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Running title: Nailfold capillary abnormalities in SSc-PAH

ABSTRACT

Objectives. To evaluate differences in nailfold videocapillaroscopy (NVC) findings between systemic sclerosis-SSc patients with and without a diagnosis of pulmonary arterial hypertension (PAH).

Methods. 110 SSc patients were enrolled in this cross-sectional, case-control, multi-centre study. Patients were divided into cases (SSc-PAH confirmed by right hearth catheterization-RHC) and controls (SSc-nonPAH with low probability of PAH). NVC patterns (early, active, and late) and morphological parameters (microvascular density, non-specific abnormalities, giant capillaries, micro-haemorrhages, avascular areas) were considered using a semiquantitative scoring system.

Results. SSc-PAH patients showed higher frequencies of late pattern ($p<0.01$), non-specific abnormalities ($p<0.01$), lower capillary density ($p<0.01$), higher avascular areas ($p<0.01$), and a higher mean NVC score ($p<0.01$). Contrarily, the early/active pattern ($p<0.01$) and a higher rate of micro-haemorrhages ($p=0.04$) were

more frequent in non-PAH patients. By the multivariate analysis, SSc-PAH patients, compared to non-PAH, had more non-specific abnormalities (27/55, 49.1% vs 10/55, 18.2%, adjusted OR: 16.89, 95%CI: 3.06-93.16), a lower capillary density (grade 3, 20/55, 36.4% vs 5/55, 9.1%, adjusted OR: 38.33, 95%CI: 2.34-367.80), and avascular areas (18/55, 32.7% vs 10/55, 18.2%, adjusted OR: 16.90, 95%CI: 2.64-44.35). A correlation was found between the mean pulmonary arterial pressure-mPAP and avascular areas ($p<0.01$), capillary density ($p<0.01$), and non-specific abnormalities ($p<0.01$). A clinical model including the NVC variables may be able to predict the diagnosis of PAH.

Conclusions: Our results indicate that the distinctive peripheral microcirculatory injury of SSc, i.e capillary loss and morphological abnormalities, appear more severe and pronounced in patients with SSc-PAH.

Key words: nailfold capillaroscopy, pulmonary arterial hypertension, systemic sclerosis

Key messages:

- Patients with SSc-PAH show the presence of specific NVC abnormalities, respect to non-PAH.
- PAH is associated with significant capillary loss and presence of nailfold capillaroscopic late pattern.
- The NVC findings may resemble the severity of the injured pulmonary circulation of SSc-PAH patients.

Introduction

Pulmonary arterial hypertension (PAH) is considered one of the most notable lung complications of systemic sclerosis (SSc) (1-6). It is included in a group of diseases distinguished hemodynamically by a mean pulmonary artery pressure (mPAP) exceeding 20-25 mmHg at rest, as assessed by right heart catheterization (RHC) (1-4). Medial smooth muscle cells hypertrophy of small pulmonary arteries, particularly the small resistance arterioles and pre-capillary vessels (lumen diameter < 150μ), widespread endothelial injury, and vascular inflammation result in pathological vascular remodelling, pulmonary vasoconstriction, and increase in pulmonary vascular resistance (5-9)

PAH is a major cause of morbidity and one of the leading causes of death in SSc (5, 6), affecting up to 15% of patients, with a high rate of mortality within 3 years from diagnosis (8). Despite the possibilities provided by new targeted therapies in delaying the progression of PAH, we still need an early and accurate diagnosis, based on new biomarkers, to improve the survival of these patients (5-9).

A systemic vascular damage is present in SSc patients and different methods can assess the microvascular involvement. The most widely used is an easy, standardized, non-invasive technique named nailfold videocapillaroscopy (NVC) (9, 10), which can provide an “in vivo” visual inspection of capillaries. Typical abnormalities reported in SSc include giant capillaries, micro-haemorrhages, neoangiogenesis, and decreased capillary density with progressive devascularisation (10). These alterations at nailfold can be qualified as NVC-SSc pattern (namely early, active, and late) and quantified by different scoring systems for both

diagnostic and prognostic purposes (11, 12), standing as a clinical biomarker of disease activity, severity, and internal organ involvement (5, 9, 10).

