

# Nailfold videocapillaroscopy and serum VEGF levels in scleroderma are associated with internal organ involvement

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## Abstract

**Purpose** Nailfold videocapillaroscopy (NVC) identifies the microvascular hallmarks of systemic sclerosis (SSc) and vascular endothelial growth factor (VEGF) and may play a pivotal role in the associated vasculopathy. The aim of the present study was to compare NVC alterations with clinical subsets, internal organ involvement, and serum VEGF levels in a cohort of selected SSc cases.

**Methods** We studied 44 patients with SSc who were evaluated within 3 months from enrollment by NVC, skin score, severity index, pulmonary function tests, carbon monoxide diffusing capacity (DLCO), echocardiography, pulmonary high-resolution computed tomography (HRCT), gastroesophageal (GE) endoscopy or manometry or X-ray, and serum autoantibodies. Serum VEGF-A levels were determined by ELISA in 72 SSc patients and 31 healthy controls.

**Results** Giant capillaries were inversely correlated with age ( $p = 0.034$ ,  $r = -0.34$ ) and to the extent of reticular pattern at HRCT ( $p = 0.04$ ,  $r = -0.5$ ). Avascular areas were directly correlated with capillaroscopy skin ulcer risk index (CSURI) ( $p = 0.006$ ,  $r = +0.4$ ) and severity index ( $p = 0.004$ ,  $r = +0.5$ ). The mean capillary density was directly correlated to the ulcer number ( $p = 0.02$ ,  $r = +0.4$ ) and to DLCO/alveolar volume ( $p = 0.02$ ,  $r = +0.4$ ) and inversely correlated with severity index ( $p = 0.01$ ,  $r = -0.4$ ) and skin score ( $p = 0.02$ ,  $r = -0.4$ ).

Serum VEGF levels were higher in the SSc population vs controls ( $p = 0.03$ ) and inversely correlated with DLCO ( $p = 0.01$ ,  $r = -0.4$ ) and directly with ground-glass and reticular pattern at HRCT ( $p = 0.04$ ,  $r = +0.4$  for both).

**Conclusions** Our data suggest the importance of NVC not only for the diagnosis, but also for the global evaluation of SSc patients. Of note, serum VEGF levels may act as a biomarker of interstitial lung involvement.

**Keywords** Autoimmunity · Biomarkers · Personalized medicine · Systemic sclerosis · Endothelium

## Introduction

Systemic sclerosis (SSc) is an immune-mediated vascular and fibrotic systemic disease [1], with striking female predominance and with unknown pathogenesis involving both genetics and environmental factors [2–5]. Microvascular alterations underlying Raynaud's phenomenon and rearrangement of the capillary network characterize the early phases of the disease. Nailfold videocapillaroscopy (NVC) can easily identify SSc microvascular hallmarks as giant capillaries and avascular areas, but also less specific and frequent changes as ectasia, microhemorrhages, bushy capillaries, and reduced capillary density. NVC is now recognized as an indispensable tool for the early diagnosis of SSc together with the presence of Raynaud's phenomenon and specific serum autoantibodies such as anti-centromere and anti-topoisomerase I (anti-Scl70) [6]. The NVC scleroderma pattern has been recently included in the last SSc classification criteria [7], but the role of NVC during the course of SSc is less defined. NVC alterations can change with disease progression and severity, and Cutolo et al. have reported three different NVC

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scleroderma patterns, i.e., early (presence of giant capillaries), active (prevalence of giant capillaries), and late pattern (prevalence of avascular areas) [8], possibly mirroring the different phases of the disease. More recently, Sebastiani et al., developed a score associated with ulcer development called capillaroscopy skin ulcer risk index (CSURI >2.94) [9], thus suggesting a role for NVC other than the early diagnosis of SSc.

While the pathogenetic mechanisms in SSc remain elusive, the vascular endothelial growth factor (VEGF) is one of the main mediators of angiogenesis and vasculogenesis, being involved in SSc vasculopathy. Whether and which changes in VEGF and its receptor levels are associated with SSc and how they contribute to scleroderma vasculopathy continue to be unclear [10–15].

The aim of this study is to investigate the correlations between NVC alterations, Cutolo's NVC patterns and CSURI with the fine clinical SSc phenotype including internal organ involvement, SSc severity score [16], and serum VEGF levels in a cohort of well-defined SSc cases.

