Abnormal plasma levels of different angiogenic molecules are associated with different clinical manifestations in patients with systemic sclerosis

V. Riccieri, K. Stefanantoni, M. Vasile, V. Macrì, I. Sciarra, N. Iannace, C. Alessandri, G. Valesini

Rheumatological Unit, Department of Internal Medicine and Clinical Specialities, University of Rome "Sapienza", Rome, Italy.

Valeria Riccieri, MD
Katia Stefanantoni, MD
Massimiliano Vasile, MD
Valeria Macrì, MD
Iiana Sciarra, MD
Nicoletta Iannace, MD
Cristiano Alessandri, MD
Guido Valesini, MD

Please address correspondence and reprint requests to:
Dr Valeria Riccieri
Divisione di Reumatologia,
Università Sapienza,
00161 Roma, Italy.
E-mail: valeria.riccieri@uniroma1.it

Received on September 21, 2010; accepted in revised form on April 21, 2011.

Clin Exp Rheumatol 2011; 29 (Suppl. 65): S46-S52.

Key words: systemic sclerosis, angiogenesis, angiogenetic factors, vascular disease.

Competing interests: none declared.

ABSTRACT

Objective. *Systemic sclerosis (SSc) is characterised by microvascular damage due to an impairment of different angiogenic and angiostatic factors. The aim of this study was to measure plasma levels of nine molecules involved in these vascular processes in a group of SSc patients, with respect to healthy controls (NC).*

Methods. *Sixty-five patients (M/F=2/63; mean age=57.29 yrs; mean disease duration=9.63 yrs) with established SSc according to ARA criteria, and sixteen age- and sex- matched NC were enrolled. Plasma levels of vascular endothelial growth factor (VEGF), angiopoietin-2 (Ang-2), platelet derived growth factor- bb (PDGF-BB), platelet endothelial cellular adhesion molecule-1 (PECAM-1), leptin, hepathocyte growth factor (HGF), follistatin, granulocyte-colony stimulating factor (G-CSF) and interleukin 8 (IL-8) were measured using commercially available immunoassay kits (Human Angiogenesis 9-Plex Panel, Bio-Rad Laboratories).*

Results. *We detected a significant increase of Ang-2 (median value 1315.4 pg/ml vs. 538.73 pg/ml; p=0.0292), HGF (median value 2886.16 pg/ml vs 1296.16 pg/ml; p=0.0001), IL-8 (median value 32.22 pg/ml vs. 16.86 pg/ml; p=0.02), leptin (median value 32589.1 pg/mg vs. 10679.61 pg/ml; p=0.0065), PDGF-BB (median value 7258.6 pg/ml vs. 2913.44 pg/ml; p=0.0005), PECAM-1(median value 21681.81 pg/ml vs. 10354.53 pg/ml; p=0.0003) and VEGF (median value 236.72 pg/ml vs. 122,905 pg/ml; p=0.0073) in patients with SSc with respect to NC.*

Higher levels of PDGF-BB (p=0.03) and PECAM-1 (p=0.05) were found in patients with digital ulcers while lower levels of PECAM-1 were found in patients with pulmonary hypertension (PH). Besides, levels of IL-8 were higher in patients with PH (p=0.04) and lower in those with pulmonary fibrosis (p=0.05), while levels of Ang-2 were higher in those with a "late" nailfold video-capillaroscopy (NVC) pattern with respect to those with an "early/active" one (p=0.05).

Moreover, plasma levels of VEGF (p=0.02) and PDGF-BB (p=0.04) were significantly higher in those patients positive for anti-topoisomerase 1 antibodies.

Conclusion. *Our findings show significantly higher circulating levels of seven angiogenic parameters in SSc patients, thus reflecting the disregulation of endothelium in this disease. Abnormal levels of these molecules may be considered an attempt for compensatory although ineffective mechanisms of vascular function, leading to the development of the main clinical manifestations of SSc.*

Introduction

Systemic sclerosis (SSc) is a connective tissue disease clearly characterised by a vascular involvement. Raynaud's phenomenon is almost always the earliest clinical feature. The involvement of both small arteries and microvessels leads to chronic tissue hypoxia and contributes to tissue fibrosis thus leading to internal organ damage (1).

Angiogenesis is a complex process regulated by many different factors with angiogenic and angiostatic properties. Normally their functions are balanced but under certain conditions, such as hypoxia and inflammation, angiogenic factors are induced, thus initiating angiogenesis.

In SSc, despite the reduced blood flow and the presence of a hypoxic condition, there is paradoxically no evidence for a sufficient angiogenetic response,

(2) This is clearly demonstrated by nailfold capillaroscopy changes during the disease evolution, where disturbed angiogenesis is related to many different morphological changes such as megacapillaries, ramification, bizarries of the capillaries. In the later stages, disturbed angiogenesis is clearly defined by capillary loss and the presence of the so-called avascular areas (3).