A number of papers have attempted to address which NVC alterations are associated with PAH (13-20). Also, NVC might be able to distinguish SSc patients with and without PAH (7, 9, 13-20). This is an important aspect, as NVC changes may reflect the overall ongoing vascular remodelling underlying disease progression and more aggressive organ involvement. Thus, NVC may be used for an earlier recognition of possible organ damage.

Therefore, we compared qualitative and quantitative NVC findings in SSc patients with and without a diagnosis of PAH according to the 2015 European Society of Cardiology/European Respiratory Society (ESC/ERS) definition (4). Moreover, we investigated the relationship between NVC findings and the value of mPAP, the main hemodynamic parameter required for the diagnosis of PAH (1, 4).

Patients and methods

Study design, cases, and controls definitions

A cross-sectional, case-control, multicentre study was conducted in five tertiary University Rheumatology centres in Italy with expertise in SSc diagnosis and management, as well as in NVC (10, 14, 18, 19). PAH assessment was performed by the local Cardiology Units with experience in RHC. All the recruited patients met the ACR/EULAR 2013 classification criteria for SSc (22). The DETECT screening algorithm for PAH was used to optimize diagnostic RHC and minimize missed PAH diagnosis (23). The DETECT algorithm can identify patients with PAH in the asymptomatic stages, using a broad panel of non-invasive variables, including clinical data, pulmonary function tests, immunological, biological, electrocardiographic, and echocardiographic parameters (24). Those with a high PAH probability underwent RHC. On the other hand, due to ethical reasons, RHC was not carried out in those with a low probability of PAH. Besides mPAP, other parameters of pre-capillary involvement, namely pulmonary artery wedge pressure (PAWP) ≤15 mmHg and pulmonary vascular resistance (PVR) >3 Wood units (WU) were considered (1, 4). PAH was diagnosed when mPAP >25 mmHg, PAWP≤15 mmHg, PVR>3 Wood Units at rest, as recommended (3, 4, 23). Patients were divided into cases (patients with SSc and PAH confirmed by RHC) and controls (patients with SSc and a low probability of PAH according to the DETECT-PAH algorithm).

SSc-PAH patients and SSc-non-PAH controls were matched for sex, age, and disease duration. Written informed consent was obtained from all participants and data were collected into a comprehensive database. Study approval was obtained from the proposing institution (Rheumatology Unit, Marche Polytechnic University) in the context of a larger database of patients with RP secondary to SSc (**RAY**naud pheno**M**en**ON** **D**atabae, RAYMOND study n. 257-2020 ID 1650) in accordance with the Declaration of Helsinki.

Demographic, clinical and laboratory data

We collected the following variables for all enrolled patients: sex, age, disease duration, type of cutaneous subset (limited/diffuse), presence of Raynaud's phenomenon (RP), modified Rodnan skin score (mRSS), other

skin involvement (subcutaneous calcinosis, telangiectasia), presence of digital ulcers-DUs, lung involvement (interstitial lung disease-ILD), oesophageal symptoms (dysphagia, reflux), cardiac involvement (heart failure, pericardial effusion, dilated cardiomyopathy), sicca syndrome (xerostomia/xeropthalmia), and joint involvement (tenosynovitis, arthritis, tendon friction rubs). Laboratory and instrumental evaluations included antinuclear antibodies (ANA), anti-extractable nuclear antigens (anti-ENA), SSc-related antibodies (anti-centromere/CENP-B, anti-topoisomerase I/Scl70, anti-RNA polymerase III) and non-SSc related (anti-U3 RNP, anti-SSA), diffusion capacity for carbon monoxide (DLCo) and high-resolution computed tomography-HRCT (16, 20, 23).

Finally, information about previous and current treatments, regarding PAH, including vasoactive/vasodilator drugs (prostanoids, endothelin receptor antagonists-ERAs, and phosphodiesterase type 5 inhibitors-PDE5Is) were collected.

