Prediction of organ involvement in systemic sclerosis by serum biomarkers and peripheral endothelial function

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ABSTRACT

Objective. *To identify prognostic factors among serum biomarkers and endothelial vasodilator function findings in patients with systemic sclerosis (SSc).*

Methods. *This is a clinical observational study. We assessed 60 consecutive SSc patients (44 limited cutaneous-type, 16 diffuse cutaneous-type). Circulating growth differentiation factor-15 (GDF-15), placenta growth factor (PlGF), endostatin, vascular endothelial growth factor (VEGF), and pentraxin 3 (PTX3) were measured by ELISA. Peripheral endothelial function was measured by forearm blood dilatation response to brachial artery occlusion using non-invasive plethysmography (EndoPAT2000), which is associated with nitric-oxide-dependent vasodilatation and yields a reactive hyperaemia index (RHI). We evaluated whether abnormalities in these values were associated with type of SSc – namely, diffuse cutaneous SSc (dcSSc) or limited cutaneous SSc (lcSSc) – or organ involvement including interstitial lung disease (ILD), digital ulcer (DU) and estimated right ventricular systolic pressure (RVSP) by echocardiography >30 mmHg.*

Results. *SSc patients showed significantly elevated serum GDF-15, PlGF, endostatin and VEGF but not PTX3 compared with controls. GDF-15 and PlGF were high in dcSSc patients. EndoPAT-RHI was low, and incidence of RVSP >30 mmHg was high in dcSSc. Multivariate analysis revealed that elevated GDF-15 was highly predictive of dcSSc, ILD or RVSP >30 mmHg. PlGF for DU was also found. Conversely, a low EndoPAT-RHI value was predictive of the presence of dcSSc, ILD or DU.*

Conclusion. *This is the first study to inclusively investigate the relationships among biomarkers, EndoPAT-RHI and organ involvement in patients with SSc. Our data suggest a complex pathological progression of SSc through fibrotic impairment and microvascular damage.*

Introduction

Systemic sclerosis (SSc) is a chronic, complex, and not yet completely understood autoimmune disease characterised by the presence of immunological events, fibrosis, and vascular alterations (1-4). The development of fibrosis seems to be a consequence of the initial ischaemic process related to endothelial injury (1, 2, 4). Many cell types, such as endothelial cells, smooth muscle cells, pericytes, fibroblasts and mononuclear cells, interactively contribute to the disease progression. The hypothesis of Vaga *et al*. regarding disease pathogenesis suggested that the disease is initiated by microvascular injury inducing local inflammation and autoimmunity (4). This local inflammation directly activates fibroblasts and induces recruitment and transdifferentiation of progenitor cells of mesenchymal origin, which further contribute to fibrosis and tissue damage (4, 5).

Circulating biomarkers have the potential to play a significant clinical role in defining the disease activity and predicting the prognosis of patients with SSc (5-8). Since the organ involvements of SSc are widely distributed, related changes in a variety of serum biomarkers involved in autoimmunity (*i.e.* autoantibodies), inflammation, fibrosis or angiogenesis have been reported (2, 3, 9). Considering the hypothesis that vascular injury with fibrosis is one of the most important and earliest clinical features of SSc, the search for vascular/fibrotic biomarkers reflecting the severity of SSc is considered particu-

larly meaningful. In this regard, previous studies have examined the roles of such biomarkers as growth differentiation factor-15 (GDF-15) (6-8], placenta growth factor (P*l*GF) (5, 10-14), endostatin (14-7), vascular endothelial growth factor (VEGF) (14, 18, 19), and pentraxin 3 (PTX3) (20, 21).

In addition to serum biomarkers, other non-invasive measures assessing endothelial vasodilator function include endo-peripheral arterial tonometry (EndoPAT). To measure digital reactive hyperaemia, PAT involves measuring arterial pulsatile volume at rest and during conditions of increased shear stress that result in a release of nitric oxide (NO) and other mediators affecting vascular tone and homeostasis (22). Although the usefulness of EndoPAT for atherosclerosis and cardiovascular disease has been previously reported (23, 24), only a few reports have used EndoPAT to demonstrate microvascular abnormalities due to peripheral endothelial dysfunction in SSc (25, 26). We previously reported that the plasma concentration of NO was low in SSc patients complicated with pulmonary hypertension (27). Therefore, peripheral endothelial dysfunction observed by EndoPAT may reflect organ involvement including pulmonary hypertension and its severity in SSc patients.

The objectives of this study were to explore the roles of serum biomarkers and endothelial function measurement by Endo-PAT in predicting organ involvement in patients with SSc.

