=0.002, *p*=0.005, *p*=0.045, *p*=0.005, *p*=0.036, *p*=0.006, respectively). TGF-β1 in patients receiving immunosuppressives (*p*=0.001), sE-selectin in patients with high acute phase response (*p*=0.028), sCD40L (pg/ml) in patients with lcSSc (*p*=0.011) and smoking habitus (*p*=0.032) were found to be significantly higher in the subgroup analysis of the patients (Table II). The distribution and median levels of MCP-1, sE-selectin, TGF-β1 and SCD40L between SSc patient groups according to organ involvement were summarised in Figure 1 a-h.

The association between biomarkers with disease activity and severity parameters were analysed. MCP-1 and sE-selectin levels were significantly but weakly correlated with disease activity scores (r=0.243, *p*=0.040 and r=0.303, *p*=0.010). MCP-1 (r=0.323, *p*=0.006) and TGF-β1 (r=0.312, *p*=0.008) were correlated with severity of pulmonary involvement.

Discussion

In this prospective study, we evaluated the relationship between biomarkers and organ involvement in a SSc cohort

Table II. Vascular biomarkers between disease characteristics of systemic sclerosis patients.

			MCP-1 (pg/ml)	sCD40L (pg/ml)	sE-selectin (ng/ml)	TGF-β1 (pg/ml)
Systemic sclerosis patients		n=72	***1302 ± 550***	27847 ± 33315	***269 ± 106***	***8277 ± 8592***
Healthy subjects		n=20	907 ± 300	24620 ± 13051	205 ± 78	2421 ± 4785
Diffuse cutaneous		n=23	***1586 ± 579****	*18494 ± 20360*	285 ± 107	9544 ± 8864
Limited cutaneous		n=49	*1169 ± 486*	***32238 ± 37284****	262 ± 106	7683 ± 8489
Flexion contractures	+	n=12	***1757 ± 646****	16768 ± 13118	279 ± 129	9068 ± 6873
	-	n=60	*1211 ± 485*	30064 ± 35687	267 ± 102	8119 ± 8937
Low DLCO (<80%)	+	n=28	***1548 ± 654****	34841 ± 49690	274 ± 116	9832 ± 8785
	-	n=44	*1145 ± 407*	23397 ± 15170	266 ± 100	7288 ± 8418
Low FVC (<80%)	+	n=19	***1537 ± 618****	23374 ± 21490	267 ± 104	10558 ± 8604
	-	n=53	*1218 ± 503*	29452 ± 36683	270 ± 108	7460 ± 8520
Pulmonary Fibrosis	+	n=18	***1526 ± 558****	26309 ± 21268	287 ± 89	8831 ± 9385
	-	n=54	*1228 ± 531*	28361 ± 36615	263 ± 111	8093 ± 8397
High acute phase response	+	n=30	***1543 ± 621****	27581 ± 20414	***300 ± 95****	9219 ± 8522
	-	n=42	*1137 ± 424*	28522 ± 40715	*247 ± 110*	7790 ± 8704
Smoking history	+	n=32	1233 ± 514	***36878 ± 45771***	281 ± 104	7627 ± 9047
	-	n=40	1358 ± 576	*20624 ± 15326*	260 ± 108	8798 ± 8290
Immunosuppressives	+	n=41	1345 ± 547	24497 ± 19379	270 ± 110	***10981 ± 9057****
	-	n=31	1246 ± 557	32279 ± 45720	268 ± 102	*4702 ± 6493*

*$p<0.05$, **$p<0.01$ When groups were compared with Mann-Whitney test.

which was mainly consisted of early patients and limited cutaneous form. One fourth of the group were treatment naive and immunosuppressive drugs were used by nearly half. The evaluation of cytokines in such an early group relatively less exposed to immunosuppressives was thought be informative about the pathogenesis of the disease.

Among the studied biomarkers, MCP-1 was shown to be prominently related to organ involvement and higher in patients with dcSSc, flexion contractures, low FVC and/or low DLCO, pulmonary fibrosis and high acute phase response. MCP-1 levels were correlated with disease activity scores and severity of pulmonary involvement in this study.

MCP-1 can be excreted by different cell types including mononuclear cells, fibroblasts, smooth muscle, epithelial and endothelal cells. MCP-1, expressed predominantly by mononuclear cells, has an important role in the chemotaxis of leucocytes in early inflammatory stages. It has been shown to be released by fibroblasts in later fibrotic stages, prompts the migration of fibrocytes to tissues and also stimulates production of large amounts of collagen (1, 7). Due to the close link, the relationship between MCP-1 and fibrosis in SSc was studied in several studies. MCP-1 was shown to be also overexpressed in non-fibrotic skin similar to involved fibrotic skin areas, so it might have a critical role in pro-fibrotic and fibrotic stages (7, 17, 18). Significantly elevated levels of MCP-1 were also found to be related to early disease, dcSSc and pulmonary fibrosis (19-22). The absence of the receptor for MCP-1 (CCR-2) was shown to be specifically protective against fibrosis (23, 24) and treatment with MCP-1 antagonists was found to prevent skin fibrosis in experimantal models (25). These data support the importance of MCP-1 in SSc fibrosis.