These microvascular abnormalities seem to be strictly related to an impairment of different angiogenic and angiostatic factors, all differently contributing to the development of the disease lesions, acting as key regulators of blood vessel formation (4, 5).

Among the different angiogenesis inducers, vascular endothelial growth factors (VEGF), angiopoietin-2 (Ang-2), platelet derived growth factor-BB (PDGF-BB), platelet endothelial cellular adhesion molecule-1 (PECAM-1), hepatocyte growth factor (HGF) and leptin seem to be highly involved in several steps of physiological and pathological angiogenic processes, such as proliferation, maturation and survival of new blood vessels (4-7).

Some of these molecules have been separately examined on SSc sera leading often to conflicting results and the lack of a sufficient response to hypoxia and the consequent vascular changes in SSc, might be explained by an impairment on those mechanisms differently mediated by these angiogenic or angiostatic factors (6-9).

The aim of this study was to measure a group of angiogenic molecules, rarely investigated in SSc patients, looking for any possible correlation with the main clinical, demographic and laboratory parameters.

Materials and methods

Sixty-five consecutive patients (63 women and 2 men; mean age = 52.7 yrs; mean disease duration = 9.63 yrs) fulfilling the SSc classification criteria proposed by the American College of Rheumatology (ACR) (10) were recruited from the Division of Rheumatology, University of Rome "Sapienza", giving their informed consent. The study was also approved by the local ethics committee. The clinical, and laboratory data reported in this study were obtained at the time the blood samples were drawn. Control samples were obtained from 16 healthy subjects, matched for sex and age, who had no evidence of Raynaud's phenomenon.

Patients had a detailed clinical assessment and their organ system involvement was defined as previously described (11): lung = bibasilar pulmonary fibrosis on chest radiography; isolated pulmonary hypertension = clinical evidence of pulmonary hypertention and increased mean pulmonary arterial pressure (>35 mmHg), indirectely assessed by echocardiography, in the absence of severe pulmonary interstitial fibrosis; oesophagus = hypomotility shown by barium radiography; joint = inflammatory polyarthralgias or arthritis; heart = pericarditis, congestive heart failure, or arhythmias requiring treatment. HRCT was performed in each case showing negative x-ray and the diagnosis of pulmonary fibrosis was done by a radiologist, blinded reading, basing on the presence either of bilateral basilar reticulo-nodular changes on x-ray or of "ground glass" appearance of the lung parenchyma on HRTC.

The cutaneous evaluation included: presence/absence of Raynaud's phenomenon, modified skin score (mSS) (12), presence of digital ulcers, defined as a loss of ephitelialisation and tissues involving the epidermid, the dermis and the subcutaneous tissue, teleangectaesia, calcinosis, defined as deposits of calcium in soft tissues eye visible or confirmed by x-ray (13).

All patients underwent a nailfold videocapillaroscopy (NVC) as previously described (14, 15).

All blood samples were stored at -20° C till used.

Antinuclear antibodies (ANA) including anti-centromere antibodies were detected by indirect immunofluorescence using HEp-2 cell line as substrate (Binding Site). Antibodies against topoisomerase I (anti-Scl70) were measured using enzyme-linked immunosorbent assays (ELISA) (Diamedix, Miami, FL).

Plasma levels of vascular endothelial growth factor (VEGF), angiopoietin-2 (Ang-2), platelet derived growth factor- bb (PDGF-BB), platelet endothelial cellular adhesion molecule-1 (PECAM-1), leptin, hepathocyte growth factor (HGF), follistatin, granulocyte-colony stimulating factor (G-CSF) and interleukin 8 (IL-8) were measured using commercially available multiplex bead-based sandwich immunoassay kits (Human Angiogenesis 9-Plex Panel, Bio-Rad Laboratories).

Assays were performed following the manufacturer's instructions. The system allows simultaneous identification of different molecules in a 96-well filter plate. Briefly, 9 distinct sets of fluorescently dyed beads loaded with capture monoclonal antibodies specific for each molecule to be tested, were used. The appropriate molecule standards (50 µl/well) and samples diluted (50 µl/well) in plasma diluents were added to a 96-well filter plate and incubated for 30 min at room temperature. After three washes, premixed streptavidin/phycoerythrin was added to each well and incubated for 10 min followed by three more washes. Then beads were resuspended with 125 µl of assay buffer, and the molecule reaction mixture was quantified using the Bio-Plex protein array reader. Data were analysed using the Bio-Plex Manager software version 4.1.1. (Bio-Rad Laboratories). Values presenting a coefficient of variation beyond 12% were discarded before the final data analysis. Samples concentration (pg/mL) of different analytes in the plasma samples were determined by using the standard curves generated in the multiplex assays. Minimum levels of detection (pg/mL) were: 3.27 for VEGF, 11.32 for Ang-2, 5.15 for PDGF-BB, 33.29 for PECAM-1, 100.05 for leptin, 3.08 for HGF, 8.41 for follistatin, 6.66 for G-CSF, 1.6 for IL-8.