Materials and methods

Patients

Sixty Japanese patients with SSc, who fulfilled the 2013 classification criteria for systemic sclerosis (28), were consecutively recruited to the present study from May 2011 to November 2014. Patients visited our hospital with referrals from the Japanese Red Cross Nagasaki Genbaku Hospital, Isahaya General Hospital, Nagasaki Medical Hospital of Rheumatology, and NHO National Nagasaki Medical Centre. Patients were grouped according to the classification system proposed by LeRoy *et al*. (29): 44 patients had limited cutaneous SSc (lcSSc) and 16 patients had diffuse cutaneous SSc (dcSSc). Five patients had been treated with low-dose corticosteroids (prednisolone <10 mg daily). None of the patients had received immunosuppressants or vasodilators such as endothelin receptor antagonists and phosphodiesterase type 5 (PDE5) inhibitors. Ten patients treated with Calcium channel blockers against hypertension and nine patients were treated with antiagrregants. All patients underwent a full medical history and physical examination. Laboratory assessment included blood tests, pulmonary function tests, carbon monoxide diffusion capacity (DLco), electrocardiogram, transthoracic echocardiography, chest x-ray and/or high-resolution computer tomography computer tomography (HRCT), and EndoPAT. Antinuclear antibody (ANA) was determined by indirect immunofluorescence using HEp-2 cells as the substrate, and autoantibody specificities were further assessed by enzyme-linked immunosorbent assay (ELISA). Serum and plasma were stored. This study was prospective and cross-sectional. The patients gave their informed consent to be subjected to the protocol, which was approved by the Institutional Review Board of Nagasaki University Hospital (approval number: 11032820).

Skin assessment

Skin thickness was scored by Japanese Dermatological Association-certified dermatologists (F.O), according to the modified Rodonan total skin score (mRTSS), which was measured by summing skin thickness measurements determined on a 0-3 scale by palpating skin in 17 body areas (30). Digital ulcer (DU) was defined as a loss of epithelialisation on the distal finger surface of ischaemic origin according to the physician (31-33). A presence of nailfold bleeding was visually confirmed by F.O.

Peripheral endothelial function measurement by the reactive hyperaemia index (RHI)

Digital pulse amplitude was measured with a PAT device by placing the probes on the tips of both index fingers (Endo-PAT 2000; Itamar Medical, Caesarea, Israel) as previously described (34). PAT signal measurement was performed with the digital probe inflation pressure set at 10 mmHg below the diastolic pressure or 70 mmHg (whichever was the lowest), as previously described in the Framingham study (35). Briefly, baseline pulse amplitude was recorded bilaterally on the tips of the index fingers for 5 minutes. This was followed by vaso-occlusion on the right side. After 5 minutes, the cuff was rapidly deflated and the PAT signal measurement was recorded for an additional 5 minutes. As the control, measurement of non-endothelial-dependent systemic changes occurring during the study was done on the contralateral finger. Mean PAT amplitudes were measured 90 seconds after the occlusion for a duration of 60 seconds. Finally, the ratio of the post-to-pre occlusion PAT amplitude of the tested arm, divided by the post-to-pre occlusion ratio of the control arm, was calculated as the reactive hyperaemia index (RHI). All PAT amplitudes and RHI values were automatically calculated by the EndoPAT device, with an RHI of <1.67 considered the cut-off defining endothelial dysfunction based on an earlier study (26).

Echocardiography

Right ventricular systolic pressure (RVSP) was estimated by trans-thoracic echocardiography for screening of pulmonary hypertension. RVSP was calculated from the peak signal velocity of the tricuspid regurgitant (TR) signal using a modified Bernoulli equation. Right atrial pressure was estimated by evaluating the size and collapsibility of the inferior vena cava and was added to the calculated TR jet velocity to obtain the estimated pulmonary artery systolic pressure.

Biomarker measurement

The serum levels of GDF-15 (R&D Systems, Minneapolis, MN), P*l*GF (R&D Systems), endostatin (R&D Systems) and VEGF (R&D Systems) and the plasma levels of PTX3 (Perseus Proteomics, Tokyo, Japan) were measured with specific ELISA kits using stored samples. These biomarkers were also measured in 25 healthy controls.

Table I. Demographic and clinical characteristics and biomarker levels of the 60 SSc patients.

