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ORIGINAL ARTICLE

Impaired angiogenesis as a feature of digital ulcers in systemic sclerosis

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Abstract Impaired angiogenesis in systemic sclerosis has a major role in tissue injury pathogenesis. Our objective was to determine whether angiogenic biomarkers (vascular endothelial growth factor (VEGF), endoglin, and endostatin) are related to microvascular damage and to determine their predictive value for new digital ulcers (DU). The main outcome of the study was the occurrence of a new digital ulcer during 3-year follow-up. This prospective longitudinal study was performed between October 2011 and December 2014. Seventy-seven patients definitely diagnosed with systemic sclerosis where divided into two groups: those with active DU at baseline and those with no DU until enrollment. Patients were matched by sex and age with healthy controls. Serum levels of VEGF, endoglin, and endostatin were measured at enrollment, and several nailfold videocapillaroscopies were performed during the 3-year follow-up. Serum levels of VEGF were lower (245.06, 158.68–347.33; $p<0.001$) and those of endoglin were higher (3.013, 1.463–7.023; $p<0.001$) in patients with active DU than those with no DU history (339.49, 202.00–730.93/1.879, 0.840–3.280), and they were higher than those found in controls (178.030, 101.267–222.102)/0.277, 0.154–0.713), respectively. No differences in endostatin levels were found between groups ($p=0.450$). Endoglin was the only biomarker significantly different ($p=0.031$) between patients with diffuse versus limited systemic sclerosis and between early, active, and late patterns ($p=0.020$). VEGF was identified as an independent predictor for the development of new DU. Our study confirmed the relationship between angiogenic vascular biomarkers and the occurrence of DU. Endoglin and VEGF serum levels are potential risk factors, and VEGF has a predictive value for the occurrence of new DU.

Keywords Capillaroscopy · Digital ulcers · Endoglin · Endostatin · Systemic sclerosis · VEGF

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Introduction

Microvascular alterations are key features of systemic sclerosis (SSc). Disease outcome depends on extent and severity of vasculopathy, and frequently, the earliest clinical symptoms are related to the peripheral vascular system [1].

The Raynaud phenomenon (RP) is the earliest clinical finding of SSc, and for many, it is considered the first manifestation of disease. Initially, the RP has only functional implications, but repeated bouts lead to structural abnormalities and digital ischaemia, which may progress to digital ulceration or, in extreme cases, to digital critical ischaemia [2].

Digital ulcers are a true burden for patients because they are very painful, heal slowly, carry high risks of infection, and are extremely disabling, leading to severe impairment of simple daily activities. In adults, 40 to 50 % [3, 4] of patients

experience at least one ulcer in the course of disease and of these, 31 to 71 % will have recurrent ulcers [5].

Endothelial cell (EC) injury results in disorganization of the EC layer favouring an early disorganized capillary architecture with loss of capillaries [1]. In early disease stages, enlarged, giant, meandering, ramified, and bushy capillaries occur in part as a result of EC injury and subsequent vascular remodelling [6]. With disease progression, capillary loss might result from an uncompensated endothelial repair through angiogenesis and vasculogenesis, leading to reduced peripheral blood flow with consequent tissue hypoxia and formation of new blood vessels from the pre-existing microvasculature [6–8].

Microvascular structural changes are easily assessed using nailfold videocapillaroscopy (NVC), a simple, non-invasive, inexpensive, and accessible tool. Capillaroscopic patterns are dynamic and probably reflect disease evolution and severity. Several qualitative, semi-quantitative, and quantitative indices have been proposed as predictors of organ involvement and, in particular, digital ulcers (DUs) [2].

Angiogenesis, the creation of new blood vessels from pre-existing ones, depends primarily on the activation, proliferation, and migration of ECs and is driven by angiogenic stimuli that also induce proteolytic enzymes that cleave the extracellular matrix [9]. It is tightly regulated by the balance between pro-angiogenic and anti-angiogenic factors [8]. This event is highly complex and requires a dynamic, temporal, and spatially regulated interaction between ECs, soluble angiogenic growth factors, and extracellular matrix molecules [9].

Several studies have reported impaired angiogenesis in SSc, suggesting immune reactions to viral or environmental factors, reperfusion injury, or to anti-endothelial antibodies [10]. Alternatively, it might be a consequence of an imbalance between angiogenic factors and angiostatic factors.