## Patients and methods

### Subjects

We enrolled 44 patients fulfilling the 2013 ACR/EULAR criteria for SSc [7] without other overlapping rheumatic diseases and for whom the following data were available: NVC images of the right and left II–V fingers; all clinical, immunologic, clinimetric, and instrumental evaluations (Table 1); and one serum sample for each SSc patient. All SSc cases were evaluated between 2001 and 2014, but serum samples, clinical, and instrumental data analyzed in the study were obtained within 3 months from NVC. The characteristics of the study population are summarized in Table 1. Serum samples of 31 female healthy subjects (median age 65 years, range 3–80 years) and additional 32 women with SSc (median age 67 years, range 40–78; median disease duration 8 years, range 1–17 years; diffuse SSc 10; ACA (+) 17 cases; anti-Sc170 (+) 8 cases) were also obtained and used as controls. All subjects enrolled signed a written informed consent for serum samples and data analysis after approval from the local ethical committee.

### NVC analysis

Two independent readers (MDS and CC), blinded to patient information, analyzed and recorded the mean capillary density per finger and the total numbers of giant capillaries, neoangiogenesis, and avascular areas on four consecutive NVC fields extending over 1 mm, in the middle of the nailfold of the right and left second, third,

**Table 1** Characteristics of the SSc cohort ( $n = 44$ )

	SSc ( $n = 44$ )
F (%) vs M (%)	42 (95.4 %) vs 2 (4.6 %)
Median age, yy(range)	66 (34–80)
Median disease duration, yy (range)	9 (1–16)
D (%) vs L (%)	10 (22.7 %) vs 34 (77.3 %)
anti-Sc170 (+) (%)	12 (27.3 %)
ACA (+) (%)	22 (50 %)
Others Abs (%) <sup>a</sup>	10 (22.7 %)
Iloprost (%)	25 (56.8 %)
Bosentan (%)	7 (15.9 %)
Ulcers (%)	15 (34.1 %)
Early NVC pattern (%)	6 (13.6 %)
Active NVC pattern (%)	20 (45.4 %)
Late NVC pattern (%)	18 (40.9 %)
GE disease (%)	36 (81.8 %)
sPAP >45 mmHg (%)	3 (6.8 %)
DLCO <50 % (%)	7 (15.9 %)
DLCO/AV <50 % (%)	1 (2.3 %)
FVC <80 % (%)	8 (18.2 %)
Honeycombing lung (%)	4 (9.1 %)
Rodnan skin score >14 (%)	9 (20.4 %)

ACA anti-centromere, *anti-Sc170* anti-topoisomerase, AV alveolar volume, D diffuse, DLCO carbon monoxide diffusing capacity, F female, FVC forced vital capacity, GE gastro-esophageal, L limited, M male, NVC nailfold videocapillaroscopy, sPAP systolic pulmonary artery pressure on echocardiography, SSc systemic sclerosis, vs versus

<sup>a</sup> 1/10 anti-Ku, 2/10 anti-RNApol III, 1/10 anti-Th/To

fourth, and fifth fingers; early, active, and late scleroderma patterns, as described by Cutolo et al. [8], and CSURI as described by Sebastiani et al. [9] were assessed.

### Clinical and imaging assessment

Age, disease duration (from the first non-Raynaud symptom), cutaneous subset, modified Rodnan skin score (mRSS), digital ulcers (current), SSc severity index [16], and treatments received were analyzed. All patients underwent echocardiography for pulmonary hypertension screening with systolic pulmonary artery pressure (sPAP) assessment (3 patients had PAPs >45 mmHg related to interstitial lung disease); pulmonary function tests for forced vital capacity (FVC) and carbon monoxide diffusing capacity (DLCO) and DLCO/alveolar volume (DLCO/AV) assessment; high-resolution computed tomography of the lung (HRCT) with the assessment of ground-glass reticular pattern and honeycombing extent as described elsewhere [17–19]. Gastro-esophageal (GE) involvement was investigated during history and assessed in symptomatic patients with esophageal manometry or X-ray or endoscopy.

## Laboratory tests

Serum was obtained from the peripheral blood of the 44 SSc patients included in the study, 32 unselected SSc patients, and 31 sex- and age-matched healthy controls. Laboratory tests for SSc cases included complete blood cell count, C-reactive protein (CRP) level, serum creatinine concentration, urine analysis, complement C3 and C4, ACA (immunofluorescence on Hep2 cells), anti-Scl70 (ELISA), protein immunoprecipitation (IP) of K562 cell extract radiolabeled with  $^{35}\text{S}$ -methionine and by RNA-IP through silver staining; specificities of autoantibodies were determined using specific reference sera [20, 21] for serum antibody assessment.

VEGF-A was measured by quantitative sandwich enzyme-linked immunosorbent assay (ELISA) (Quantikine kits; R&D). Concentrations were calculated using a standard curve generated with specific standards provided by the manufacturer.