Nailfold videocapillaroscopy and image analysis

All patients underwent NVC within 3 months before/after the RHC and the use of the DETECT algorithm, using a videocapillaroscope with a 200x magnification optical contact probe. All fingers of both hands, excluding thumbs, were examined. Two contiguous fields of 1 mm in the middle of the nailfold were captured from each finger at least, according to the current method (10, 11, 12, 16, 21, 25). The corresponding digital images were stored and the same experienced investigator for each Rheumatology centre (RDA, VR, FI, SB, DG), blinded for the clinical data, reviewed, and rated the NVC images. The following parameters were considered, according to previous categorizing methods (10, 11, 12, 16, 21, 25): disorganization of the vascular bed, non-specific morphologic abnormalities (10) (tortuous capillaries, except for those described as "normal" having a convex tip, ramified/bushy capillaries, bizarre loops), giant capillaries (width of limbs >50 μm), micro-haemorrhages, impaired capillary flow (appearing as granular/sludge), visibility of the subpapillary venular plexus (SPVP). These parameters were scored using a semi-quantitative rating scale: 0 = no changes; 1 = < 33%; 2 = 33–66% 3 = >66% of changes on the total number of capillaries (10, 16, 25). The mean score combining each item was obtained from a dynamic and complete examination of the fingers (10, 11, 12, 16, 20, 25). The degree of capillary density was 0 when capillaries were >9/mm, 1 when 7–9 capillaries/mm were present, 2 when 4–6 capillaries/mm and 3 in case of < 4 capillaries/mm. The rating for avascular areas was as follows: grade 0 = no obvious avascular areas; grade 1 = mild (one or two discrete areas of vascular deletion); grade 2 = moderate (more than two discrete areas of vascular deletion); grade 3 = severe (presence of large, confluent avascular areas) (10, 16, 20, 25). A consensus concerning image acquisition and analysis, scoring systems and reliability of image acquisition and interpretation had already been reached by the authors (10, 16, 20, 21, 25).

Finally, in each patient, NVC findings were classified in one of the following qualitative "scleroderma" patterns: (i) early (few giant capillaries, few micro-haemorrhages, quite unaltered capillary distribution, no obvious capillary loss); (ii) active (frequent giant capillaries and micro-haemorrhages, moderate capillary loss with rare avascular areas, mild disorganization of the capillary bed, with or without few ramified capillaries);

(iii) late (irregular enlarged capillaries, few or absent giant capillaries, lack of haemorrhages, severe capillary loss with confluent avascular areas, severe disorganization of the capillary array, frequent ramified/bushy capillaries) (10, 11, 12, 16, 21, 25) (Figure.1).

Statistical analysis

Qualitative variables were reported using the absolute frequencies and/or their corresponding percentage. Quantitative variables were reported using the mean and standard deviation (SD). Baseline demographic, laboratory, and disease-related data were compared among cases and controls using the Chi-Square test (for qualitative variables) and Mann-Whitney U test (for quantitative variables). Multivariable statistical analysis was also performed by using a logistic regression model adjusted for sex, age, disease duration, serological status (anti-Scl70 vs anti-centromere vs others anti-ENA antibodies), skin subset (diffuse vs limited), use of prostanoids, ERAs, PDE5Is, and presence of DUs. These variables were selected "a priori" since they had shown an association with SSc and PAH (17, 18, 20). The diagnostic performance of NVC findings was reported using sensitivity, specificity and accuracy and their 95% confidence intervals (95%CI). The correlation between hemodynamic and NVC findings was evaluated using the Spearman's rank correlation coefficient. Finally, to assess the additional value of NVC findings over clinical findings in predicting the diagnosis of PAH, we compared the area under the receiver operating characteristic (ROC) curves (AUC) of a "clinical model" including sex, age, disease duration, serological status (anti-Scl70 vs anti-centromere vs others anti-ENA antibodies), skin subset (diffuse vs limited), use of prostanoids, ERAs, PDE5Is, telangiectasias, and presence of DUs, a "NVC model" (including NVC findings that showed a significant association with PAH) and a clinical+NVC model (including the variables in the "clinical model" and those in the "NVC model"). A p value<0.05 was considered significant. The analyses were carried out using STATA v.14.

Results

Fifty-five patients with SSc and a diagnosis of PAH by RHC (SSc-PAH, cases) and 55 patients with SSc and a low probability of PAH according to the DETECT algorithm (SSc-non-PAH, controls) were enrolled.

SSc-PAH patients showed a higher frequency of limited cutaneous involvement (46/55, 83.6% vs 37/55, 67.3%, p=0.05) than non-PAH, and were prescribed more frequently with ERAs (39/55, 70.9% vs 23/55, 41.8%, p<0.01) and PDE5Is (25/55, 45.4% vs 14/55, 25.4%, p=0.03). Contrarily, non-PAH patients were more often anti-Scl70 positive (21/55, 38.2% vs 10/55, 18.5%, p=0.02), displayed DUs (30/55, 54.5% vs 17/55, 30.9%, p=0.01) and were treated with prostanoids (34/55, 65.3% vs 22/55, 40%, p=0.02). Table 1 provides a description of patients' characteristics.

