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ORIGINAL ARTICLE

Serum level of endostatin and digital ulcers in systemic sclerosis patients

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Abstract

Patients with systemic sclerosis (SSc) are at a high risk of the development of ischaemic digital ulcers (DUs) that can be complicated with infections, gangrene, and osteomyelitis. The aim of this study is to evaluate the role of endostatin in scleroderma DUs.In total, 90 SSc patients were enrolled in this study. Serum endostatin levels and DU assessment were determined in all SSc patients. The serum levels of endostatin significantly increased with progression of capillaroscopic damage ($P < .01$). The serum levels of endostatin are significantly ($P < .05$) higher in SSc patients with new DUs than in SSc patients without new DUs (127 ± 31.1 ng/mL vs 116.3 ± 39.7 ng/mL). The Receiver Operating Characteristic (ROC) curves demonstrated good accuracy of new DU prediction for the serum level of endostatin (0.70, $P < .01$ [95% confidence interval (CI) 0.59-0.81]). Using a cut-off value of 116 ng/mL, the odds ratio was 2.609 (CI 1.075-6.330, $P < .05$). The serum levels of endostatin are significantly ($P < .01$) higher in SSc patients with infected DUs than in SSc patients without infected DUs (139.2 [114.6-340.91] ng/mL vs 117.5 [64.3-163.9] ng/mL). Serum levels of endostatin are higher in patients with DUs, especially in those with infected DUs.

KEYWORDS

angiogenesis, digital ulcers, endostatin, systemic sclerosis, vasculopathy

1 | INTRODUCTION

Systemic sclerosis (SSc) is a connective tissue disease characterised by endothelial dysfunction and fibrosis of both skin and internal organs.[1] Vascular dysfunction is 1 of the hallmarks of SSc and involves both the macro- and microvasculature. The local tissue response to vascular injury involves activation of matrix metalloproteinases, leading to the extracellular matrix breakdown, and release of angiogenic growth factors, such as fibroblast growth factors and vascular endothelial growth factors. Systemic factors influencing local tissue angiogenesis include circulating growth factors and inhibitors of angiogenesis, such as endostatin and angiostatin. Endostatin is higher in patients with scleroderma and correlate positively with vascular damage.[2] Digital ulcers (DUs) are major vascular skin complications, and they are present in approximately 30% of SSc patients per year.[3,4] DUs are typically very painful, with possible evolution towards impaired hand function. Usually, the ulcers found on the fingertips are recurrent, numerous, and often complicated by local infection, osteomyelitis, gangrene, and amputation.[3,5] Major risk factors for the development of infected DUs are immunosuppressive drugs, defective immune system activity, scleroderma vasculopathy, and fibrosis.[5]

The aim of this study is to evaluate the association between serum levels of endostatin and DUs in SSc patients.

2 | METHODS

In total, 90 consecutive patients (79 female and 11 males; mean age 54.4 ± 13.6 years), fulfilling the American College of Rheumatology/European League criteria for classification of SSc, were enrolled in this study;[6] 43 patients had limited cutaneous SSc (lcSSc) and 47 diffuse cutaneous SSc (dcSSc) as defined by Le Roy et al.[7]

At enrolment, all SSc patients underwent treatment with calcium channel blockers (nifedipine 30 mg/day); 36 patients were treated with bosentan at a dose of 125 mg twice daily. Patients with pulmonary hypertension, heart failure, interstitial lung disease (ILD), significant gas exchange abnormalities (diffusion lung capacity for CO [DL_{CO}] ≤ 60% of predicted value), history of uncontrolled systemic hypertension, dyslipidaemia, diabetes mellitus, peripheral vascular diseases, coagulopathy, and pregnant or breastfeeding women were excluded. None of the patients was treated with immunosuppressive agents (eg, cyclophosphamide or mycophenolate mofetil or corticosteroids therapy at an equivalent dose of prednisone ≥10 mg/day), ACE inhibitors or angiotensin receptor antagonists, and phosphodiesterase 5 inhibitors.

Written consent was obtained from the participants according to the Declaration of Helsinki, and the study was conducted in agreement with local ethics committee's directives.