## Statistical analysis

All data are presented as median and range for continuous variables. The two-sample Mann–Whitney’s test and contingency tables were used as appropriate. The Spearman’s test was used for correlations.  $P$  values  $<0.05$  were considered to be significant.

## Results

Our analysis was performed on 44 SSc cases, 32 unselected SSc patients, and 31 sex- and age-matched healthy controls. The number of giant capillaries was inversely correlated with age ( $p = 0.034$ ,  $r = -0.34$ ; Fig. 1a) and to the extent of reticular pattern on HRCT ( $p = 0.04$ ,  $r = -0.5$ , Fig. 1b). The number of giant capillaries was higher in patients without serum anti-Scl70 or ACA and in those with skin score  $>14$  ( $p = \text{ns}$ ; Table 2). As expected, the

number of giant capillaries was significantly higher in patients with active NVC pattern ( $p < 0.0001$ ; Table 2).

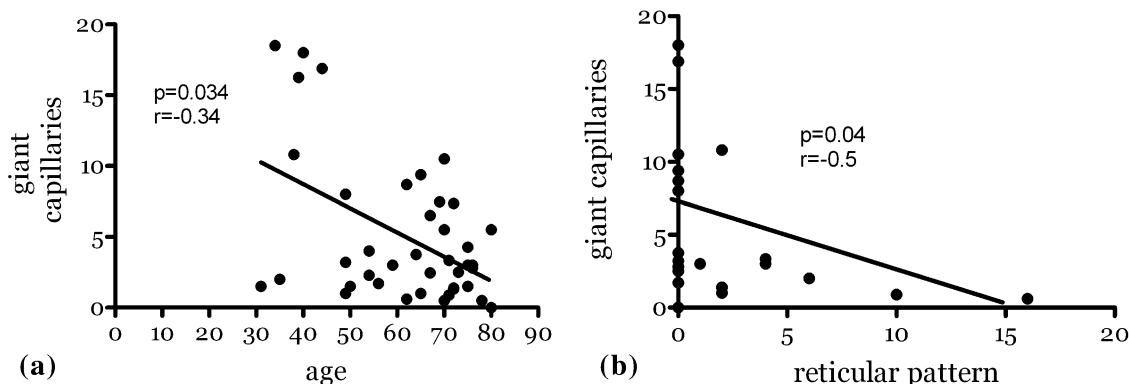
The degree of neoangiogenesis was higher in SSc patients with GE involvement, honeycombing, DLCO  $<50\%$ , sPAP  $>45$  mmHg, higher VEGF levels (Table 2), and in SSc patients treated with bosentan ( $p = 0.016$ ; Table 2).

The number of avascular areas was directly correlated to severity index ( $p = 0.004$ ,  $r = +0.5$ ; Fig. 2a) and inversely to DLCO/AV ( $p = 0.02$ ,  $r = -0.4$ ; Fig. 2b). Moreover, the number of avascular areas was higher in SSc patients with anti-Scl70 or ACA ( $p = \text{ns}$ ; Table 2) and in those with higher sPAP ( $p = 0.03$ ; Table 2) or needing iloprost ( $p = 0.04$ ; Table 2) or bosentan ( $p = \text{ns}$ ; Table 2) for digital ulcers. As expected, the number of avascular areas was significantly higher in patients with late NVC pattern ( $p < 0.0001$ ; Table 2) and CSURI  $>2.94$  ( $p = 0.01$ ; Table 2).

The mean capillary density was directly correlated with the number of digital ulcers ( $p = 0.02$ ,  $r = +0.4$ ) and to DLCO/AV ( $p = 0.02$ ,  $r = +0.4$ ), and inversely with the severity index ( $p = 0.01$ ,  $r = -0.4$ ; Fig. 3a) and skin score ( $p = 0.02$ ,  $r = -0.4$ ; Fig. 3b). Moreover, the mean capillary density was lower in patients with anti-Scl70, skin score  $>14$ , ( $p = \text{ns}$ ; Table 2), and, as expected, in patients with late NVC pattern ( $p = 0.0007$ ; Table 2) and CSURI  $<2.94$  ( $p = 0.047$ ; Table 2). The highest mean capillary density was found in patients with early NVC pattern ( $p = 0.005$ ; Table 2).