The two groups significantly differed in NVC findings (Table 2). Particularly, SSc-PAH patients showed a higher frequency of late pattern (30/55, 54.5%, vs 4/55, 7.3%, p<0.01), that includes as per definition and here confirmed such non-specific abnormal shaped capillaries (neoangiogensis) found in higher rate (27/55, 49.1% vs 10/55, 18.2%, p<0.01), a lower capillary density (20/55, 36.4% vs 5/55, 9.1%, p<0.01), high percentages

of avascular areas (grade 2, 22/55, 40.0% vs 8/55, 14.5%, p<0.01 and grade 3, 18/55, 32.7% vs 10/55, 18.2%, p<0.01), and a higher mean NVC score (26/55, 47.3%, vs 5/55, 9.1%, p<0.01). On the contrary, the early/active pattern (51/55, 91.7% vs 24/55, 43.6%, p<0.01) and a higher rate of micro-haemorrhages (20/55, 36.3% vs 11/55, 20%, p=0.04) were more frequent in non-PAH patients (Table 2).

Notably, specific NVC findings were significantly associated with the diagnosis of PAH in multivariate regression analysis, after adjusting for sex, age, disease duration, serological status (anti-Scl70 vs anti-centromere vs others anti-ENA antibodies), skin subset (diffuse vs limited), use of prostanoids, ERAs, PDE5Is, and presence of DUs (Table 2). In particular, SSc-PAH patients, compared to non-PAH, had more non-specific capillary abnormalities (grade 3, adjusted OR: 16.89, 95%CI: 3.06-93.16), low capillary density (grade 3, adjusted OR: 38.33, 95%CI: 2.34-367.80), and presence of severe avascular areas (grade 2, adjusted OR: 17.78, 95%CI 2.56-86.74; grade 3, adjusted OR: 16.90, 95%CI: 2.64-44.35). Finally, the mean NVC score was significantly associated with a diagnosis of PAH. Indeed, grade 3 was found in 26/55 (47.3%) cases vs 5/55 controls (9.1%), with an adjusted O of 47.53 (95%CI: 4.87-464.13).

Data regarding the diagnostic performance of the capillaroscopic findings are reported in Table 3. The NVC pattern had the best AUC (AUC: 0.80, 95%CI 0.73-0.88), followed by non-specific abnormalities (AUC: 0.71, 95%CI 0.62-0.80), capillary density (AUC: 0.72, 95%CI 0.64-0.80), avascular areas (AUC: 0.70, 95%CI 0.61-0.80) and the mean NVC score (AUC: 0.74, 95%CI 0.66-0.82). Highest specificity values pertain to the late pattern (92.7, 95%CI 82.4-98.0), non-specific abnormalities (grade 3: 81.8, 95% CI 69.1-90.9), low capillary density (grade 3: 90.9, 95%CI 80.1-97.0), avascular areas (grade 2:85.5, 95%CI 73.3-93.5; grade 3: 81.8, 95%CI 69.1-90.9) and mean NVC score (grade 3: 90.9, 95%CI 80.1-97.0).

A strong correlation was found between RHC values of mPAP and the mean NVC score (rho=0.40, p<0.01), whereas avascular areas (rho=0.34, p<0.01), capillary density (rho=0.34, p<0.01), and non-specific morphological capillary abnormalities (rho=0.35, p<0.01) showed a moderate correlation with mPAP.

The inclusion of NVC findings (i.e., NVC pattern, non-specific abnormalities, capillary density, avascular areas, NVC score) increased the predictive ability of a clinical model (i.e., sex, age, disease duration, serological status-anti-Scl70 vs anti-centromere vs other anti-ENA antibodies, skin subset-diffuse vs limited, use of prostanoids, ERAs, PDE5Is, telangiectasias, and DUs) in estimating the risk of PAH (Table 4).

Discussion

To the best of our knowledge, this study analysed the largest existing sample of patients with SSc-PAH diagnosed by RHC (11-18). Our results clearly highlight the presence of specific NVC abnormalities in patients with PAH, respect to non-PAH.