Angiogenesis biomarkers have been extensively studied in SSc patients and investigated as possible putative biomarkers of organ involvement. Vascular endothelial growth factor (VEGF) is the most widely researched and understood angiogenic mediator; it is a potent angiogenic factor that stimulates migration, proliferation, and survival of ECs and endothelial pre-cursor cells [11].

Distler et al. [8] found increased levels of VEGF in SSc patients without fingertip ulcers, suggesting it has a protective effect. Farouk et al. [12] also demonstrated a significantly higher serum VEGF level in patients in the early phases of SSc without digital ischemic manifestations when compared to those with ischemic manifestations. Avouac et al. [1] reported increased levels of VEGF in the late phases of the disease, suggesting that its upregulation might be an insufficient compensatory mechanism to stimulate angiogenesis and an inverse correlation between capillary density and VEGF.

Endoglin (CD105 or ENG) is a co-receptor for TGF-β predominantly expressed on cell surfaces of ECs. In SSc patients, this angiostatic biomarker has been measured and correlated primarily with pulmonary arterial hypertension. Wipff et al. [13] analysed serum levels of sENG in SSc patients and found that they were higher in those with SSc with a vascular phenotype that integrates the presence of DUs.

Endostatin is a C-terminal 20-kDa fragment of the basement protein collagen type XVIII that strongly inhibits angiogenesis and tumour growth by reducing EC proliferation and migration [14]. Hebbar et al. [15] demonstrated greater endostatin serum levels in SSc patients than in healthy controls and greater mean endostatin concentrations in SSc patients with cutaneous ulcers or scars than in those without cutaneous ulcerations. Farouk et al. [12] found significantly higher levels of serum endostatin in the late stages of SSc in patients with ischemic manifestations. Distler et al. [8] found no association between endostatin levels and fingertip ulcers, but did find an association between endostatin levels and the presence of giant capillaries.

We hypothesize that angiogenic biomarkers (VEGF, endoglin, and endostatin) reflecting disturbances of angiogenesis are related to microvascular damage objectified in NVC. Decreased angiogenic factors and/or increased angiostatic factors may be associated with the presence and development of new ischemic fingertip DUs during a 3-year follow-up of SSc patients. In this study, our primary outcome was the occurrence of a new DU during the 3-year follow-up.

Patients and methods

Patients

A prospective, longitudinal observational study was carried out between October 2011 and December 2014. Seventy-seven SSc patients (72 women; mean age 52.95 ± 12.6 years; range, 14–79) attending the Multidisciplinary Raynaud Clinics of the Clinical Immunology Unit at Centro Hospitalar do Porto in Portugal were followed. All patients fulfilled the 2013 classification criteria for SSc of the American College of Rheumatology [16]. According to the Leroy classification [17], 13 (16.9 %) had diffuse SSc (dcSSc), and 64 (83.1 %) had limited SSc (lcSSc). A group of 34 (sex and age matched) healthy controls were invited (29 women; mean age 47.1 ± 10.96 years; range, 23–66). They were non-obese and without self-reported cardiovascular risk factors (smoking, hypertension, diabetes, or hyperlipidaemia). No control was on vasoactive medications. Three patients died during the study period and were excluded.

At enrollment, patients were divided into two groups. The DU group comprised 38 patients with an active ulcer at baseline, with or without a past history of DUs (34 women; mean age 52.7 ± 14.8 years; range, 14–75). The non-DU group comprised 39 patients with no DU in the course of disease until inclusion (38 women; mean age 53.2 ± 10.3 years; range, 30–79). Onset of disease was defined as the first episode of RP. All patients were

on vasodilators, either the calcium channel blocker nifedipine or the angiotensin II receptor antagonist losartan, and a washout of these drugs was done before inclusion in the study.

This study was approved by the institutional review board (including the Ethical for Health Committee of Centro Hospitalar do Porto). All patients or parents, in the cases of the two children, signed informed consents before inclusion in the study. Data were collected by analysis of clinical file data and by clinical interview.

Nailfold videocapillaroscopy

Nailfold videocapillaroscopy was performed using a KK technology videocapillaroscope with a ×200 magnification lens. The same operator performed all exams.

With the patient in a quiet room at a controlled temperature (21 to 24 °C), the nailfold distal row capillaries of eight fingers (second, third, fourth, and fifth of both hands) were examined.