Cutolo’s NVC late pattern was significantly more prevalent in SSc with higher sPAP ( $p = 0.025$ ; Table 3) and honeycombing ( $p = 0.037$ ; Table 3), in patients receiving iloprost ( $p = 0.039$ ; Table 3) or bosentan ( $p = 0.017$ ; Table 3) for digital ulcers, and in those with the lowest VEGF levels ( $p = 0.02$ ; Table 3). Moreover, the late pattern was significantly less frequent in patients with CSURI  $<2.94$  ( $p = 0.02$ ; Table 3).

CSURI directly correlated with the number of avascular areas ( $p = 0.006$ ,  $r = +0.4$ ) and was higher in patients with late NVC pattern ( $p = 0.02$ ; Table 3).



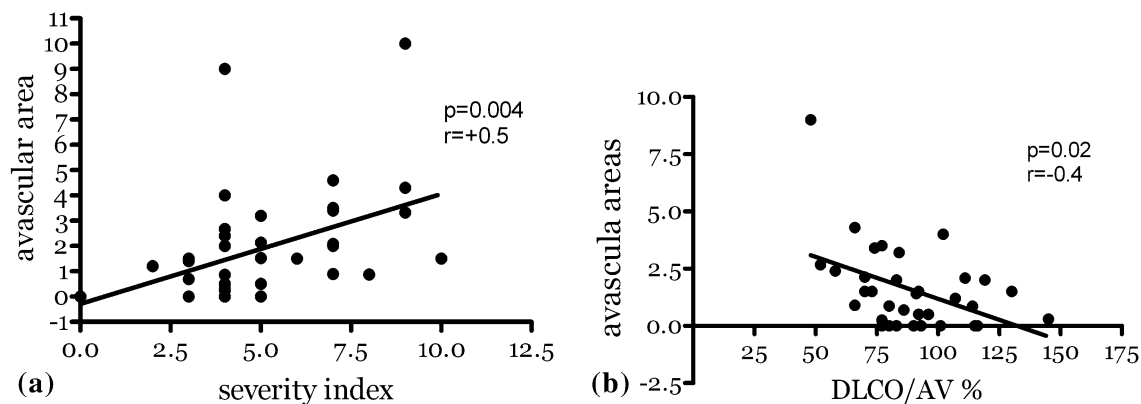
**Fig. 1** Giant capillaries inversely correlated with age and reticular pattern on high-resolution computed tomography