Overall, SSc-PAH patients showed a high frequency of the late pattern, that accordingly includes low degree of capillary density (<4 loops/mm), along with a high grade of vascular deletion, namely avascular areas, and a higher mean of NVC score, as well as high degree of non-specific abnormalities. This association was confirmed in a multivariable model, adjusted for the main disease-related features. The same findings exhibited a high specificity, hence their identification should induce clinician to consider the presence of PAH. Non-

PAH patients showed an early/active pattern more often, and higher rates of micro-haemorrhages, regularly observed with this type of qualitative classification (11, 12). Early and active patterns are recognized to be associated with a less severe disease (9, 20). Furthermore, strong correlation was found between values of mPAP both with the NVC pattern and the mean NVC score, whereas non-specific capillary abnormalities, capillary density, and avascular areas showed a moderate correlation. Additionally, a model including both NVC significant variables and clinical data performed significantly better than clinical data alone in predicting PAH in SSc patients.

Taken together, our results indicate that the distinctive peripheral microcirculatory injury of SSc, i.e the capillary loss, as indicated by the late pattern, reduced capillary density and avascular areas, combined with a striking alteration of capillary morphology, appear more severe and pronounced in patients with PAH, and that these NVC findings significantly correlate with mPAP. These specific capillaroscopic changes, together with typical clinical characteristics of the disease, may improve the predictive ability to diagnose PAH in SSc patients.

Not surprisingly, SSc-PAH patients displayed a limited cutaneous subset (15, 16, 19, 26), a lower frequency of Scl-70 antibodies (16, 20), and a cardiac involvement (13, 18). They used more ERAs and PDI5, as recommended for the treatment of PAH (6, 20).

A few studies, whose population consisted of small groups of patients (13-19), have been carried out investigating NVC abnormalities in SSc-PAH patients diagnosed by means of RHC, without applying the DETECT algorithm, mostly confirming the presence of low capillary density, both in case-control (15, 19, 20) and observational reports (14, 17). NVC findings reported to be associated with PAH were higher avascular scores (16, 19, 20) and/or active-late pattern (16, 17, 20). Moreover, some authors found an increase in capillary width (17, 19) and neoangiogenesis (17, 18) that seem to be somewhat different to our observations. However, both the presence of variously shaped neoangiogenic loops and the marked increase in diameter may fall under the description of "non-specific abnormalities" (10), which was more significantly frequent in our SSc-PAH patients. The neoformation of capillaries and/or their widening represent an attempt to counteract capillary damage and dropout before the appearance of avascular areas (10, 21, 25).

A certain amount of capillary loss appears to be a common feature of both the nailfold and the pulmonary bed in SSc (5, 9), and a more prominent loss of capillaries may be due to PAH itself, a condition that increase**s** the reduction in vascularity, resulting in tissue ischemia, fibrosis, and eventually organ failure (5, 16, 19, 27). Therefore, the NVC results seem to show what occurs in the pulmonary circulation of SSc patients, and the greater capillary loss in PAH-SSc patients, together with the alteration in loops' morphology, meaning vascular remodelling, may be a marker defining the severity of pulmonary disease (5, 26, 27, 28, 29). Our observations are reinforced by the relationship found between mPAP and the degree of capillary density. Rarefaction in pulmonary microcirculation as reflected by peripheral NVC is directly related to the value of mPAP (15, 19, 20, 29).

The lack of association with ILD was expected, as from a hemodynamic and pathogenetic viewpoint our SSc-PAH patients undergoing RHC belonged to group I, according to PH classification (1-4, 26). Nevertheless,

progressive loss of capillaries over time by NVC was significantly associated with PAH and not with ILD development, when the two condition coexist (30).

Finally, the missing association in PAH-SSc patients with DUs needs further investigation. A comparison between patients with SSc-DU and without DU could not find a clear link between DU and internal organ vasculopathy, even suggesting a trend to a lower incidence of PAH (31). Moreover, the possibility of an improvement of pre-existing DUs induced by PAH treatments may have acted as a confounder (32).