	SSc (n=60)	dcSSc (n=16)	lcSSc (n=44)	*p* (dcSS *vs* lcSSc)
Age (yrs[a])	64 (57-69)	64 (58-67)	64 (55-69)	*NS*
Gender (female)	56 (93.3)	15 (93.8)	41 (93.2)	*NS*
Duration of disease (years)	2.0 (0.5-7.3)	3.8 (0.5-10.0)	2.0 (0.5-5.5)	*NS*
Cutaneous type (diffuse / limited)	16 (26.7) / 44 (73.3)	-	-	-
Raynaud's phenomenon	51 (82.3)	15 (93.8)	36 (81.8)	*NS*
Nailfold bleeding	32 (53.3)	12 (75.0)	20 (45.5)	0.043
mRTSS (points)	5 (4-8)	11 (6-14)	4 (3-6)	<0.001
Organ involvement				
Interstitial lung disease	24 (40.0)	15 (93.8)	9 (20.5)	<0.0001
Pulmonary hypertension (including borderline)	4 (6.7)	1 (6.3)	3 (6.8)	*NS*
Heart involvement	1 (1.7)	0 (0)	0 (0)	*NS*
Renal involvement	0 (0)	0 (0)	0 (0)	*NS*
Digital ulcer (presence or history)	10 (16.7)	8 (50)	2 (4.5)	<0.0001
Autoantibodies				
Anti-nuclear antibody	56 (93.3)	15 (93.8)	41 (93.2)	*NS*
Anti-topoisomerase I	15 (25.0)	10 (62.5)	5 (11.4)	<0.0001
Anti-centromere	31 (51.7)	3 (18.8)	28 (63.6)	0.0021
Anti-U1-RNP	6 (10.0)	3 (18.8)	3 (6.8)	*NS*
Anti-RNA polymerase III	3 (5.0)	2 (16.7)	1 (2.6)	0.069
KL-6 (U/ml)	317 (200-754)	960 (473-1750)	262 (177-428)	<0.001
NT-proBNP (pg/ml[a])	95.2 (44.6-182)	88.4 (54.1-224.3)	99.3 (40.5-178.6)	*NS*
GDF-15 (pg/ml)	1117 (676-1823)	2801 (1732-3766)	784 (629-1401)	<0.001
P*l*GF (pg/ml)	13.2 (8.2-17.9)	18.6 (13.2-20.8)	9.5 (7.5-16.2)	0.0016
VEGF (pg/ml)	384 (191-561)	376 (223-429)	391 (166-580)	*NS*
PTX3 (ng/ml)	2.79 (2.1-3.5)	3.0 (1.6-3.8)	2.8 (2.2-3.4)	*NS*
EndoPAT-RHI	1.56 (1.24-2.02)	1.18 (0.96-1.48)	1.58 (1.45-2.09)	0.023
RVSP estimated by echocardiography	29 (24-37)	35 (30-39)	28 (23-33)	0.015
RVSP>30mmHg	24 (40.0)	10 (62.5)	14 (31.8)	0.032

Data are expressed as the median (interquartile range) or a number (percentage).
Mann-Whitney U-test and χ^2 test were used for the statistical analysis (and Fisher's exact probability test when appropriate).
mRTSS: modified Rodnan Total Skin Score; GDF-15: growth differentiation factor 15; P*l*GF: placenta growth factor; VEGF: vascular endothelial growth factor; PTX3: pentraxin 3; RHI: reactive hyperaemia index; RVSP: right ventricular systolic pressure; SSc: systemic sclerosis; NS: not significant.

Serum levels of KL-6 (normal range, <500U/ml) and NT-proBNP (normal range, <125pg/ml) were measured in clinical practice.

Statistical analyses

Statistical analysis was performed using JMP Pro statistical software, version 11.0 (business unit of SAS Institute). Within-group comparisons were made using Mann-Whitney U-test and the χ^2 test, or Fisher's exact probability test when appropriate. Correlations were assessed with Spearman's correlation coefficient. We tried to find the variables associated with cutaneous type or organ involvement from serum biomarkers and EndoPAT-RHI using multivariate logistic regression analysis. Variables with *p*-values less than 0.05 were used in multivariate models. The overall significance level for statistical analysis was 5% (two-sided). *P*-values less than 0.05 were considered statistically significant.

Results

Demographic and clinical characteristics of the 60 SSc patients

Demographic and clinical characteristics of the 60 SSc patients are shown in Table I. The median (interquartile range, IQR) of the patients' ages was 64 (57–69) years, and that of their disease duration was 2.0 (0.5–7.3) years. The rate of the limited cutaneous type was higher than that of the diffuse cutaneous type (73.3% *vs*. 26.7%). The positivity of anti-centromere antibody (51.7%) was higher than those of anti-topoisomerase I (25.0%), anti-U1-RNP (10.0%), and anti-RNA polymerase III antibodies (5.0%). The median (IQR) value of mRTSS was 5 (4-8). Twenty-four (40.0%) patients were complicated with interstitial lung disease (ILD). HRCT was examined in 56 patients (93.3%). In the remaining 4 patients, chest x-ray and pulmonary function test were normal. Ten (16.7%) patients had DUs. The incidences of pulmonary hypertension, heart involvement, and renal involvement were low in our cohort, probably due to the preponderance of relatively early disease. However, the mean value of EndoPAT-RHI was 1.56 in SSc patients, which may indicate the existence of microvascular endothelial vasodilator dysfunction.

The characteristics of dcSSc were quite different from those of lcSSc: namely, the former showed significantly higher mRTSS, ILD, DU, anti-topoisomerase I antibody, KL-6, and RVSP estimated by echocardiography but low anti-centromere antibody. Compared to healthy controls, SSc patients showed significantly elevated serum GDF-15 ($p<0.0001$), P*l*GF ($p<0.0001$), endostatin ($p<0.001$) and VEGF ($p<0.001$) but not PTX3 ($p=0.10$). (Supplementary Table I). As shown in Table I, GDF-15 and P*l*GF were high in dcSSc patients. GDF-15 significantly correlated with mRTSS (rs=0.30, $p=0.020$), but there was no correlation between other biomarkers and mRTSS. In addition, EndoPAT-RHI was low and the incidence of RVSP >30 mmHg was high in dcSSc.