Statistical analysis

Data are presented as median (range) and the two-tailed Mann-Whitney U-test and χ^2 (or Fisher's exact test when appropriate) test were performed. Among the scleroderma subjects, Spearman's rank correlation, simple and multivariate analysis (linear regression and ordered logistic regression) was used to analyse correlations between angiogenic factors and clinical manifestations. Statistical significance was set at $p<0.05$.

Table I. Main clinical-demografic and laboratory parameters of 65 SSc patients.

Parameter	Value
Sex (M/F)	2/63
Mean age (yrs)(range)	57.3 (20–81)
Mean disease duration (months) (range)	93 (2–696)
Form (lcSSc - dcSSc)(n / %)	33/51–32/49
Raynaud's phenomenon (n / %)	65/100
Digital ulcers (n / %)	33/51
Telangectasias (n / %)	14/45
mRSS≥14 (n / %)	22/34
Pulmonary fibrosis (n / %)	31/48
Pulmonary hypertension (n / %)	22/33.8
NVC pattern: early (n / %)	22/34
active (n / %)	26/40
late (n / %)	17/26
Anti-centromere antibodies +ve (n / %)	29/44.61
Anti-Topoisomerase 1 antibodies +ve (n / %)	25/38.46

Table II. Comparison of the median (range) plasma levels of 9 different angiogenic molecules in 65 SSc patiens and in 16 NC.

Angiogenetic Factors	Median (range) Plasma levels (SSC) (pg/ml)	Median (range) Plasma levels (NC) (pg/ml)	*p*<
VEGF	236.72 (30.71–1028.66)	122.905 (32.84–546.7)	0.0073
ANG-2	1315.4 (57–9198.16)	538.73 (229.33–2201.99)	0.0292
PDGF-BB	7258.6 (679.88–33210.16)	2913.44 (76.57–16301.8)	0.0005
PECAM-1	21681.81 (3639.57–105306.7)	10354.53 (3337.14–25641.17)	0.0003
Leptin	32589.1 (943.45–82240.69)	10679.61 (1296.96–70272.74)	0.0065
HGF	2886.16 (668.99–9630.09)	1296.16 (645.11–3535.02)	0.0001
IL-8	32.22 (4.6–10943.6)	16.86 (2.37–858.7)	0.02
Follistatin	540.78 (37.64–3140.76)	595.255 (129.16–1342.67)	N.S.
G-CSF	32.055 (6.64–376.82)	50.305 (2.94–467.34)	N.S.

Results

The main clinical-demographic and laboratory parameters of our 65 SSc patients are shown in Table I.

Thirty-two (49.23%) of them had a diffuse cutaneous form of SSc (dcSSc) and 33 (50.77%) had a limited cutaneous SSc (lcSSc). All the patients complained of Raynaud's phenomenon. No patient was an active smoker. Twenty-two patients (33.85%) had a modified skin score (mSS) ≥15 while pulmonary fibrosis was found in 31 cases (47.69%) and isolated pulmonary hypertension (PH) in 22 (33.85%), confirmed by right heart catheterisation in 12 cases. The mean sPAP in echocardiography was 41.3 mmHg with a SD of 5.8. Digital ulcers were present in 33 (50.77%). Anti-topoisomerase I antibodies were present in 25 cases (38.46%), anti-centromere antibodies in 29 (44.61%). An "early" NVC pattern was observed in 22 SSc patients (33.85%), an "active" pattern was found in 26 patients (40%) and a "late" pattern was recognised in 17 cases (26.15%).

We detected a significant increase of VEGF (median value 236.72 pg/ml *vs*. 122.905 pg/ml; p=0.0073), Ang-2 (median value 1315,4 pg/ml *vs*. 538.73 pg/ml; p=0.0292), PDGF-BB (median value 7258.6 pg/ml *vs*. 2913.44 pg/ml; p=0.0005), PECAM-1(median value 21681.81 pg/ml *vs*. 10354.53 pg/ml; p=0.0003),leptin (median value 32589.1 pg/mg *vs*. 10679.61 pg/ml; p=0.0065), HGF (median value 2886.16 pg/ml *vs*. 1296.16 pg/ml; p=0.0001), and IL-8 (median value 32.22 pg/ml *vs*. 16.86 pg/ml; p=0.02), in the 65 SSc patients with respect to NC (Table II, Fig. 1).