Our study is limited by the cross-sectional design, which prevents establishing a causal relationship between the presence of NVC abnormalities and the subsequent development of PAH. The diagnosis of PAH by RHC was based on 2016 ESC/ERS guidelines, but not on the new hemodynamic definition revised in 2022 (mPAP>20 mmHg and PVR>2 WU) (3). However, the latest guidelines emphasized that the efficacy of drugs approved for PH had been demonstrated in patients with mPAP $\geqslant$ 25 mmHg and PVR>3 WU (3, 4, 23).

In conclusion, we found specific NVC findings in SSc-PAH patients, dominated by severe capillary loss as usually observed in presence of the late NVC pattern, and marked morphological abnormalities, significantly associated with mPAP, suggesting a more widespread microvasculopathy in these subjects.

Given the ability to predict PAH of a model consisting of NVC and clinical variables, our research agenda will be to plan a well-defined prospective, multicenter study to evaluate whether NVC findings could allow an earlier diagnosis of PAH and provide an additional value when combined with the DETECT algorithm in the risk estimation of PAH in SSc patients (9, 14, 24, 30, 33, 34).

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EC performed the statistical analysis, RDA the writing of the original draft.
All authors critically revised it for interpretation of results and important intellectual content.
All authors approved the final version of the manuscript.

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Figure. 1

Nailfold videocapillaroscopy x 200 magnification. A) “early pattern”; B) “active” pattern; C-D) “late” pattern. Arrows: giant capillaries. Arrowheads: abnormal morphology (neoangiogenesis) . Rhombus: micro-hemorrhage. Circle: avascular area.

Table 1. Demographic, clinical and laboratory data of SSc patients with and without PAH

	SSc-PAH (n=55)	SSc-non-PAH (n=55)	P value
Sex - Female, n (%)	53 (96.4)	53 (96.4)	0.51
Age, mean (SD)	66.7 (9.0)	64.6 (10.8)	0.10
Disease duration - months, mean (SD)	210.7 (164.7)	165.8 (102.9)	0.49
sPAP, mean (SD)	59.9 (16.9)		
mPAP, mean (SD)	39.1 (12.2)		
PVR (Wood Units), mean (SD)	8.6 (5.1)		
PAWP, mean (SD)	10.8 (4.4)		
Raynaud's phenomenon, n (%)	55 (100)	55 (100)	0.99
Limited cutaneous involvement, n (%)	46 (83.6)	37 (67.3)	0.05*
Diffuse cutaneous involvement, n (%)	9 (16.4)	18 (32.7)	
Modified Rodnan skin score, mean (SD)	7.9 (8.1)	7.0 (4.6)	0.51
Calcinosis, n (%)	12 (21.8)	10 (18.2)	0.63
Teleangectasias, n (%)	41 (74.5)	32 (58.2)	0.07
Digital ulcers, n (%)	17 (30.9)	30 (54.5)	0.01*
Interstitial lung disease, n (%)	30 (54.5)	21 (38.2)	0.09
Gastroinstestinal symptoms, n (%)	38 (69.1)	37 (67.3)	0.84
Cardiac involvement, n (%)	27 (49.1)	16 (29.1)	0.03*
Sicca syndrome, n (%)	51 (92.7)	48 (87.3)	0.88
Joint involvement, n (%)	10 (18.2)	15 (27.3)	0.26
ANA positive, n (%)	54 (98.2)	55 (100)	0.85
Anti-centromere, n (%)	36 (65.4)	27 (49.1)	0.08
Anti-Scl70, n (%)	10 (18.2)	21 (38.2)	0.02*
Anti-RNA Polimerase III, n (%)	4 (7.3)	5 (9.1)	0.73
Others (anti-U3 RNP, anti-SSA), n (%)	4 (7.3)	2 (3.6)	0.24
Vasoactive/Vasodilatators use, n (%)	55 (100)	37 (67.3)	<0.01*
- **Prostanoids use, n (%)**	22 (40.0)	34 (61.8)	0.02*
- **ERAs use, n (%)**	39 (70.9)	23 (41.8)	<0.01*
- **PDE-5Is use, n (%)**	25 (45.4)	14 (25.4)	0.03*

ERAs, Endothelin receptor antagonists.
PDE5Is, Phosphodiesterase inhibitors.
sPAP, Systolic pulmonary arterial pressure.
mPAP, Mean pulmonary arterial pressure.
PAWP, Pulmonary Artery Wedge Pressure.
Significant p values*

Table 2. Nailfold capillaroscopic characteristics in SSc patients with and without pulmonary arterial hypertension