Capillaroscopic findings were described according to the qualitative classification of scleroderma microangiopathy damage as proposed by Cutolo [18]: early, active, or late pattern. The early pattern was characterized by the presence of a small number of giant capillaries and microhaemorrhages, no avascular areas, and a relatively well preserved capillary distribution. The active pattern was characterized by the presence of numerous giant capillaries and microhaemorrhages, moderate capillary loss (20–30 %), and a mildly disorganized capillary architecture with few branched capillaries. The late pattern was characterized by a near-absence of giant capillaries and microhaemorrhages, presence of extensive avascular areas (50–70 % capillary loss), presence of many branched and ramified bushy capillaries (neoangiogenesis), and a complete disorganization of the capillary array [18].

Biomarkers

Fasting venous blood samples were collected at enrollment into a serum tube and a tube containing sodium heparin (Vacuette; Greiner-Bio-One, Austria). Serum was allowed to clot at room temperature and then separated from the cells within 60 min and stored at −70 °C until analysis for endoglin, endostatin, and VEGF-A.

VEGF assessment

Serum VEGF-A was measured using an enzyme-linked immunosorbent assay (IBL International GMBH, Germany). The resulting values were reported in picogram per milliliter.

Endoglin and endostatin assessment

Serum endoglin and endostatin were measured using enzyme-linked immunosorbent assay (Uscn; Life Science Inc., Wuhan). The resulting values were reported in nanogram per milliliter.

Follow-up

Ischemic digital fingertip ulcerations in the stage 3 peripheral vascular category according to the Medsger Disease Severity scale [19] were considered to be DUs.

When included in the study cohort, patients were seen on a regular basis at 3- to 6-month intervals, as indicated by disease severity. The final observation was made in the fourth quarter of 2014.

Outcome measures

Our main objectives were to analyse the roles of angiogenic and angiostatic factors, to determine their associations with active ulcers at enrollment, and to establish whether they are predictors of developing new DUs during the 3-year follow-up period.

Statistical analysis

For comparisons of normally distributed scale variables, we used unpaired or paired two-sided Student *t* tests or analysis of variance (ANOVA). In these cases, data were described using mean ± standard deviation. Normal distribution was tested using Q-Q plots. In cases of non-normally distributed variables, we used the non-parametric Mann-Whitney and Kruskal-Wallis tests, and data were described using median and interquartile interval (Q_1–Q_3), where Q_1 represents the first quartile (corresponding to 25 % of data) and Q_3 represents the third quartile (corresponding to 75 % of data). When using ANOVA, if homogeneity of variance was not satisfied, we used the Welch test. For comparing categorical variables, we used chi-square or Fisher exact probability tests. Predictors of digital ulcers were evaluated using univariate and multivariate logistic regressions. We applied survival analysis to determine the probability of freedom from new DUs during the study period and evaluated the effects of VEGF, endoglin, and endostatin in that probability using the Kaplan-Meier method and the Cox regression. When $p \leq 0.05$, significance was recognized. Data were analysed using SPSS software (v.22.0, SPSS, Chicago, IL).

Results

Study population

Demographic and clinical characteristics are described in Table 1. Significant differences in the disease subset ($p < 0.001$) and capillaroscopic patterns ($p < 0.001$) were found between the DU and non-DU groups. The former predominantly showed the late pattern while the early and active

Table 1 Comparison between DU and non-DU groups at enrolment

Variables	DU ($n=38$)	Non-DU ($n=39$)	p value
Age (years), mean ± SD (min–max)	52.7 ± 14.8 (14–75)	53.2 ± 10.3 (30–79)	0.845[d]
Gender *women n* (%)	34 (89.5)	38 (97.4)	0.2[b]
dcSSc/lcSSc			0.001[a,*]
Limited, *n* (%)	26 (68.4)	38 (97.4)	
Diffuse, *n* (%)	12 (31.6)	1 (2.6)	
Disease duration (years), *Median* (Q_1–Q_3)	10.00 (5.00–23.00)	10.00 (7.00–20.00)	0.602[c]
Onset of the first ulcer (years), *Median* (Q_1–Q_3)	5 (3–13.25)	NA	NA
Autoantibodies			
ACA positive, *n* (%)	22 (57.9)	27 (69.2)	0.301[a]
Anti-scleroderma-70 positive, *n* (%)	12 (31.6)	6 [4, 15]	0.093[a]
Endoglin ng/ml, *Median* (Q_1–Q_3)	3.013 (1.463–7.023)	1.879 (0.84–3.28)	0.017[c,*]
Endostatin ng/ml, *Median* (Q_1–Q_3)	0.695 (0.26–1.73)	0.429 (0.16–0.8)	0.129[c]
VEGF pg/ml, *Median* (Q_1–Q_3)	245.06 (158.68–347.33)	339.49 (202.00–730.93)	0.009[c,*]
NVC pattern			
Early, *n* (%)	0 (0)	13 (33.3)	<0.001[a,*]
Active, *n* (%)	11 (28.9)	22 (56.4)	
Late, *n* (%)	27 (71.1)	4 (10.3)	