Clinical parameters of the scleroderma patients were examined to see if any specific disease manifestations correlated with any of the measured factors. We did not find any association between the clinical parameter and the measured factors with univariate and multivariate analysis. But when clinical characteristics were dichotomised and the two groups were compared with Mann-Whitney test, we found significantly higher levels of PDGF-BB (p=0.03) and PECAM-1 (p=0.05) in those patients with digital ulcers (Fig. 2a and 2b). Moreover, plasma levels of IL-8 were higher in patients with PH (p=0.04) (Fig. 2c) and lower in those with pulmonary fibrosis (p=0.05) (Fig. 2d). Lower levels of PECAM-1 were found in patients with PH (p=0.04) (Fig. 2e) while plasma levels of Ang-2 were significantly higher in patients with a "late" NVC pattern respect to those with an "early/active" NVC pattern (p=0.05) (Fig. 2f). Moreover plasma levels of VEGF (p=0.02) and PDGF-BB (p=0.04) were significantly higher in those patients positive for anti-topoisomerase 1 antibodies (Fig. 2g and 2h). We did not find any significant association concerning age, disease duration, cutaneous form, mSS or treatments.

Discussion

The presence of abnormal vascularity is a well-known feature of SSc. The significant progressive loss of capillaries, as noted by NVC, is probably due to a defect of vascular repair with a decrease of vascular growth. Many pro- and anti-angiogenetic factors have been extensively studied in ScS demonstrating their different involvement in the disease (7, 16).

We investigated 9 different molecules, most of which are rarely investigated in SSc, and we clearly demonstrated abnormal plasma levels of several of them. The elevated levels of three different growth factors such as VEGF, PDGF-BB and HGF, of other angiogenic molecules, Ang-2, leptin, PECAM-1 and of IL-8 cytokine, seem to be an attempt for compensatory although uneffective mechanisms to improve vascular function and to prevent the tissue damage in our SSc patients.

Looking separately at the literature, many of these factors seem to have a role as marker of endothelial cell activity or injury. Some of them have already been found to be raised in patients with SSc and their levels seem to correlate with disease activity, being regulated by therapy in some cases (8, 17).

In fact VEGF is a well-known endothelial stimulating molecule, showing higher levels mainly at the earliest stages of the disease in the absence of digital ulcers (6, 17). The different expression of VEGF in the different stages of SSc may be related to compensatory mechanisms and may have deleterious effects on the vascular network as shown by the inverse association of higher serum VEGF levels with the capillary density on NVC (18, 19).