2.1 | Digital ulcers assessment

The DUs were assessed according to Amanzi et al. As the first step, the digital lesions were observed and classified at the time of presentation as a digital pitting scar (DPS), DU, calcinosis, gangrene, etc. Then, the DUs were divided into subsets according to their origin and main features. A cutaneous swab for microbiological examinations was performed on DUs with signs of local bacterial infections. Infected DUs could be presented with purulent exudate, swelling, and nocturnal throbbing pain. All swabs were performed with a sterile cotton tip at the level of pure DUs, according to Amanzi classification,[8,9] of both the wound bed and margins. Then, the material was sent to a laboratory to evaluate the presence of microorganism using agglutination tests with antibodies specific for bacterial surface antigens, using protein or DNA sequencing. Medications were administered in the hospital with rigorous asepsis. Then, all patients were informed about how to perform daily home medications, with particular emphasis on hand hygiene of both patients and relatives in order to minimise infectious complications.

2.2 | Laboratory parameters

Laboratory investigations included serum creatinine (sCr) (normal range: 0.5-0.9 mg/dL), blood urea nitrogen (normal range: 10.20-49.80 mg/dL), and serum uric acid (UA) (normal range: 3.40-7.20 mg/dL). sCr was measured using a Jaffe alkaline picrate assay (Abbott Aeroset analyser). Glomerular filtrate rate (GFR) was calculated using the CKD-EPI equation, already validated in SSc patients.[10] Serum UA was measured with an automatic analyser (7700 series; Hitachi, Tokyo, Japan).

Key Messages

- SSc is a connective tissue disease and vascular dysfunction is one of the hallmarks of SSc
- Ischaemic digital ulcers (DUs) are serious complications in the course of SSc and may be complicated by infections, gangrene, and osteomyelitis
- endostatin is higher in patients with SSc and correlates positively with vascular damage
- in SSc patients with new DUs serum levels of endostatin are significantly higher than in SSc patients without new DUs
- the serum levels of endostatin increase with progression of capillaroscopic damage and are significantly higher in SSc patients with infected DUs than in SSc patients without infected DUs

2.3 | Nailfold videocapillaroscopy

Nailfold videocapillaroscopy (NVC) was performed with a videocapillaroscope (Pinnacle Studio Version 8) equipped with a 500× optical probe. The nailfold of the second, third, fourth, and fifth finger was examined in each patient. According to Cutolo et al,[11] patterns identified within the "SSc pattern" include: early, active, and late.

2.4 | Clinical assessment

Modified Rodnan total skin score (mRSS) was chosen as the method to assess skin induration in SSc. The score is determined at a standardised location of 17 different sites of the body with a standardised pinching method, and it is scored from 0 to 3.[12] Disease activity and disease severity were measured using the Disease Activity Index (DAI) and Medsger Disease Severity Scale (DSS), respectively.[13,14]

2.5 | Endostatin

Serum endostatin levels were determined in SSc patients using a commercial ELISA kit (Human Endostatin, Quantikine ELISA, R&D Systems, Minneapolis MN), with a sensitivity of 0.063 ng/mL and an assay range of 0.3-10 ng/mL according to the instructions provided by the manufacturer.

2.6 | Statistical analysis

The results were expressed as mean and standard deviation (SD) or median and range, as appropriate. Commercial software (SPSS version 23·0) was used for statistical analysis. The coefficient of skewness and the coefficient of kurtosis were used to evaluate the normal distribution of data. Multiple regression analysis was applied to evaluate the relationship between endostatin and demographic and clinical features (age, duration of disease, mRSS, DAI, DSS, serum uric acid). Pearson product–moment or Spearman correlation coefficient (r) were used to test for bivariate analysis. Group comparisons were made by Student's unpaired 2-tailed *t* test or the Kruskal-Wallis test, as appropriate. A receiver operating characteristic (ROC) curve analysis was performed to analyse the prognostic accuracy of serum level of endostatin with regards to ulcer development. Odds ratio and 95% confidence intervals (95% CIs) are reported. The chi-square test or Fisher's exact test were used to compare categorical variables. *P*-values less than .05 were considered significant.