Distribution of biomarkers in SSc patients with or without organ involvements

Table II summarises the data. Among biomarkers examined, GDF-15 was significantly high in SSc patients with ILD, DU, or RVSP >30 mmHg. Also, P*l*GF was significantly high in SSc patients with ILD or DU, and PTX3 and endostatin in those with DU. However, no difference in VEGF was found between the presence and absence of organ involvement. EndoPAT-RHI was low in SSc patients with ILD or DU. DUs were present at fingertip in 7 patients and other sites such as nailbed in 1 patient, extensor area of distal inter-

Table II. Comparison of biomarkers and endothelial function between patients with and without organ involvements.

	ILD (+) n=24	ILD (-) n=36	*p*	DU (+) n=10	DU (-) n=50	*p*	RVSP>30mmHg n=24	RVSP≤30mmHg n=36	*p*
Anti-topoisomerase I	12 (50.0)	3 (8.3)	0.0003	5 (50.0)	10 (20.0)	0.046	8 (33.3)	7 (19.4)	*NS*
Anti-centromere	5 (20.8)	26 (72.2)	<0.0001	2 (20.0)	29 (58.0)	0.028	9 (37.5)	22 (61.1)	0.073
Anti-U1-RNP	4 (16.7)	2 (5.6)	*NS*	1 (10.0)	5 (10.0)	*NS*	2 (8.3)	4 (11.1)	*NS*
Anti-RNA polymerase III	2 (8.3)	1 (2.8)	*NS*	1 (10.0)	2 (4.0)	*NS*	2 (8.3)	1 (2.8)	*NS*
GDF-15 (pg/ml)	1911 (1306-3307)	766 (618-1338)	<0.0001	2315 (1785-2999)	1076 (647-1668)	0.0094	1667 (780-2672)	856 (624-1581)	0.018
P*l*GF (pg/ml)	17.0 (13.2-20.3)	9.2 (7.2-14.4)	0.0010	19.5 (14.1-21.3)	10.8 (7.6-16.6)	0.0044	14.1 (8.6-19.4)	13.0 (7.0-17.0)	*NS*
VEGF (pg/ml)	385 (234-552)	338 (157-567)	*NS*	385 (287-588)	384 (160-557)	*NS*	376 (149-538)	385 (228-561)	*NS*
PTX3 (ng/ml)	2.8 (2.1-3.5)	2.8 (2.1-3.5)	*NS*	3.45 (2.65-3.91)	2.73 (1.97-3.29)	0.0058	2.89 (2.32-3.73)	2.79 (2.0-3.2)	*NS*
EndoPAT-RHI	1.24 (1.06-1.48)	1.64 (0.72)	0.006	1.06 (0.63-1.10)	1.58 (1.45-2.19)	0.0005	1.46 (1.07-1.94)	1.61 (1.39-2.12)	*NS*

Data expressed as median (interquartile range) or as number (percentage).
Mann-Whitney's U test and $\chi 2$ test (Fisher's exact probability test when appropriate).
DU: digital ulcer; GDF-15: growth differentiation factor 15; ILD: interstitial lung disease; P*l*GF: placenta growth factor; VEGF: vascular endothelial growth factor; PTX3: pentraxin 3; RHI: reactive hyperaemia index; RVSP: right ventricular systolic pressure; NS: not significant

Table III. Multivariate logistic analysis.

		dcSSc			ILD			DU			RVSP>30		
		OR	95%CI	*p*	OR	97%CI	*p*	OR	98%CI	*p*	OR	99%CI	*p*
GDF-15	100 increase	1.19	1.07-1.39	0.0003	1.13	1.03-1.26	0.0050	1.08	0.98-1.21	*NS*	1.08	1.02-1.15	0.0054
P*l*GF	1 increase	1.01	0.89-1.15	*NS*	1.03	0.93-1.15	*NS*	1.21	1.00-1.61	0.048			
PTX3	1 increase							1.57	0.79-3.73	*NS*			
EndoPAT-RHI	1 decrease	5.46	1.58-27.3	0.0059	3.01	1.12-9.52	0.028	72.4	6.51-6785	<0.0001			

DU: digital ulcer; GDF-15: growth differentiation factor 15; ILD: interstitial lung disease; PlGF: placenta growth factor; VEGF: vascular endothelial growth factor; PTX3: pentraxin 3; RHI: reactive hyperaemia index; RVSP: right ventricular systolic pressure; dcSSc: diffuse cutaneous systemic sclerosis; NS: not significant.

phalangeal joint in 1 patient, and lateral aspect in 1 patient. There was no difference of biomarkers and EndoPAT-RHI by this distribution of DUs or treatment such as Calcium channel blockers and antiaggregants. In addition, GDF-15, P*l*GF, and EndoPAT-RHI respectively correlated with abnormalities of pulmonary function (GDF-15 *vs*. %VC; rs = -0.42, *p*=0.0009, GDF-15 *vs*. %DLco; rs = -0.43, *p*=0.0008: P*l*GF *vs*. %VC; rs=-0.14, *p*=0.30, P*l*GF *vs*. %DLco; rs = -0.39, *p*=0.0028: EndoPAT-RHI *vs*. %VC; rs=0.30, *p*=0.026, EndoPAT-RHI *vs*. %DLco; rs = 0.34, *p*=0.011).