Moreover, PDGF-BB expression is

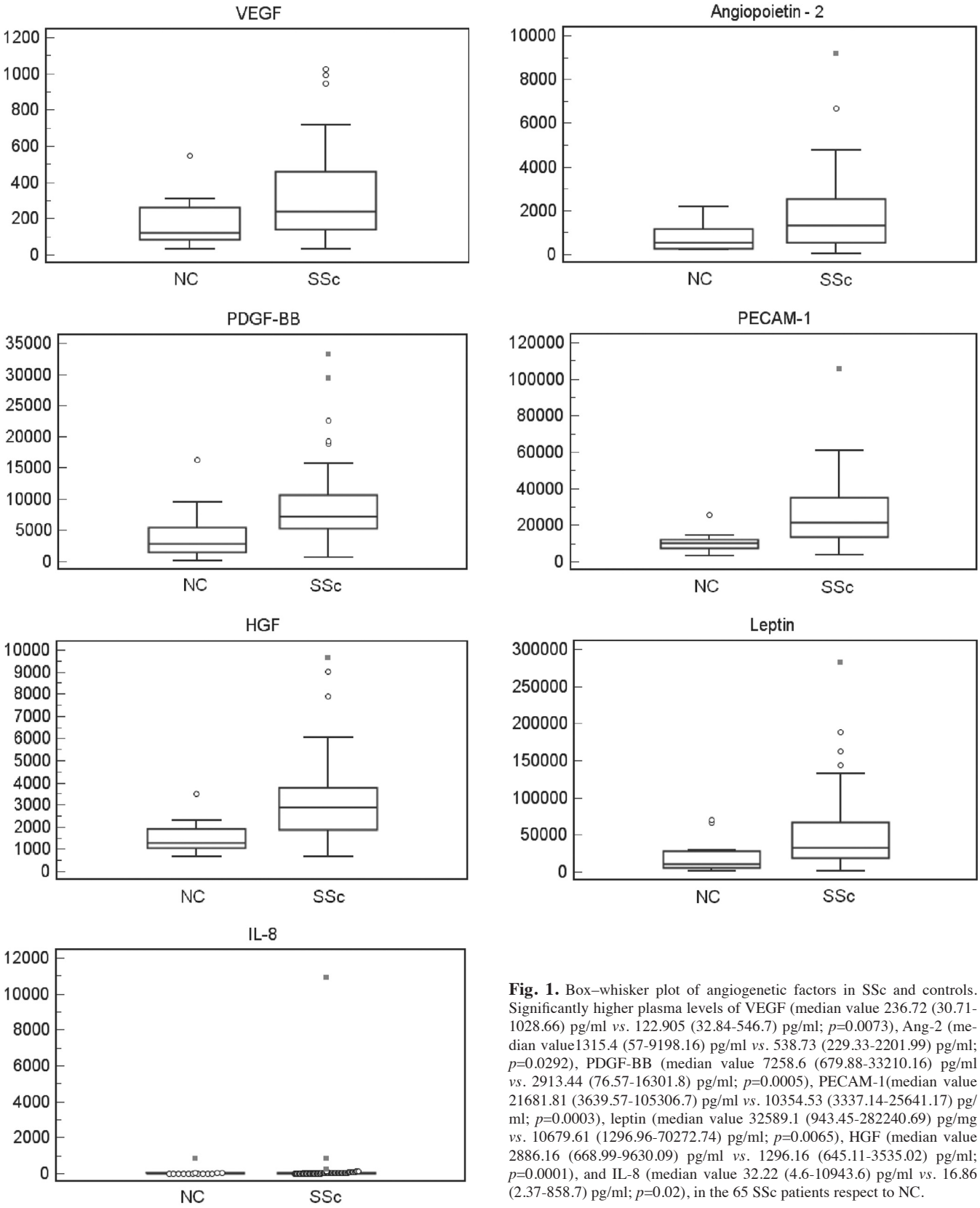

Fig. 1. Box–whisker plot of angiogenetic factors in SSc and controls. Significantly higher plasma levels of VEGF (median value 236.72 (30.71-1028.66) pg/ml *vs*. 122.905 (32.84-546.7) pg/ml; p=0.0073), Ang-2 (median value1315.4 (57-9198.16) pg/ml *vs*. 538.73 (229.33-2201.99) pg/ml; p=0.0292), PDGF-BB (median value 7258.6 (679.88-33210.16) pg/ml *vs*. 2913.44 (76.57-16301.8) pg/ml; p=0.0005), PECAM-1(median value 21681.81 (3639.57-105306.7) pg/ml *vs*. 10354.53 (3337.14-25641.17) pg/ml; p=0.0003), leptin (median value 32589.1 (943.45-282240.69) pg/mg *vs*. 10679.61 (1296.96-70272.74) pg/ml; p=0.0065), HGF (median value 2886.16 (668.99-9630.09) pg/ml *vs*. 1296.16 (645.11-3535.02) pg/ml; p=0.0001), and IL-8 (median value 32.22 (4.6-10943.6) pg/ml *vs*. 16.86 (2.37-858.7) pg/ml; p=0.02), in the 65 SSc patients respect to NC.

regulated by VEGF, it acts in concert with Ang-2 and is essential for capillary stabilisation during the angiogenic process (20, 21).

Higher HGF levels seem to reduce the expression of pro-fibrotic substances such as type I collagen and matrix metalloproteinase-I in SSc dermal fibroblasts while HGF production by the same SSc fibroblasts seems to be increased in an attempt of an anti-fibrotic response (22, 23).