SSc systemic sclerosis, *DU* digital ulcer, *dcSSc* diffuse systemic sclerosis subset, *lcSSc* limited systemic sclerosis subset, *ACA* autoantibody anti-centromere, *NVC* nailfold videocapillaroscopy, *VEGF* vascular endothelial growth factor. *NA* not applicable, *SD* standard deviation, *Q* quartile

[a] Chi-square test

[b] Fisher's exact test

[c] Mann-Whitney test

[d] Student's t test

*Statistical significance for a level of 5 %

patterns were more frequent in the latter. No differences were found in age, sex, disease duration (RP onset), or presence of auto-antibodies.

The DU group had lower VEGF serum levels 245.06 (158.68–347.33) pg/ml ($p<0.001$) and higher endoglin serum levels 3.013 (1.463–7.023) ng/ml ($p<0.001$) compared to non-DU group 339.49 (202.00–730.93) pg/ml/1.879 (0.840–3.280) ng/ml. See Table 2. In contrast, patients with active DUs had higher serum levels of VEGF and endoglin when compared to the control group 178.030 (101.267–222.102) pg/ml/0.277 (0.154–0.713) ng/ml, respectively ($p<0.001$). No significant differences were found in endostatin levels between the groups ($p=0.450$). See Fig. 1.

No significant differences were found between those classified with dcSSc and lcSSc in terms of VEGF (344.50, 267.54–379.56/269.26, 192.74–453.82 pg/ml; $p=0.492$) or endostatin (0.46, 0.28.1.70/0.53, 0.17–1.07 ng/ml; $p=0.355$). Endoglin serum levels were higher among patients with dcSSc (3.21, 2.20–17.51/2.06, 1.06–3.96 ng/ml; $p=0.031$).

Endoglin levels were significantly different between those with early, active, or late pattern NVC ($p=0.020$), but VEGF and endostatin levels were not ($p=0.318$ and $p=0.074$, respectively).

Using univariate logistic regression, baseline VEGF ($p=0.007$) and endoglin ($p=0.028$) were identified as risk factors for digital ulcers. Multivariate regression analysis

Table 2 Comparison of pro-angiogenic VEGF and angiostatic endostatin and endoglin between groups at enrollment

	Control	Non-DU group	DU group	p value
Endoglin, *Median* (Q_1–Q_3)	0.277, 0.154–0.713	1.879, 0.840–3.280	3.012, 1.462–7.022	<0.001*
Endostatin, *Median* (Q_1–Q_3)	0.565, 0.350–0.770	0.429, 0.160–0.770	0.695, 0.260–1.727	=0.450
VEGF, *Median* (Q_1–Q_3)	178.030, 101.267–222.102	339.49202.00–730.93	245.060, 158.675–347.327	<0.001*