**Table 2** NVC alterations in the SSc cohort (*n* = 44)

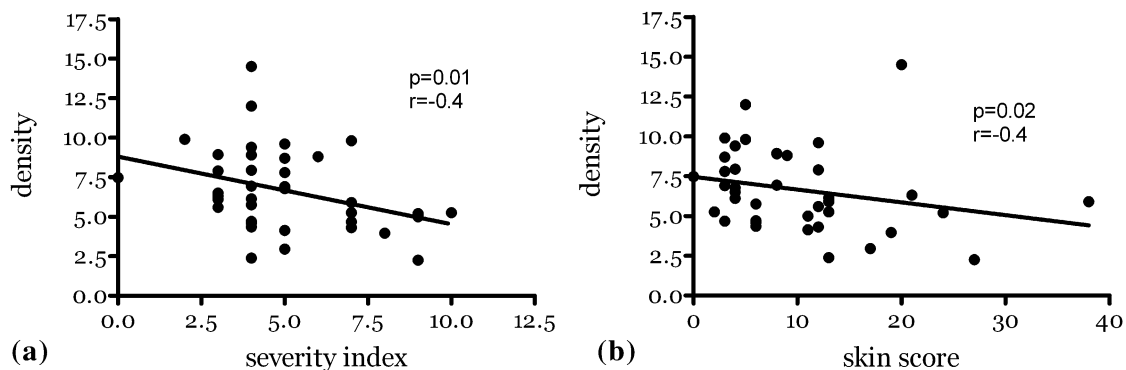
	Giant capillaries	Neoangiogenesis	Avascular areas	Density
Females vs males	3.0 (1-19) vs 6.0 (3-9)	2.5 (0-10) vs 4.2 (0-8)	1.5 (0-10) vs 1.1 (0-3)	6.2 (2.3-14.5) vs 6.9 (5.9-7.9)
Diffuse vs limited	3.0 (1-11) vs 3.1 (1-19)	1.0 (0-8) vs 2.7 (0-10)	2.0 (0-10) vs 1.2 (0-9)	5.2 (2.3-14.5) vs 6.5 (3.0-12)
Anti-ScI70 vs ACA	1.8 (1-11) vs 3.4 (1-19)	2.8 (0-8) vs 2.7 (0-10)	1.8 (0-10) vs 1.5 (0-9)	4.9 (2.3-8.8) vs 6.1 (2.4-14.5)
Anti-ScI70 vs others	1.8 (1-11) vs 8.7 (1-19)	2.8 (0-8) vs 1.4 (0-10)	1.8 (0-10) vs 0.5 (0-2)	4.9 (2.3-8.8) vs 7.5 (4.0-14.5)
ACA vs others	3.4 (1-19) vs 8.7 (1-19)	2.7 (0-10) vs 1.4 (0-10)	1.5 (0-10) vs 0.5 (0-2)	6.1 (2.3-14.5) vs 7.5 (4.0-14.5)
No ulcers vs ulcers	3.2 (1-18) vs 3.0 (1-19)	2.7 (0-8) vs 2.0 (0-10)	1.5 (0-10) vs 1.4 (0-5)	6.1 (2.3-14.5) vs 5.9 (4.0-9.8)
No GE vs GE	5.7 (1-17) vs 2.8 (1-19)	1.6 (0-3) vs 3.0 (0-10)	1.0 (0-4) vs 1.5 (0-10)	6.9 (3.0-9.4) vs 6.0 (2.3-14.5)
sPAP < 45 mmHg vs > 45 mmHg	3.3 (1-19) vs 2.0 (1-3)	2.4 (0-10) vs 6.5 (0-8)	1.2 (0-9) vs 4.3 (2-10)*	6.3 (2.4-14.5) vs 5.2 (2.3-5.9)
DLCO < 50 % vs > 50 %	2.5 (1-7) vs 4.1 (1-19)	5.0 (0-8) vs 2.7 (0-10)	1.5 (0-4) vs 1.2 (0-10)	5.3 (4.6-14.5) vs 6.1 (2.3-12)
FVC < 80 % vs > 80 %	3.4 (1-19) vs 3.0 (2-9)	2.7 (0-10) vs 2.4 (0-8)	0.9 (0-9) vs 2.1 (0-10)	6.5 (2.3-14.5) vs 5.3 (2.3-7.9)
No honeycombing vs honeycombing	3.6 (1-19) vs 2.3 (1-3)	1.5 (0-10) vs 5.0 (1-6)	0.7 (0-4) vs 1.2 (0-10)	6.3 (4.0-9.9) vs 5.0 (2.3-14.5)
Skin score < 14 vs > 14	3.0 (1-19) vs 8.0 (1-11)	2.7 (0-10) vs 1.2 (0-8)	1.4 (0-9) vs 1.8 (0-10)	6.6 (2.4-12.0) vs 4.6 (2.3-14.5)
No iloprost vs iloprost	3.6 (1-19) vs 3.0 (1-16)	2.0 (0-8) vs 3.0 (0-10)	0.7 (0-3.4) vs 1.5 (0-10)*	6.1 (4.0-9.9) vs 6.1 (2.3-14.5)
No bosentan vs bosentan	3.4 (1-19) vs 2.0 (1-6)	1.8 (0-10) vs 5.8 (3-8)*	0.9 (0-10) vs 1.8 (2-4)	6.3 (2.3-14.5) vs 5.6 (5.0-8.8)
Early pattern vs others	0.5 (1-4) vs 3.3 (1-19)*	3.1 (1-10) vs 2.2 (0-10)	0.5 (0-1) vs 1.5 (0-10)*	9.5 (9.0-10.8) vs 5.9 (2.3-14.5)*
Active pattern vs others	8.0 (1-19) vs 1.6 (1-8)*	2.0 (0-10) vs 3.0 (0-10)	0.7 (0-3) vs 2.0 (0-10)*	6.9 (4.0-14.5) vs 5.3 (2.3-12.0)
Late pattern vs others	2.1 (1-7) vs 6.3 (1-19)*	2.7 (0-8) vs 2.4 (0-10)	2.1 (1-10) vs 0.5 (0-3)*	5.0 (2.3-9.8) vs 7.5 (4.0-14.5)*
VEGF-A <0.8U vs >0.8U	3.0 (1-19) vs 3.8 (1-9)	2.0 (0-10) vs 4.5 (0-6)	1.5 (0-10) vs 1.5 (0-3)	6.4 (2.3-12.0) vs 4.7 (4.0-14.5)
CSURI >2.94 vs <2.94	3.0 (1-19) vs 3.1 (1-11)	1.8 (0-8) vs 2.6 (0-10)	1.5 (0-10) vs 0.3 (0-1)*	5.8 (2.3-14.5) vs 8.3 (4.0-12.0)*

ACA anti-centromere, anti-ScI70 anti-topoisomerase I, DLCO carbon monoxide diffusing capacity, FVC forced vital capacity, GE gastro-esophageal, NYC nailfold videocapillaroscopy, sPAP systolic pulmonary artery pressure on echocardiography, SSc systemic sclerosis, vs versus