3 | RESULTS

Table 1 shows SSc patients' epidemiological and clinical features. The mean values of endostatin for SSc patients are 117 ± 38 ng/mL. The serum levels of endostatin significantly ($P < .01$) increased with progression of NVC damage: early (98.9 ± 40.8 ng/mL), active (114.7 ± 35.5 ng/mL), and late (133.8 ± 34.5 ng/mL) (Figure 1A). The serum levels of endostatin are significantly ($P < .05$) higher in SSc patients with new DUs than in SSc patients without new DUs (127 ± 31.1 ng/mL vs 116.3 ± 39.7 ng/mL) (Figure 1B).

The ROC curves demonstrated good accuracy of new DU prediction for serum levels of endostatin (0.70, $P < .01$ [95% CI 0.59-0.81]). Using a cut-off value of 116 ng/mL, the odds ratio was 2.609 (CI 1.075-6.330, $P < .05$).

Any significant ($P > .05$) difference in serum levels of endostatin was observed between males and females (121.4 ± 34.4 ng/mL vs 117.4 ± 39.3 ng/mL) or dcSSc and lcSSc (115.5 ± 36 ng/mL vs 120.4 ± 4.6 ng/mL). In the multiple regression analysis, we analysed serum levels of endostatin as the dependent variable (Table 2). In the analysis, we observed only a positive correlation between serum levels of endostatin and serum uric acid (r = 0.25, $P < .05$) (Figure 1C).

Thirteen SSc patients (40.6%) with new DUs have infected DUs. The main agents detected were *Staphylococcus aureus* ($n = 8$, 61.5%), *Staphylococcus haemolyticus* ($n = 3$, 23.1%), *Staphylococcus epidermitis* ($n = 1$, 7.7%), and *Staphylococcus warneri* ($n = 1$, 7.7%). The serum levels of endostatin are significantly ($P < .01$) higher in SSc patients with infected DUs than in SSc patients without infected DUs (139.2 [114.6-340.91] ng/mL vs 117.5 [64.3-163.9] ng/mL) (Figure 1D). Any significant ($P > .05$) differences in median value of age, serum UA, mRSS, DAI, DSS, and intimal media thickness (IMT) were observed between SSc patients with infected new DUs or without infected new DUs. Nine (69.2%) SSc patients with infected new DUs have a dcSSc and 4 (30.8%) lcSSc. Seven (53.8%) SSc patients with infected new DUs have an active capillaroscopic pattern and 6 (46.2%) a late capillaroscopic pattern.

In 3 patients (23%), there were re-infected DUs; 2 patients were particularly positive for *Staphylococcus aureus* and 1 for *Pseudomonas aeruginosa.* No significant differences of serum levels of endostatin were observed in SSc patients with reinfected DUs or without reinfected DUs.

TABLE 1 SSc patients' epidemiological and clinical features

Gender, female/male	79/11
Age (years)	54.4 ± 13.6
Disease duration (years)	9 ± 6
mRSS	11.3 ± 6.3
DAI	2.8 ± 2.4
DSS	5 ± 3.2
dcSSc/lcSSc	47/43
Digital ulcers history	50 (55.5%)
New digital ulcers	32 (35.6%)
Digital ulcers infected	13 (14.4%)
SSc-specific autoantibodies, *n* (%)	
Anti-topoisomerase I	50 (55.6)
Anticentromere	36 (40)
None	4 (4.4)
Capillaroscopic pattern, *n* (%)	
Early	22 (24.4)
Active	35 (38.9)
Late	33 (36.7)
Endostatin (ng/mL)	117 ± 38
Serum uric acid (mg/dL)	4.2 ± 1.1
sCr (mg/dL)	0.74 ± 0.18
GFR (mL/min)	94.1 ± 19
IMT (mm)	0.80 ± 0.15
PAPs (mm Hg)	31 ± 8
DL_{CO} (% of predicted)	71 ± 10

DAI, Disease Activity Index; dcSSc, diffuse cutaneous SSc; DL_{CO}, diffusion lung capacity for carbon monoxide; DSS, Disease Severity Scale; GFR, glomerular filtrate rate; IMT, intimal media thickness; lcSSc, limited cutaneous SSc; mRSS, modified Rodnan total skin score; PAPs, systolic pulmonary artery pressure.