DU digital ulcer, *VEGF* vascular endothelial growth factor

*Statistical significance for a level of 5 %

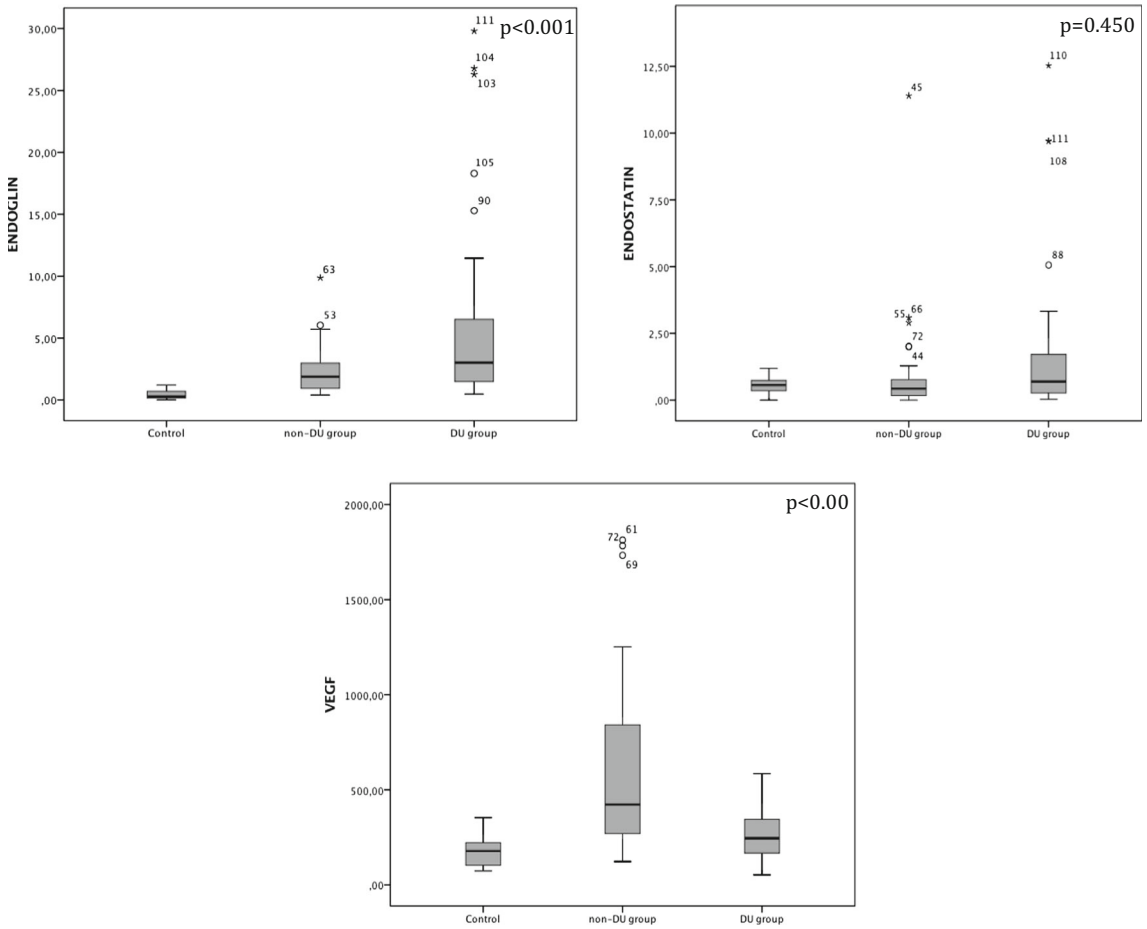

Fig. 1 Serum levels of vascular endothelial growth factor(VEGF), endostatin, and endoglin in 77 SSc patients with and without digital ulcers (DU) compared to 34 healthy controls. Serum levels of endoglin were increased in patients with DU 3.012 (1.462–7.022) compared to controls 0.277 (0.154–0.713) and patients without DU 1.879 (0.840–3.280) ($p < 0.001$). VEGF serum levels were increased in patients without DU 339.49 (202.00–730.93) ($p < 0.001$) suggesting that increased levels of VEGF are protective for new DU development. No significant difference between groups regarding endostatin ($p = 0.450$)

confirmed both as independent risk factors for DU ($p = 0.006$ and $p = 0.028$, respectively).

Occurrence of a new DU during the 3-year follow-up

In the 3-year clinical follow-up, 40 (51.95%) patients developed new ischaemic digital ulcers (Table 3). Those with dcSSc had significantly more new DUs (76.92 %) than those with lcSSc (46.88 %), $p = 0.048$. No differences in disease duration were found ($p = 0.101$). Median time to the occurrence of a new DU was 4.50 months (range, 1.25–16.25).

Using univariate analysis, VEGF was the only predictive risk factor identified for the occurrence of at least one new DU during the 3-year follow-up ($p = 0.018$). The Kaplan-Meyer analysis of freedom of DU for VEGF, endostatin, and endoglin is shown in Figs. 2 a–c. Those with low VEGF serum levels (<422.47 pg/mL) had significantly more DUs ($p = 0.028$) in the 3-year follow-up period. While not significant, a trend toward increased serum levels of endoglin (>4.215 ng/mL) was associated with a new DU ($p = 0.053$). No predictive value was found for endostatin ($p = 0.130$). Multivariate Cox analysis confirmed low VEGF as an independent predictor for the development of new DUs ($p = 0.002$).