\* *p* < 0.05



**Fig. 2** Avascular areas directly correlated with severity index and inversely with DLCO/AV



**Fig. 3** Mean capillary density correlated inversely with severity index e with skin score

Serum VEGF-A was higher in SSc vs healthy controls (median 0.5, range 0.02–3.05 vs median 0.4, range 0.2–0.5, respectively;  $p = 0.03$ ). Among SSc cases, VEGF-A was inversely correlated with DLCO ( $p = 0.01$ ,  $r = -0.4$ ; Fig. 4b), and directly with ground-glass and reticular pattern extent on HRCT ( $p = 0.04$ ,  $r = +0.4$  for both; Fig. 4b). Moreover, VEGF-A levels were lower in the serum of SSc patients with evidence of interstitial lung disease (median 0.39, range 0.1–3.05 vs median 0.48, range 0.14–1.70, respectively;  $p = 0.44$ ).

## Discussion

The hallmarks of the NVC scleroderma pattern include giant capillaries and avascular areas, albeit poorly specific for SSc, as they are also observed in other connective tissue diseases characterized by Raynaud's phenomenon or interstitial lung disease such as dermatomyositis and mixed connective tissue disease [22]. Nevertheless, almost all scleroderma patients had one or both of those alterations, while the association of scleroderma pattern to other connective tissue diseases is not quite clear, especially for rare

diseases as anti-synthetase syndrome [23, 24]. The present study was aimed at a clear definition of the NVC patterns and fine phenotyping in well-defined SSc clinical subtypes in association with serum VEGF-A levels. Our data cumulatively support the finding that NVC may be helpful not only for the diagnosis, but also for the global and longitudinal evaluation of patients with SSc. Further, serum VEGF-A levels may represent a biomarker to predict interstitial lung involvement in SSc.

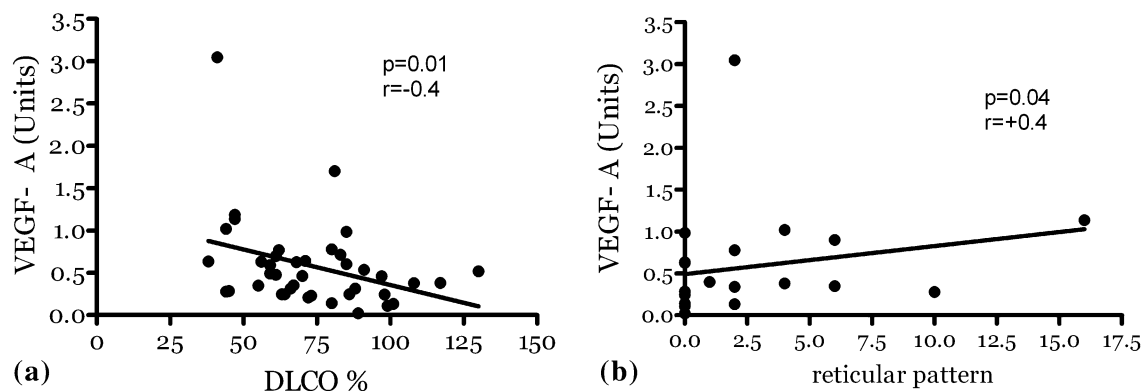
In our cohort, the crude prevalence of giant capillaries and avascular areas appears to be related to the severity of the disease as indicated by the correlations with interstitial lung disease, sPAP, higher skin score, or SSc severity index. When confirmed in larger cohorts, these data may suggest a helpful role of this simple counting score as marker of internal organ involvement. Moreover, it would be interesting to test it in cases of SSc *sine* scleroderma or in other connective tissue diseases with NVC scleroderma pattern and risk of interstitial lung disease or pulmonary arterial hypertension. Another interesting point is to explore NVC in SSc patients with rare autoantibodies, since in our cohort SSc patients with rare autoantibodies seem to have more giant capillaries compared to avascular