*Multivariate analysis predicting dcSSc and organ involvements of SS*c

Multivariate analysis was performed to determine whether serum variables or EndoPAT-RHI are predictive of the presence of dcSSc or organ involvements of SSc. As shown in Table III, the increment of GDF-15 was highly predictive toward dcSSc, ILD or RVSP >30 mmHg. In addition, P*l*GF for DU was also found. Conversely, a decrease of EndoPAT-RHI was predictive of the presence of dcSSc, ILD or DU, but the PTX3 level was not predictive.

Correlations among biomarkers

Table IV shows the correlations among biomarkers including GDF-15, P*l*GF, endostatin, PTX3, VEGF and Endo-PAT-RHI. There was a significant positive correlation between GDF-15 and P*l*GF or endostatin. In addition, we identified non-significant trends of a negative correlation of GDF-15 with EndoPAT-RHI and of P*l*GF with Endo-PAT-RHI. No correlations were found among other biomarkers.

Discussion

Regarding serum or plasma biomarkers, we focused on the factors that are considered to be deeply involved in microvascular injury in patients with SSc (2, 5-9, 11-21) Overall, we found high serum concentrations of GDF-15, P*l*GF, endostatin and VEGF in SSc patients compared with healthy controls. Plasma concentrations of PTX3 also tended to be high in SSc patients. Impairment in the hyperemic response calculated by EndoPAT-RHI has been shown to correlate or predict coronary artery endothelial dysfunction or cardiovascular death in atherosclerosis patients (36-38); in addition, this is the first analysis of EndoPAT-RHI in SSc patients in relation with biomarkers. We obtained the following new insights highlighting the association of these markers with organ involvement. First, among the biomarkers examined, GDF-15 is considered the principal biomarker associated with the pathological processes of SSc, since GDF-15 has been well correlated with organ involvement. GDF-15, also known as macrophage inhibitory cytokine-1, is a member of the transforming growth factor β (TGFβ) superfamily. Recently, GDF-15 has been reported to be associated with the development of pulmonary hypertension (6) and cutaneous and pulmonary fibrosis (7, 8) in SSc patients. Since our present data indicate the association of serum GDF-15 concentrations with dcSSc, ILD or RVSP >30, GDF-15 appears to reflect a general role in the fibrotic process of

Table IV. Correlations among biomarkers.

	P*l*GF		VEGF		PTX3		EndoPAT-RHI	
	rs	*p*	*rs*	*p*	*rs*	*p*	*rs*	*p*
GDF-15	0.43	0.0008	-0.13	0.31	0.05	0.73	-0.18	0.18
P*l*GF	-	-	0.01	0.97	0.09	0.51	-0.16	0.23
VEGF	-	-	-	-	-0.04	0.74	-0.09	0.50
PTX3	-	-	-	-	-	-	0.01	0.95

Correlations were assessed with Spearman's correlation coefficient test.
GDF-15: growth differentiation factor 15; P*l*GF: placenta growth factor; VEGF: vascular endothelial growth factor; PTX3: pentraxin 3; RVSP: right ventricular systolic pressure.

SSc. Lambrecht *et al*. suggested that increased GDF-15 in the blood originates from affected organs such as the lung and skin (7) However, they also found that GDF-15 is not indispensable for fibrosis development (7), which might explain the lack of significant relation of GDF-15 with the DU of the present data despite GDF-15 being higher in subsets of the DU+ group.

P*l*GF was also recently studied as a predictor of vascular complications (5, 12) P*l*GF, a secreted dimeric glycoprotein very similar to VEGF, has been shown to be chemotactic and mitogenic for endothelial cells *in vitro* (39) and proangiogenic for endothelial cells *in vivo* (40) Consistent with a previous report (12), the multivariate logistic analysis of the present study confirmed the previous observation that the P*l*GF level predicts the presence of DU. Although the P*l*GF level was high in our patients with dcSSc as in previous reports (10, 13), the association by multivariate logistic analysis did not reach statistical significance. However, there was a clear positive correlation of P*l*GF with GDF-15, identified as a predictor of dcSSc. Thus, P*l*GF might be important in skin fibrosis such as dcSSc.