The VEGF ($p = 0.024$) and endoglin ($p = 0.020$) levels were significantly different between those with early or active patterns and those with late pattern NVCs. No difference was found in endostatin levels ($p = 0.151$). See Fig. 3.

Only VEGF levels were significantly different ($p = 0.027$) between the DU and non-DU groups 267 (134.570–357.302) vs 415.230 (207.525–863.165) in terms of developing a new DU during the follow-up period.

Discussion

Disparities seen in the results of various studies are likely the result of different SSc patient populations, disease stages, or clinical presentations as well as diverse organ involvement, non-uniform DU classification, and a small number of SSc

Table 3 Primary outcome: new digital ulcer occurrence during the 3-year follow-up

Variables		DU ($n=40$)	Non-DU ($n=37$)	p value
dcSSc/lcSSc				0.048[a,*]
	Limited, *n* (%)	30 (75.0)	34 (91.9)	
	Diffuse, *n* (%)	10 (25.0)	3 (8.1)	
Disease duration		9.50	13.00	0.101[d]
Median (Q_1–Q_3)		(4.25–20.00)	(7.50–22.00)	
History of DU, *n* (%)		30 (75.0)	8 (21.6)	<0.001[a,*]
Time to new DU occurrence		4.50	NA	NA
Median (Q_1–Q_3)		(1.25–16.25)		
Autoantibodies				
ACA				0.464[a]
	Positive, *n* (%)	26 (65.0)	22 (59.5)	
Anti-scleroderma-70				0.012[a,*]
	Positive, *n* (%)	14 (35.0)	4 (10.8)	
NVC pattern baseline				
	Early, *n* (%)	2 (5.0)	11 (29.7)	<0.001[a,*]
	Active, *n* (%)	12 (30.0)	21 (56.8)	
	Late, *n* (%)	26 (65.0)	5 (13.5)	
Endoglin		2.57	2.14	0.153[d]
Median (Q_1–Q_3)		(1.39–4.73)	(1.03–3.33)	
Endostatin		0.695	0.429	0.142[d]
Median (Q_1–Q_3)		(0.273–1.755)	(0.16–0.77)	
VEGF		279.94	335.17	0.018[d,*]
Median (Q_1–Q_3)		(142.37–360.29)	(207.53–717.01)	

DU digital ulcer, *dcSSc* diffuse systemic sclerosis subset, *lcSSc* limited systemic sclerosis subset, *ACA* autoantibody anti-centromere, *NVC* nailfold videocapillaroscopy, *VEGF* vascular endothelial growth factor

[a] Chi-square test

[b] Fisher's exact test

[c] Mann-Whitney test

[d] Student's t test

*Statistical significance for a level of 5 %

patients in most clinical and investigational research cohorts. Furthermore, most of the studies were not designed to find predictable risk factors for DU on their own, but information and lessons may be learned from these publications.

Our study shows that circulating levels of VEGF are increased in SSc patients with early or active NVC patterns and without DUs, suggesting it has a protective role against digital ulcers. Reduced VEGF levels in late NVC patterns suggest that ineffective angiogenesis may contribute to the avascular areas observed in this pattern, largely responsible for the ischemic territory underlying digital ulcers. This insufficient angiogenesis in the late pattern also might be related to increased levels of angiostatic factors, which are reportedly increased in the late stages of SSc [20]. Another explanation is that a prolonged, uncontrolled, and chronic overexpression of VEGF in SSc may have a deleterious effect on the vascular network, resulting in a chaotic vascular morphology with reduced blood flow in the newly formed vessels [1, 20]. Our study was not designed to analyse any association between VEGF and capillary morphology, but our results confirmed those found by Distler et al. [8] and Farouk et al. [12], finding significantly higher serum VEGF levels in the early phases of the disease when digital ischemic manifestations are lacking. In contrast, Avouac et al. [1] reported increased levels of VEGF in the late pattern as well, suggesting that this upregulation might be an insufficient compensatory mechanism to stimulate angiogenesis and an inverse correlation between capillary density and VEGF level.

We found similar levels of endoglin as that reported by Wipff et al. [13]. Greater serum levels in patients with the SSc vascular phenotype were associated with the presence of digital ulcers. High levels of sENG in the sera of SSc patients highlight a possible contribution of this antiangiogenic protein in tSSc vascular disturbances. Greater serum levels of endoglin in the subset with dcSSc are expected, given the important profibrotic role of endoglin in SSc fibroblasts [21, 22].