TGF-β signalling in the fibrotic process has been well-known and become one of the striking therapeutic target in this process (1, 13, 26-29). In our study TGF-β1 was only found to be higher in SSc patients taking immunosuppressives and correlated with the severity of pulmonary involvement that might suggest higher levels in severe SSc patients, but we could not show any relationship with any organ involvement independently. sE-selectin, which was found to be related to extent of organ involvement in SSc (30), was higher in patients with high acute phase response and correlated with disease activity in this study. sCD40L was found to be higher in patients with lcSSc and smoking habitus who were also have higher severity scores of peripheral vascular involvement in our study. Previously, sCD40L was shown to be higher in patients with early disease course, lcSSc, digital ulcers and pulmonary arterial hypertension and correlated with pulmonary arterial pressure (31, 32). We did not find any relationship with sCD40L and vascular manifestations.

Our study has some limitations because of the cross-sectional nature and the course of biomarkers at different stages of SSc could not be observed. Comorbidities and concomitant treatments might have a confounding effect on the cytokine profile. The power of the analysis of the subgroups was limited due to the small number of patients. Collected serum might be contaminated by platelets due to short delays in isolation, this might influence the levels of related biomarkers.

In conclusion, MCP-1 was the prominent biomarker related to fibrotic involvements of the skin, lungs and tendons. It was also correlated with disease activity due to fibrosis and

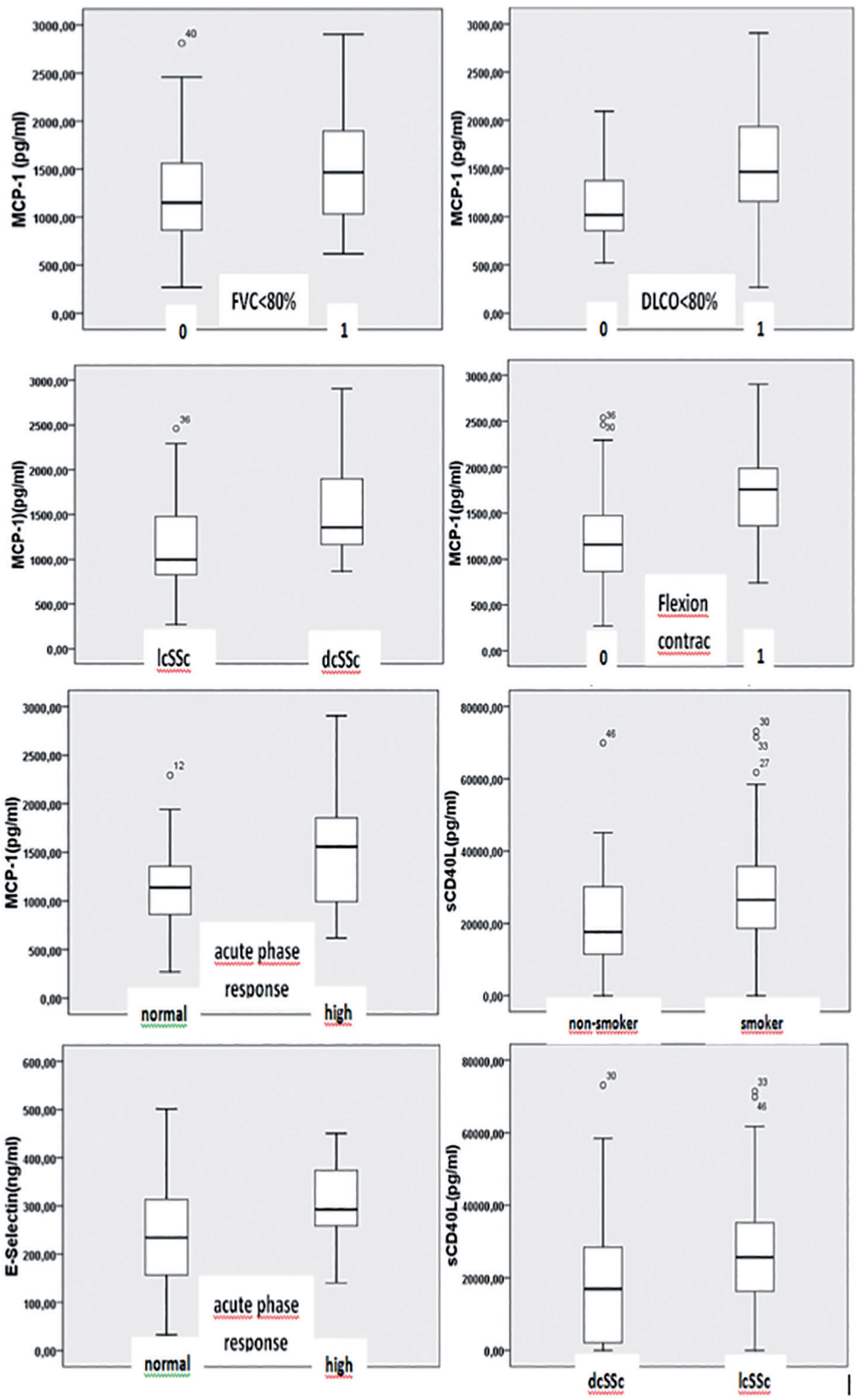

Fig. 1 a-h. The distribution and median levels of biomarkers among SSc patient groups according to organ involvement.

severity of pulmonary involvement. MCP-1 may be a surrogate marker for the evaluation of fibrosis in SSc. Treatment and smoking may have an effect on the cytokine profile. Vascular biomarkers can be used to predict the characteristics and severity of SSc warranting prospective studies.

Acknowledgement
The authors thank Actelion Turkey for providing laboratory kits.

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