4 | DISCUSSION

The results of our study demonstrated that serum levels of endostatin are significantly higher in SSc patients with new DUs than in SSc patients without new DUs. The serum levels of endostatin increased with progression of capillaroscopic damage. No significant association was observed

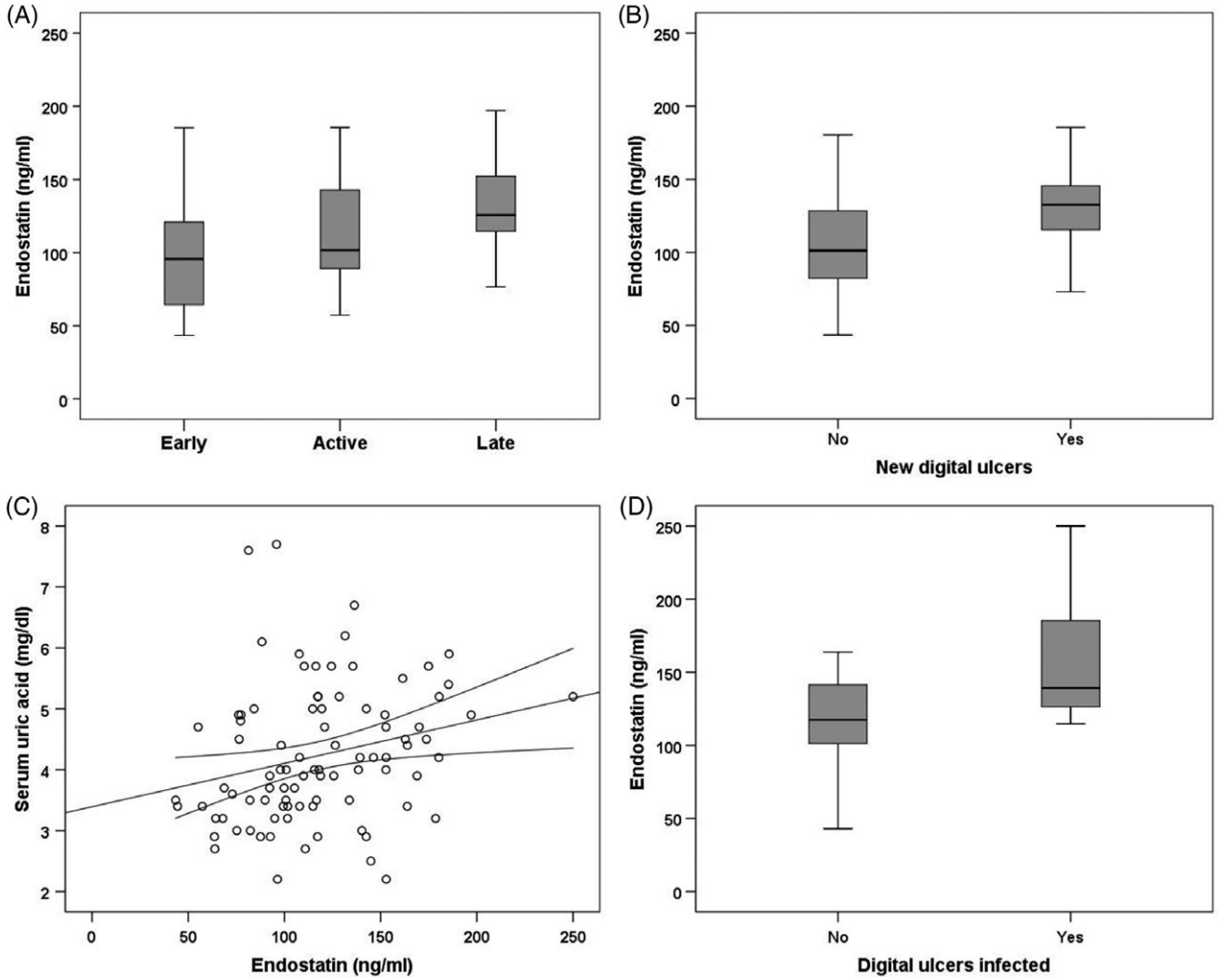

FIGURE 1 A, Serum levels of endostatin (ng/mL) in 3 capillaroscopic groups. B, Serum levels of endostatin (ng/mL) in SSc patients with new digital ulcers and in SSc patients without new digital ulcers. C, Correlation between serum levels of endostatin (ng/mL) and serum uric acid. D, Serum levels of endostatin (ng/mL) in SSc patients with infected new digital ulcers and in SSc patients without infected new digital ulcers