Conflicting results have been reported regarding endostatin in SSc patients. Our results confirm those of Distler et al. [8],

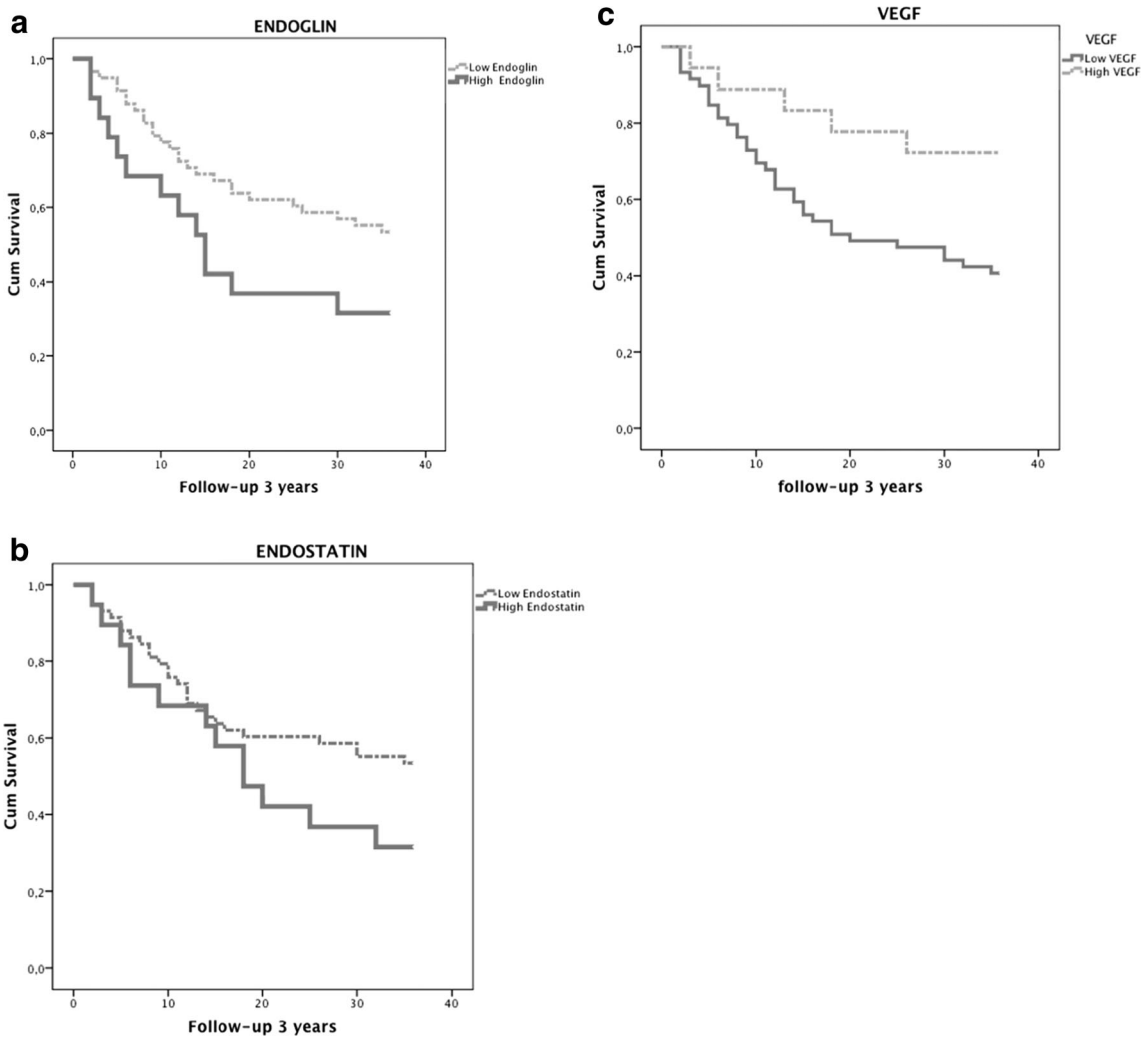

Fig. 2 **a**–**c** Kaplan-Meier analyses of freedom from new digital ulcers in 36 months follow-up of 77 systemic sclerosis patients. Curves are shown for **a** patients who had endoglin serum levels ≥4.215 ng/ml or <4.215 ng/ml ($p = 0.05$), **b** patients who had endostatin serum levels ≤1.246 ng/ml or >1.246 ng/ml ($p = 0.130$), and **c** patients who had VEGF serum levels ≤422.47 pg/ml or >422.47 pg/ml ($p = 0.028$)

finding no association between endostatin levels and DU, yet this contrasts with results reported by Hebbar et al. [15] and Farouk et al. [12], who reported associations between endostatin and the presence of DU. Additionally, we identified greater endostatin serum levels in SSc patients than in the healthy controls, as did Hebbar et al. [15]. Endostatin plays an important role in microvascular changes and balance in SSc patients, and its actions are more probably mediated by several