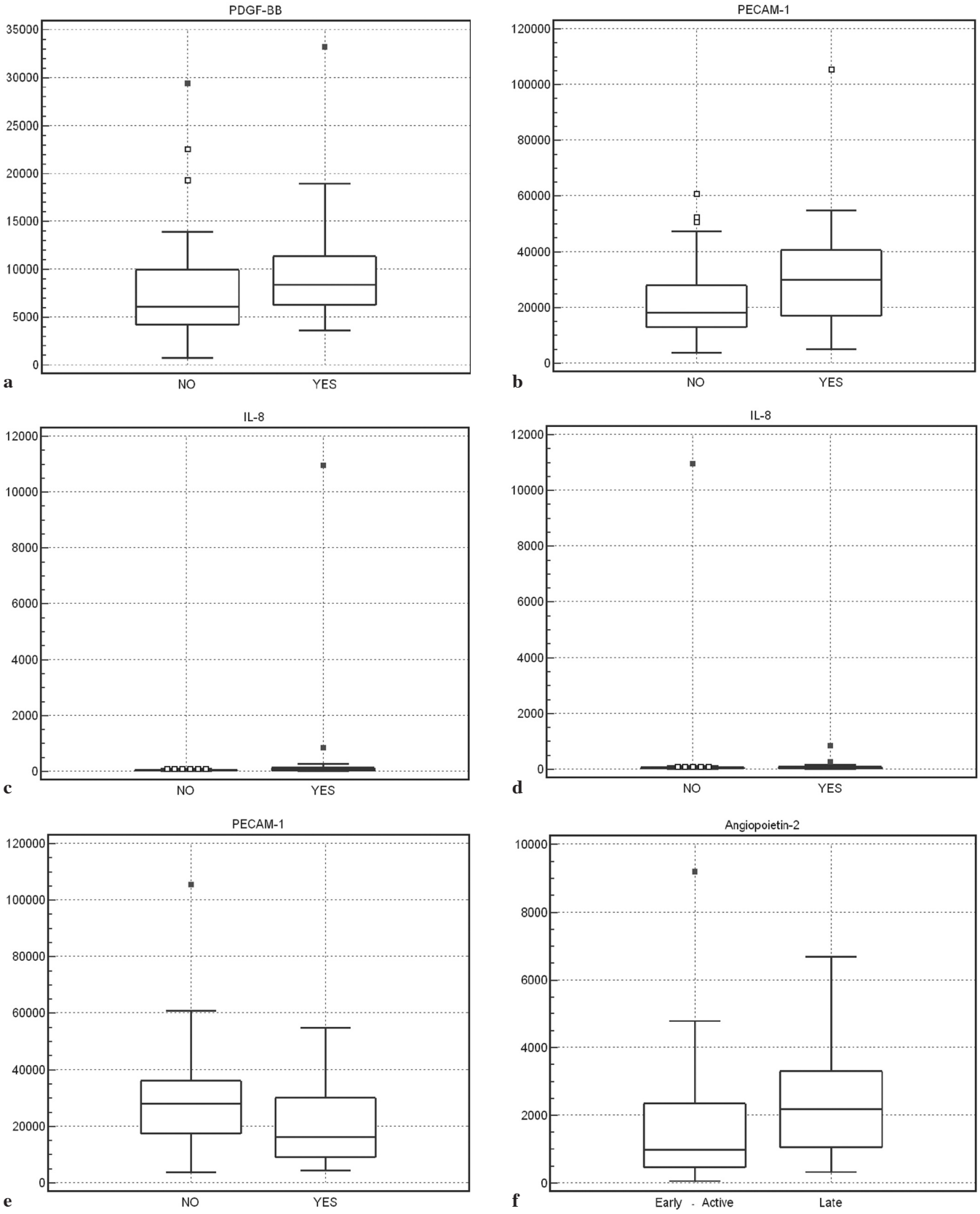

Fig. 2. a-b. Significantly higher plasma levels of PDGF-BB (p=0.03) and PECAM-1 (p=0.05) in patients with digital ulcers.
c. Significantly higher plasma levels of IL-8 in patients with PH (p=0.04).
d. Significantly lower plasma levels of IL-8 in patients with pulmonary fibrosis (p=0.05).
e. Significantly lower levels of PECAM-1 in patients with PH (p=0.04).
f. Significantly higher plasma levels of Ang-2 in patients with a "late" NVC pattern respect to those with an "early/active" (p=0.05).