**Table 3** Cutolo's NVC patterns, CSURI and VEGF levels in the SSC cohort ( $n = 44$ )

	Early pattern	Active pattern	Late pattern	CSURI	VEGF-A
Females vs males	6 (14.3 %) vs 0 (0 %)	18 (42.9 %) vs 2 (100 %)	18 (42.9 %) vs 0 (0 %)	9.6 (0.14–80.0) vs 23.2 (19.0–27.5)	0.49 (0.02–3.05) vs 0.25 (0.11–0.38)
Diffuse vs limited	1 (10 %) vs 5 (14.7 %)	5 (50.0 %) vs 15 (44.1 %)	4 (40 %) vs 14 (41.7 %)	9.1 (2.4–50.5) vs 10.5 (0.14–80.0)	0.42 (0.11–3.05) vs 0.49 (0.02–0.07)
Anti-Sc170 vs ACA	0 (0 %) vs 2 (9.1 %)	5 (41.7 %) vs 11 (50 %)	7 (58.3 %) vs 10 (45.4 %)	11.4 (3.6–20.7) vs 11.9 (0.51–80.0)	0.46 (0.13–1.14) vs 0.49 (0.02–1.70)
Anti-Sc170 vs others	0 (0 %) vs 4 (40 %)	5 (41.7 %) vs 4 (40 %)	7 (58.3 %) vs 1 (10 %)	11.4 (2.4–50.5) vs 9.2 (0.14–27.5)	0.46 (0.13–1.14) vs 0.43 (0.02–3.05)
ACA vs others	2 (9.1 %) vs 4 (40 %)	11 (50 %) vs 4 (40 %)	10 (45.4 %) vs 1 (10 %)	11.9 (0.51–80.0) vs 9.2 (0.14–27.5)	0.49 (0.02–1.7) vs 0.43 (0.11–3.05)
No ulcers vs ulcers	3 (10.3 %) vs 2 (13.3 %)	13 (29.5 %) vs 7 (46.7 %)	12 (41.4 %) vs 6 (40 %)	11.9 (0.14–68.7) vs 9.1 (0.51–80.0)	0.47 (0.02–3.05) vs 0.50 (0.11–1.14)
No GE vs GE	2 (22.2 %) vs 4 (11.4 %)	4 (44.4 %) vs 16 (45.7 %)	3 (33.3 %) vs 15 (42.5 %)	7.0 (0.51–68.7) vs 12.0 (0.14–80.0)	0.37 (0.13–1.0) vs 0.49 (0.02–3.05)
sPAP < 45 vs > 45 mmHg	6 (14.6 %) vs 0 (0 %)	20 (48.8 %) vs 0 (0 %)	17 (41.4 %) vs 3 (100 %)*	9.9 (0.14–80.0) vs 13 (3.9–15.1)	0.49 (0.02–3.05) vs 0.28 (0.21–0.35)
DLCO < 50 % vs > 50 %	0 (0 %) vs 6 (15.8 %)	2 (33.3 %) vs 18 (47.3 %)	4 (66.6 %) vs 11 (28.9 %)	5.9 (3.9–13.7) vs 11.3 (0.14–80.0)	0.48 (0.28–1.14) vs 0.49 (0.02–3.05)
FVC < 80 % vs > 80 %	0 (0 %) vs 6 (16.2 %)	3 (42.8 %) vs 17 (45.9 %)	4 (57.1 %) vs 14 (37.8 %)	9.1 (0.14–80.0) vs 13 (3.3–27.5)	0.54 (0.02–3.05) vs 0.38 (0.11–1.0)
No honeycombing vs honeycombing	5 (12.8 %) vs 1 (20 %)	19 (48.7 %) vs 1 (20 %)	15 (38.4 %) vs 3 (60 %)*	5.9 (0.14–80.0) vs 11.8 (5.1–15.1)	0.34 (0.02–3.05) vs 0.68 (0.28–1.14)
Skin score < 14 vs > 14	6 (16.2 %) vs 0 (0 %)	16 (43.2 %) vs 4 (57.1 %)	16 (43.2 %) vs 3 (42.8 %)	10.9 (0.14–80.0) vs 5.5 (2.1–68.7)	0.46 (0.02–1.7) vs 0.52 (0.11–3.05)
No iloprost vs iloprost	3 (15.8 %) vs 3 (12 %)	11 (57.9 %) vs 9 (36 %)	5 (26.3 %) vs 13 (52 %)*	10.5 (0.51–68.7) vs 9.2 (0.14–80.0)	0.38 (0.02–3.05) vs 0.49 (0.13–1.70)
No bosentan vs bosentan	6 (16.2 %) vs 0 (0 %)	19 (51.3 %) vs 1 (14.2 %)	12 (32.4 %) vs 6 (85.7 %)*	10.9 (0.14–80.0) vs 8.3 (3.9–22.3)	0.50 (0.02–3.05) vs 0.28 (0.21–0.58)
Early pattern vs others	–	–	–	4.2 (0.14–11.3) vs 11.2 (0.51–80.0)	0.63 (0.02–0.77) vs 0.39 (0.11–3.05)
Active pattern vs others	–	–	–	5.9 (1.4–68.7) vs 12.1 (0.14–80.0)	0.48 (0.11–3.05) vs 0.43 (0.02–1.07)
Late pattern vs others	–	–	–	13.7 (0.51–80.0) vs 5.9 (0.14–68.7)*	0.35 (0.21–1.70) vs 0.58 (0.02–3.05)
VEGF-A < 0.8U vs > 0.8U	5 (13.5 %) vs 1 (14.3 %)	16 (43.2 %) vs 4 (57.1 %)	16 (43.2 %) vs 2 (28.5 %)	10.2 (0.14–80.0) vs 9.0 (3.3–13.7)	–
CSURI > 2.94 vs < 2.94	4 (11.1 %) vs 2 (25 %)	15 (41.6 %) vs 5 (62.5 %)	17 (47.2 %) vs 1 (12.5 %)*	–	0.46 (0.02–3.05) vs 0.58 (0.13–0.71)