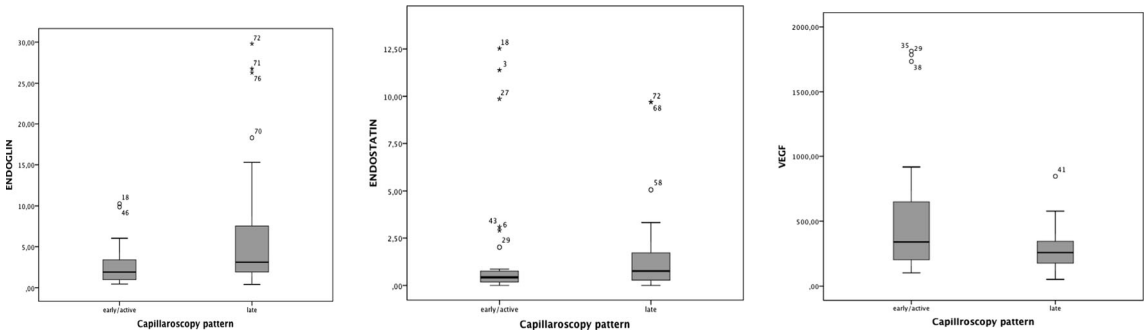

Fig. 3 Serum levels of vascular endothelial growth factor (VEGF), endoglin, and endostatin according to progression of nailfold capillaroscopic pattern in 3-year follow-up. VEGF ($p = 0.024$) and endoglin ($p = 0.020$) had significant differences when comparing early/active with late pattern in NVC. No differences were found relative to endostatin ($p = 0.151$)

other angiostatic factors that remain to be identified. Larger observational studies are required to define the relationships between angiostatic biomarkers, DUs, and other clinical manifestations of vasculopathy in SSc patients.

Limitations of this study are its small patient sample, all recruited from the same centre, and the number of patients with active DU at the time of enrollment. Additional longitudinal studies with larger cohorts are needed to validate predictive risk factors, thus enabling a better understanding of the progression of vascular damage and angiogenesis in the aetiology of DU. It would be interesting to study angiopoietins and VEGF-A isoforms and analyse their predictive roles. Angiopoietins are known to be involved in the development, remodelling, and stability of blood vessels [23]. Altered expressions of Angiopoietin 1 (Ang-1), Angiopoietin 2 (Ang-2) [23], and Angiopoetin-like Protein 3 (ANGPTL3) [24] have been studied as biomarkers of SSc-related microangiopathy. Manetti et al. [25] described proangiogenic VEGF165 and antiangiogenic VEGF165b isoforms, finding the first evidence of a switch from proangiogenic to antiangiogenic VEGF-A isoforms and their crucial roles in the defective angiogenic and vascular repair processes that characterize SSc.

Conclusion

Our study confirms the relationship between some angiogenic vascular biomarkers and the occurrence of DU and determines which of them could be predictors of these disabling complications of the disease. In SSc patients, reduced levels of VEGF, after an initial increase in the early stages of disease, independently predict development of new DUs. We identified a trend toward high serum endoglin levels as a predictor of DUs. Increased endoglin is present in dcSSc and the late NVC scleroderma pattern. We found that endostatin is not an independent risk factor for active DUs nor a predictor of the occurrence of new DUs. The molecular analysis of the vascular mediators associated with SSc, particularly with peripheral microangiopathy, may identify new therapeutic targets that lead to the prevention of further vascular injury or the improvement of SSc disease.

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Compliance with ethical standard This study was approved by the institutional review board (including the Ethical for Health Committee of Centro Hospitalar do Porto). All patients or parents, in the cases of the two children, signed informed consents before inclusion in the study. Data were collected by analysis of clinical file data and by clinical interview.

Disclosures None.

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