Endostatin is a proteolytic fragment of type XVIII collagen that acts as an angiogenesis inhibitor. Endostatin exerts its anti-angiogenetic effect by its interaction with several endothelial cell surface receptors as it competes for the receptors of VEGF (41). The present study showed that endostatin is higher in patients with SSc, especially in patients complicated with DU, supporting previous studies (15-17, 42). Endostatin might participate in the occurrence of ischaemic manifestations in SSc (15). Endostatin might negatively regulate against excessive angiogenesis due to VEGF. Also, previous investigations have revealed an increased serum concentration of VEGF or PTX3 in patients with SSc (18, 20, 21). Our present data also showed a high serum concentration of VEGF in SSc patients compared with controls but no clear association of VEGF with the organ damages of SSc. The association of VEGF with organ involvement of SSc has been controversial; thus, larger sample numbers with prospective observations are necessary to reveal the role of VEGF in the pathologic manifestations of SSc. PTX3 is a pleiotropic pattern-recognition protein that acts as an antiangiogenic factor by binding to fibroblast growth factor 2 (FGF-2) and inhibiting FGF-2-dependent EC proliferation and neovascularisation (20, 43, 44) PTX3 in the present study tended to be higher in SSc patients compared with controls, but not significantly so. Previous studies have found associations of PTX3 concentration with mRTSS, ILD, DU, and cardiac involvements of SSc (20, 21), and these associations could have been responsible for differences in the patient characteristics within the population studied.

Nailfold capillaroscopy is widely accepted as a diagnostic tool for SSc and is included in the 2013 classification criteria for SSc (28). Abnormalities detected by nailfold capillaroscopy reflect morphological alterations, whereas EndoPAT-RHI reflects changes of endothelial function (22). Our present data showed low EndoPAT-RHI in SSc patients as found in previous studies (25, 26); moreover, it was the first to show that low EndoPAT-RHI predicts the presence of organ involvement of SSc. These EndoPAT-RHI data may support the consensus that SSc is an immune-mediated vascular and fibrotic disease. Since an association of nailfold capillaroscopy abnormalities and internal organ involvement of SSc has been previously reported (45), further studies investigating the relation of EndoPAT-RHI with nailfold capillaroscopy abnormalities in conjunction with biomarkers are required. Our preset data showed EndoPAT-RHI was low in patients with DU. Machin *et al*. reported SSc patients with history of DU had endothelial dysfunction measured by flow-mediated dilatation (FMD) (46).

As a limitation of this study, a frequency of patient with lcSSc was much higher than that of patient with dcSSc (44 lcSSc *vs*. 16 dcSSc). This result may be affected by shorter disease duration without extension of scleroderma of patients with SSc in this study. In previous report of 405 Japanese SSc patients with disease durations of 13-15 years, lcSSc was twice as frequent as dcSSc (47). Although the positivity of anti-centromere antibody was higher in this study, distribution of autoantibodies was not much different from that in the previous report (47). Endothelial dysfunction markers such as soluble adhesion molecules associate with vasculopathy in SSc (48, 49). If we have measured these markers, we may have additional information. Also, nailfold videocapillaroscopy (NVC) is a useful diagnostic tool in SSc (28, 50). If we have examined NVC, we could have analysed associations between NVC alterations and indicators examined in this study in detail.

In conclusion, this is the first study to inclusively investigate the relationship among biomarkers, EndoPAT-RHI and organ involvement in patients with SSc. Our data have demonstrated the complex pathological progression of SSc through fibrotic impairment and microvascular damage.