between serum levels of endostatin and other variables of disease except for serum UA. A linear positive correlation was observed between endostatin and serum UA. Endostatin is a powerful inhibitor of angiogenesis, and high levels are reported in SSc patients when compared with healthy control group.[15] Elevated levels correlate with skin sclerosis, cutaneous scars, DUs, giant capillaries in NVC,[16] pulmonary arterial hypertension, and scleroderma renal crisis.[17] Farouk et al evaluated the possible role of angiogenesis imbalance in the pathogenesis of SSc in 25 SSc patients. The authors have demonstrated that endostatin could participate in SSc ischaemic manifestations inhibiting the assembly of human endothelial cells in complex vessels.[18] High endostatin levels are associated with larger cutaneous DUs, and some authors suggest that endostatin could reduce endothelial E-selectin expression that is activated in response to vascular injury.[17] Kim et al demonstrated that endostatin interferes with the development of basal membrane of the blood vessels.[18]

TABLE 2 Linear regression analysis models of correlations between serum levels of endostatin and other variables of diseases

Doppler indices (dependent)	Others variables (independent)	Standardised β-coefficient	*P* value
Endostatin	Age	.236	>.05
	IMT	.033	>.05
	UA	.217	<.05
	DAI	.093	>.05
	DSS	.165	>.05
	mRSS	−.123	>.05
	PAPs	−.023	>.05
	DL_{co}	−.166	>.05

DAI, Disease Activity Index; DL_{CO}, diffusion lung capacity for carbon monoxide; DSS, Disease Severity Scale; IMT, intimal media thickness; mRSS, modified Rodnan total skin score; PAPs, systolic pulmonary artery pressure; UA, serum uric acid; VEGF, vascular endothelial growth factor.

UA represents a marker of inflammation and endothelial dysfunction. UA is associated with impaired endothelium-mediated relaxation and vascular stiffness.[19] Hyperuricemia may represent a marker or an independent risk factor for cardiovascular disease, including chronic kidney disease, in addition to hypertension, diabetes, and obesity.[20] In the

DETECT study, serum UA represents 1 of the 6 non-echocardiographic variables to evaluate individual risk of pulmonary arterial hypertension development in SSc patients.[21] Gigante et al have demonstrated that serum UA concentration in SSc patients increased with progression of capillaroscopic damage.[19]

For the first time, we demonstrated that serum levels of endostatin are significantly higher in SSc patients with infected DUs than in patients without infected DUs. About 40% of SSc patients with new DUs have infected DUs. The main agents detected were *Staphylococcus aureus.* In our study, we did not detect *Escherichia coli* infection. Few reports of infected scleroderma DUs are reported in the literature. Giuggioli et al showed infected scleroderma DUs in 51.2% of cases in 82 SSc patients. Surprisingly, compared with *Staphylococcus aureus*, intestinal bacteria were detected in 26% of 42 patients.[5] The reason for the high incidence of faecal pathogens like *Escherichia coli* and *Enterococcus faecalis* was hand hygiene of patients and relatives. In our study, no patients had intestinal bacteria on microbiological findings. We can hypothesise that methodical education on hand hygiene of patients and the use of gloves during home medications have prevented the presence of faecal pathogen. DUs are often recurrent and have a longer healing time, with pulp loss and high risk of infections.[5] Continuous vasospasm promotes local ischaemia, with the formation of avascular and atrophic poorly oxygenated tissue, promoting infections.[3] As it is well known in literature that endostatin correlates with DUs and avascular tissue,[16] it is not surprising that infected DUs in this study are associated with the highest endostatin levels. In fact, the atrophic tissue is poor in nutrients, with reduced capillary blood flow and oxygen and a lack of compensative angiogenesis.

We can conclude that in SSc patients with DUs, serum levels of endostatin are significantly higher than in patients without DUs. In addition, serum levels of endostatin are significantly higher in SSc patients with infected DUs than in patients without infected DUs. Larger prospective studies are needed to evaluate the role of endostatin in DUs development.

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