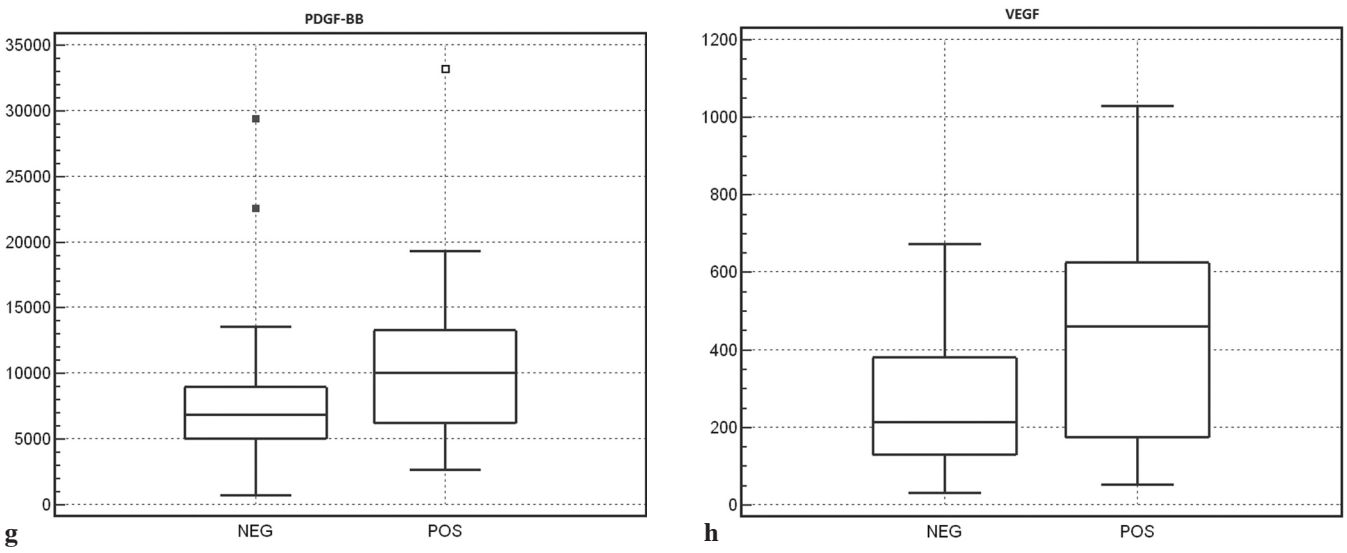

Fig. 2. g-h. Significantly higher plasma levels of VEGF (p=0.02) and PDGF-BB (p=0.04) in those patients positive for anti-topoisomerase 1 antibodies.

ANG-2 is an essential factor for the vascular formation and is normally expressed in the endothelial remodelling tissues, while it is higher expressed during hypoxic processes (5).

Leptine is a polypeptide mainly produced by white fat tissue and its receptors are expressed on the endothelial cells. In fact leptine is able to induce the endothelial cell proliferation and to stimulate neovascularisation (24).

PECAM-1 is overexpressed on the activated endothelial layer and sPECAM-1 levels were higher in SSc sera felling to normal values after anti-endothelin antagonist therapy (17).

Finally, IL-8 is one of the main inflammatory cytokines able to stimulate angiogenesis, and it has been already found elevated in SSc sera, associating with internal organ involvement, mainly with pulmonary fibrosis (25, 26).

In the present study we also found that circulating levels of some angiogenic molecules were associated with more severe vascular manifestations, such as digital ulcers, PH and a "late" capillaroscopy pattern or with the presence of an important marker of more diffuse SSc form, such as anti-topoisomerase 1 antibody.

These findings may implicate a future role for these factors as possible useful prognostic markers of SSc. In particular the association with more severe capillaroscopy abnormalities, as revealed by a "late" NVC pattern is also of interest, considering that this instrumental tool has not been used in so much studies measuring different vascular molecules (6, 18, 27), whereas other authors confirm our findings of advanced capillary changes in those patients presenting higher levels of other vascular markers such as endothelin-1 (27).

The present scarcity of data on the different SSc vascular subtypes does not allow a conclusion on the possible role for pro and anti angiogenic markers in the progression of the endothelial damage. We can only suggest that different molecules act in many different ways for the development and maintenance of this vascular damage in SSc. The cooperation among these angiogenic factors may be essential for the control of all the vascular processes, suggesting that these molecules can be considered as important indicators of endothelial impairment.

To conclude, the abnormal process leading to vascular injury and repair in SSc is presumably due to many differently involved molecules, including those examined in the present study. These molecules are known to act physiologically stimulating vasculogenesis, accelerating maturation and differentiation of endothelial cells, fibroblasts and smooth muscle cells, having a critical role in the maintenance of a stable vascularity. We only described their statistically significant differences in SSc patients *versus* normal subjects, thus hypothesizing that in SSc there may be an imbalance of their regulation and function that leads to vascular injury and to consequent clinical manifestations.

References

1. FLAVAHAN NA, FLAVAHAN S, MITRA S, CHOTANI MA: The vasculopathy of Raynaud's phenomenon and scleroderma. *Rheum Dis Clin North Am* 2003; 29: 275-91.
2. LEROY EC: Systemic sclerosis. A vascular perspective. *Rheum Dis Clin North Am* 1996; 22: 675-94.
3. MARICQ HR: Widefield capillary microscopy: technique and rating scale for abnormalities seen in scleroderma and related disorders. *Arthritis Rheum* 1981; 24: 1159-65.
4. GRUNEWALD M, AVRAHAM I, DOR Y *et al*.: VEGF-induced adult neovascularization: recruitment, retention, and role of accessory cells. *Cell* 2006; 124: 175-89.
5. THOMAS M, AUGUSTIN HG: The role of the angiopoietins in vascular morphogenesis. *Angiogenesis* 2009; 12: 125-37.
6. DISTLER O, DEL ROSSO A, GIACOMELLI R *et al*.: 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 Ther* 2002; 4: R11.
7. HUMMERS LK, HALL A, WIGLEY FM, SIMONS M: Abnormalities in the regulators of angiogenesis in patients with scleroderma. *J Rheumatol* 2009; 36: 576-82.
8. KURYLISZYN-MOSKAL A, KIMIUK 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.
9. SOLANILLA A, VILLENEUVE J, AUGUSTE P *et al*.: The transport of high amounts of vascular endothelial growth factor by blood platelets

underlines their potential contribution in systemic sclerosis angiogenesis. *Rheumatology* 2009; 48: 1036-44.