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References

1. DESBOIS AC, CACOUB P: Systemic sclerosis: An update in 2016. *Autoimmun Rev* 2016; 15: 417-26.
2. DENTON CP, ONG VH: Targeted therapies for systemic sclerosis. *Nat Rev Rheumatol* 2013; 9: 451-64.
3. HARRIS ML, ROSEN A: Autoimmunity in scleroderma: the origin, pathogenetic role, and clinical significance of autoantibodies. *Curr Opin Rheumatol* 2003; 15: 778-84.
4. VARGA J, ABRAHAM D: Systemic sclerosis: a prototypic multisystem fibrotic disorder. *J Clin Invest* 2007; 117: 557-67.
5. MCMAHAN Z, SCHOENHOFF F, VAN EYK JE, WIGLEY FM, HUMMERS LK: Biomarkers of pulmonary hypertension in patients with scleroderma: a case-control study. *Arthritis Res Ther* 2015; 17: 201.
6. MEADOWS CA, RISBANO MG, ZHANG L *et al*.: Increased expression of growth differentiation factor-15 in systemic sclerosis-associated pulmonary arterial hypertension. *Chest* 2011; 139: 994-1002.
7. LAMBRECHT S, SMITH V, DE WILDE K *et al*.: Growth differentiation factor 15, a marker of lung involvement in systemic sclerosis, is involved in fibrosis development but is not indispensable for fibrosis development. *Arthritis Rheumatol* 2014; 66: 418-27.
8. YANABA K, ASANO Y, TADA Y, SUGAYA M, KADONO T, SATO S: Clinical significance of serum growth differentiation factor-15 levels in systemic sclerosis: association with disease severity. *Mod Rheumatol* 2012; 22: 668-75.
9. HASEGAWA M: Biomarkers in systemic sclerosis: Their potential to predict clinical courses. *J Dermatol* 2016; 43: 29-38.
10. DUNNE JV, KEEN KJ, VAN EEDEN SF: Circulating angiopoietin and Tie-2 levels in systemic sclerosis. *Rheumatol Int* 2013; 33: 475-84.
11. CARMELIET P, MOONS L, LUTTUN A *et al*.: Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. *Nat Med* 2001; 7: 575-83.
12. AVOUAC J, MEUNE C, RUIZ B *et al*.: Angiogenic biomarkers predict the occurrence of digital ulcers in systemic sclerosis. *Ann Rheum Dis* 2012; 71: 394-9.
13. HAMAGUCHI Y, HASEGAWA M, TANAKA C *et al*.: Elevated serum placenta growth factor (PlGF) levels in patients with systemic sclerosis: a possible role in the development of skin but not lung fibrosis. *J Dermatol Sci* 2010; 58: 229-31.
14. MOSTMANS Y, CUTOLO M, GIDDELO C *et al*.: The role of endothelial cells in the vasculopathy of systemic sclerosis: A systematic review. *Autoimmun Rev* 2017; 16: 774-86.
15. REISETER S, MOLBERG Ø, GUNNARSSON R *et al*.: Associations between circulating endostatin levels and vascular organ damage in systemic sclerosis and mixed connective tissue disease: an observational study. *Arthritis Res Ther* 2015; 17: 231.
16. HEBBAR M, PEYRAT JP, HORNEZ L, HATRON PY, HACHULLA E, DEVULDER B: Increased concentrations of the circulating angiogenesis inhibitor endostatin in patients with systemic sclerosis. *Arthritis Rheum* 2000; 43: 889-93.
17. ALMEIDA I, OLIVEIRA GOMES A, LIMA M, SILVA I, VASCONCELOS C: Different contributions of angiostatin and endostatin in angiogenesis impairment in systemic sclerosis: a cohort study. *Clin Exp Rheumatol* 2016; 34 (Suppl. 100): S37-42.
18. DISTLER O, DEL ROSSO A, GIACOMELLI R *et al*.: Angiogenic and angiostatic factors in systemic sclerosis: increased levels of vascular endothelial growth factor are a feature of the earliest disease stages and are associated with the absence of fingertip ulcers. *Arthritis Res* 2002; 4: R11.
19. KURYLISZYN-MOSKAL A, KLIMIUK PA, SIERAKOWSKI S: Soluble adhesion molecules (sVCAM-1, sE-selectin), vascular endothelial growth factor (VEGF) and endothelin-1 in patients with systemic sclerosis: relationship to organ systemic involvement. *Clin Rheumatol* 2005; 24: 111-6.
20. SHIRAI Y, OKAZAKI Y, INOUE Y *et al*.: Elevated levels of pentraxin 3 in systemic sclerosis: associations with vascular manifestations and defective vasculogenesis. *Arthritis Rheumatol* 2015; 67: 498-507.
21. IWATA Y, YOSHIZAKI A, OGAWA F *et al*.: Increased serum pentraxin 3 in patients with systemic sclerosis. *J Rheumatol* 2009; 36: 976-83.
22. BLUM A, NAHIR M: Future non-invasive imaging to detect vascular plaque instability and subclinical non-obstructive atherosclerosis. *J Geriatr Cardiol* 2013; 10: 178-85.
23. HAMBURG NM, KEYES MJ, LARSON MG *et al*.: Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham Heart Study. *Circulation* 2008; 13: 2467–74.
24. TORIMOTO K, OKADA Y, MORI H, TANAKA Y: Relationship between fluctuations in glucose levels measured by continuous glucose monitoring and vascular endothelial dysfunction in type 2 diabetes mellitus. *Cardiovasc Diabetol* 2013; 12: 1.
25. PELED N, SHITRIT D, FOX BD *et al*.: Peripheral arterial stiffness and endothelial dysfunction in idiopathic and scleroderma associated pulmonary arterial hypertension. *J Rheumatol* 2009; 36: 970-5.
26. DOMSIC RT, DEZFULIAN C, SHOUSHTARI A *et al*.: Endothelial dysfunction is present only in the microvasculature and microcirculation of early diffuse systemic sclerosis patients. *Clin Exp Rheumatol* 2014; 32 (Suppl. 86): S154-60.
27. KAWASHIRI SY, UEKI Y, TERADA K, YAMASAKI S, AOYAGI K, KAWAKAMI A: Improvement of plasma endothelin-1 and nitric oxide in patients with systemic sclerosis by bosentan therapy. *Rheumatol Int* 2014; 34: 221-5.
28. VAN DEN HOOGEN F, KHANNA D, FRANSEN J *et al*.: 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League against Rheumatism collaborative initiative. *Arthritis Rheum* 2013; 65: 2737-47.
29. LEROY EC, BLACK C, FLEISCHMAJER R *et al*.: Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. *J Rheumatol* 1988; 15: 202-5.
30. CLEMENTS PJ, LACHENBRUCH PA, SEIBOLD JR *et al*.: Skin thickness score in systemic sclerosis: an assessment of interobserver variability in 3 independent studies. *J Rheumatol* 1993; 20: 1892-6.
31. HUGHES M, HERRICK AL: Digital ulcers in systemic sclerosis. *Rheumatology* (Oxford) 2017; 56: 14-25.
32. KORN JH, MAYES M, MATUCCI-CERINIC M *et al*.: Digital ulcers in systemic sclerosis: prevention by treatment with bosentan, an oral endothelin receptor antagonist. *Arthritis Rheum* 2004; 50: 3985-93.
33. HACHULLA E, HATRON PY, CARPENTIER P *et al*.: Efficacy of sildenafil on ischaemic digital ulcer healing in systemic sclerosis: the placebo-controlled SEDUCE study. *Ann Rheum Dis* 2016; 75: 1009-15.
34. HAMBURG NM, PALMISANO J, LARSON MG *et al*.: Relation of brachial and digital measures of vascular function in the community: the Framingham heart study. *Hypertension* 2011; 57: 390-6.
35. MULUKUTLA SR, VENKITACHALAM L, BAMBS C *et al*.: Black race is associated with digital artery endothelial dysfunction: results from the Heart SCORE study. *European Heart J* 2010; 31: 2808-15.
36. KUVIN JT, PATEL AR, SLINEY KA *et al*.: Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. *Am Heart J* 2003; 146: 168-74.
37. BONETTI PO, PUMPER GM, HIGANO ST, HOLMES DR JR, KUVIN JT, LERMAN A: Noninvasive identification of patients with early coronary atherosclerosis by assessment of digital reactive hyperemia. *J Am Coll Cardiol* 2004; 44: 2137-41.
38. HAMBURG NM, KEYES MJ, LARSON MG *et al*.: Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham Heart Study. *Circulation* 2008; 117: 2467-74.
39. MAGLIONE D, GUERRIERO V, VIGLIETTO G, DELLI-BOVI P, PERSICO MG: Isolation of a human placenta cDNA coding for a protein related to the vascular permeability factor. *Proc Natl Acad Sci USA* 1991; 88: 9267-71.
40. ZICHE M, MAGLIONE D, RIBATTI D *et al*.: Placenta growth factor-1 is chemotactic, mitogenic, and angiogenic. *Lab Invest* 1997; 76: 517-31.
41. SKOVSETH DK, VEUGER MJ, SORENSEN DR,