anti-Sc170 anti-topoisomerase I, CSURI capillaroscopic skin ulcer risk index, D diffuse, DLCO carbon monoxide diffusing capacity, FVC forced vital capacity, L limited, GE gastro-esophageal, NVC nailfold videocapillaroscopy, VEGF-A vascular endothelial growth factor A, sPAP systolic pulmonary artery pressure on echocardiography, sSc systemic sclerosis; vs: versus, ACA anti-centromere

\*  $p < 0.05$



**Fig. 4** VEGF-A inversely correlated with DLCO and directly with reticular pattern on high-resolution computed tomography

areas; however, lower SSc disease duration was found in this small group of patients.

Assessing Cutolo's pattern is time sparing, but does not allow a quantitative analysis of microvasculopathy. On the other hand, CSURI is time-consuming and provides limited information. Based on our results, the most helpful and simple data to collect is the mean capillary density. In fact, this parameter not only correlates with an important pulmonary function index (DLCO/AV), but also with SSc skin score, SSc severity index, and with the presence of digital skin ulcers.

The scleroderma NVC pattern and, particularly, the presence of giant capillaries usually predate skin fibrosis as observed in patients reporting with Raynaud's phenomenon. In fact, vasculopathy is thought to precede and possibly cause fibrosis in SSc. The exact mechanism leading to giant capillary development and then to avascular areas appearance is not known. It has been reported that the chronic overexpression of VEGF can lead to enlarged capillary development [25] and that higher levels of VEGF characterize the early phase of SSc when giant capillaries are often the first manifestation of SSc [26]. However, in our study, serum VEGF-A levels did not show any association with NVC alterations. A large study enrolling early SSc is necessary to explore this point, especially when aiming at defining biomarkers for early diagnosis and treatment of SSc [27].

Interstitial lung disease is frequent in SSc patients and in a not well-defined subset of subjects is fatally progressive, being the leading cause of death [28]. We have previously reported lower bronchoalveolar lavage fluid VEGF-A levels in SSc patients with lung involvement, particularly in those with alveolitis on bronchoalveolar lavage fluid analysis and ground-glass on lung HRCT [29]. In healthy adults, an excess of VEGF in the lung compared to other tissues contributes to normal lung tissue homeostasis and endothelial tropism [30]. Nevertheless, in SSc an imbalance between compartments seems to characterize patients with interstitial lung disease, but the pathogenic

significance of these data remains to be elucidated. Several reports demonstrated lower VEGF levels in different interstitial lung diseases as lung fibrosis and sarcoidosis [31], but bronchoalveolar lavage fluid analysis is uncomfortable for patients and is not recommended as a routine examination in lung diseases, but in lower airway infection diagnosis [32]. When confirmed in larger SSc cohorts, serum VEGF levels together with pulmonary function tests could be a useful biomarker of interstitial lung disease, maybe better identifying patients needing lung HRCT; moreover, it would be interesting to explore if serum VEGF level changes during the course of SSc, i.e., mirroring lung function decline or predicting progression of SSc interstitial lung disease on HRCT.

## Conclusions

Our data support the importance of NVC not only for the early diagnosis of SSc, but also in the evaluation and possibly prediction of the severity and organ involvement of SSc. We further suggest that prospective studies, ideally multicentric, may confirm the role of serum VEGF levels as putative biomarkers of SSc pulmonary interstitial involvement.

## Compliance with ethical standards

**Conflict of interest** MDS and AC received travel support from Actelion. The other authors declare that they have no conflict of interest.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

**Informed consent** All subjects enrolled signed a written informed consent for serum samples and data analysis after approval from the local ethical committee.

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