10. SUBCOMMITTEE FOR SCLERODERMA CRITERIA OF THE AMERICAN RHEUMATISM ASSOCIATION DIAGNOSTIC AND THERAPEUTIC CRITERIA COMMITTEE: Preliminary criteria for the classification of systemic sclerosis (scleroderma). *Arthritis Rheum* 1980; 23: 581-90.
11. STEEN VD, POWELL DL, MEDSGER TA JR: Clinical correlations and prognosis based on serum autoantibodies in patients with systemic sclerosis. *Arthritis Rheum* 1988; 31: 196-203.
12. KAHALED MB, SULTANY GL, SMITH EA: A modified scleroderma skin scoring method. *Clin Exp Rheumatol* 1986; 4: 367-9.
13. AMANZI L, BRASCHI F, FIORE G *et al*.: Digital ulcers in scleroderma: staging, characteristics and sub-setting through observation of 1614 digital lesions. *Rheumatology* 2010; 49: 1374-82.
14. CUTOLO M, SULLI A, PIZZORNI C, ACCARDO S: Nailfold videocapillaroscopy assessment of microvascular damage in systemic sclerosis. *J Rheumatol* 2000; 27: 155-60.
15. RICCIERI V, GERMANO V, ALESSANDRI C *et al*.: More severe nailfold capillaroscopy findings and anti-endothelial cell antibodies. Are they useful tools for prognostic use in systemic sclerosis? *Clin Exp Rheumatol* 2008; 26: 992-7.
16. MULLIGAN-KEHO MJ, SIMONS M: Current concepts in normal and defective angiogenesis: implication for systemic sclerosis. *Curr Rheumatol Rep* 2007; 9: 173-9.
17. IANNONE F, RICCARDI MT, GUIDUCCI S *et al*.: Bosentan regulates the expression of adhesion molecules on circulating T cells and serum soluble adhesion molecules in systemic sclerosis-associated pulmonary arterial hypertension. *Ann Rheum Dis* 2008; 67: 1121-26.
18. CHOI JJ, MIN DJ, CHO ML *et al*.: Elevated vascular endothelial growth factor in systemic sclerosis. *J Rheumatol* 2003; 30: 1529-33.
19. KOCH AE, DISTLER O: Vasculopathy and disordered angiogenesis in selected rheumatic diseases: rheumatoid arthritis and systemic sclerosis. *Arthritis Res Ther* 2007; 9: S3.
20. ONIMARU M, YONEMITSU Y, FUJII T *et al*.: VEGF-C regulates lymphoangiogenesis and capillary stability by regulation of PDGF-B. *Am J Physiol Heart Circ Physiol* 2009; 297: 1685-96.
21. HELLBERG C, OSTMAN A, HELDIN CH: PDGF and vessel maturation. *Recent Results Cancer Res* 2010; 180: 103-14.
22. KAWAGUCHI Y, HARIGAI M, FUKASAWA C, HARA M: Increased levels of hepatocyte growth factor in sera of patients with systemic sclerosis. *J Rheumatol* 1999; 26: 1012-3.
23. JINNIN M, IHN H, MIMURA Y, ASANO Y, YAMANE K, TAMAKI K: Effects of hepatocyte growth factor on the expression of type I collagen and matrix metalloproteinase-1 in normal and scleroderma dermal fibroblasts. *J Invest Dermatol* 2005; 124: 324-30.
24. ANANGOUSTOULIS S, KARAYIANNAKIS AJ, LAMBROPOULOU M, EFTHIMIADOU A, POLYCHRONIDIS A, SIMOPOULOS C: Human leptin induces angiogenesis *in vivo*. *Cytokine* 2008 42: 353-7.
25. FUROSE S, FUJII H, KABURAGI Y *et al*.: Serum concentrations of the CXC chemokines interleukin 8 and growth-regulated oncogene-alpha are elevated in patients with systemic sclerosis. *J Rheumatol* 2003; 30: 1524-8.
26. BRAT DJ, BELLAIL AC, VAN MEIR EG: The role of intereukin-8 and its receptors in gliomagenesis and tumoral angiogenesis. *Neuro Oncol* 2005; 7: 122-33.
27. SULLI A, SOLDANO S, PIZZORNI C *et al*.: Raynaud's phenomenon and plasma endothelin: correlations with capillaroscopy patterns in systemic sclerosis. *J Rheumatol* 2009; 36: 1235-9.