DE ANGELIS PM, HARALDSEN G: Endostatin dramatically inhibits endothelial cell migration, vascular morphogenesis, and perivascular cell recruitment *in vivo*. *Blood* 2005; 105: 1044-51.

42. FAROUK HM, HAMZA SH, EL BAKRY SA *et al*.: Dysregulation of angiogenic homeostasis in systemic sclerosis. *Int J Rheum Dis* 2013; 16: 448-54.
43. MANTOVANI A, GARLANDA C, DONI A, BOTTAZZI B: Pentraxins in innate immunity: from C-reactive protein to the long pentraxin PTX3. *J Clin Immunol* 2008; 28: 1-13.
44. RUSNATI M, CAMOZZI M, MORONI E *et al*.: Selective recognition of fibroblast growth factor-2 by the long pentraxin PTX3 inhibits angiogenesis. *Blood* 2004; 104: 92-9.
45. DE SANTIS M, CERIBELLI A, CAVACIOCCHI F *et al*.: Nailfold videocapillaroscopy and serum VEGF levels in scleroderma are associated with internal organ involvement. *Auto Immun Highlights* 2016; 7: 5.
46. MACHIN DR, CLIFTON HL, RICHARDSON RS, WRAY DW, DONATO AJ, FRECH TM: Acute oral tetrahydrobiopterin administration ameliorates endothelial dysfunction in systemic sclerosis. *Clin Exp Rheumatol* 2017; 35 (Suppl. 106): S167-72.
47. HASHIMOTO A, ENDO H, KONDO H, HIROHATA S: Clinical features of 405 Japanese patients with systemic sclerosis. *Mod Rheumatol* 2012; 22: 272-9.
48. COSSU M, ANDRACCO R, SANTANIELLO A *et al*.: Serum levels of vascular dysfunction markers reflect disease severity and stage in systemic sclerosis patients. *Rheumatology* (Oxford) 2016; 55: 1112-6.
49. REHBERGER P, BECKHEINRICH-MROWKA P, HAUSTEIN UF, STICHERLING M: Prostacyclin analogue iloprost influences endothelial cell-associated soluble adhesion molecules and growth factors in patients with systemic sclerosis: a time course study of serum concentrations. *Acta Derm Venereol* 2009; 89: 245-9.
50. CUTOLO M, SMITH V: State of the art on nailfold capillaroscopy: a reliable diagnostic tool and putative biomarker in rheumatology? *Rheumatology* (Oxford) 2013; 52: 1933-40.