	SSc-PAH (n=55)	SSc-non-PAH (n=55)	P value [a]	Unadjusted OR (95%CI)	Adjusted OR (95%CI) [b]
NVC pattern					
Non-specific, n (%)	1 (1.8)	0	<0.01	/	/
Early, n (%)	5 (9.1)	17 (30.9)		/	/
Active, n (%)	19 (34.5)	34 (61.8)		/	/
Late, n (%)	30 (54.5)	4 (7.3)		/	/
Disorganization of the vascular bed					
0	1 (1.8)	6 (10.9)	0.09	Reference	Reference
1	6 (10.9)	9 (16.4)		4.00 (0.38-42.18)	0.61 (0.03-14.06)
2	29 (52.7)	23 (41.8)		7.57 (0.85-67.37)	2.36 (0.12-45.85)
3	19 (34.5)	17 (30.9)		6.71 (0.73-61.49)	2.67 (0.12-59.40)
Non-specific capillary abnormalities					
0	0	1 (1.8)	<0.01	/	/
1	6 (10.9)	21 (38.2)		Reference	Reference
2	22 (40.0)	23 (41.8)		3.35 (1.14-9.85)	4.74 (0.98-22.88)
3	27 (49.1)	10 (18.2)		9.45 (2.96-30.20)**	16.89 (3.06-93.16)**
Giant capillaries					
0	7 (12.7)	9 (16.4)	0.10	Reference	Reference
1	13 (23.6)	21 (38.2)		0.80 (0.24-2.66)	0.40 (0.08-2.17)
2	31 (56.4)	23 (41.8)		1.73 (0.56-5.34)	1.15 (0.24-5.41)
3	4 (7.3)	2 (3.6)		2.57 (0.36-18.33)	1.22 (0.10-15.30)
Capillary density					
0	1 (1.8)	7 (12.7)	<0.01	Reference	Reference
1	3 (5.5)	13 (23.6)		1.62 (0.13-18.58)	0.80 (0.04-15.54)
2	31 (56.4)	30 (54.5)		7.23 (0.84-62.38)	8.43 (0.51-80.65)
3	20 (36.4)	5 (9.1)		28.00 (2.77-282.97)**	38.33 (2.34-367.80)**
Avascular areas					
0	5 (9.1)	19 (34.6)	<0.01	Reference	Reference
1	10 (18.2)	18 (32.7)		2.11 (0.60-7.38)	3.13 (0.49-19.83)
2	22 (40.0)	8 (14.5)		10.45 (2.92-37.39)**	17.78 (2.56-86.74)**
3	18 (32.7)	10 (18.2)		6.84 (1.96-23.93)	16.90 (2.64-44.35)**
Microhaemorrhages					
0	18 (32.7)	13 (23.6)	0.04	Reference	Reference
1	26 (47.3)	22 (40.0)		0.85 (0.34-2.12)	3.74 (0.93-15.06)
2	10 (18.2)	15 (27.3)		0.48 (0.16-1.41)	1.09 (0.24-4.93)
3	1 (1.8)	5 (9.1)		0.14 (0.02-1.39)	0.26 (0.02-3.79)
Subpapillary plexus visibility					
0	9 (16.4)	10 (18.2)	0.08	Reference	Reference
1	11 (20.0)	17 (30.9)		0.72 (0.22-2.33)	0.53 (0.09-3.28)
2	26 (47.3)	27 (49.1)		1.07 (0.37-3.06)	2.42 (0.45-13.12)
3	9 (16.4)	1 (1.8)		10.0 (1.06-95.23)	19.69 (0.88-278.95)
Capillary flow					
0	2 (3.6)	0	0.08	/	/
1	7 (12.7)	21 (38.2)		Reference	Reference
2	30 (54.5)	22 (40.0)		4.09 (1.48-11.31)	4.28 (0.95-19.28)
3	16 (29.1)	12 (21.8)		4.00 (1.28-12.46)	9.47 (1.57-57.07)
Mean NVC score					
0	0	0	<0.01	/	/
1	3 (5.5)	15 (27.3)		Reference	Reference
2	26 (47.3)	35 (63.6)		3.73 (0.98-14.29)	4.39 (0.62-30.90)
3	26 (47.3)	5 (9.1)		24.27 (5.04-116.86)**	47.53 (4.87-464.13)**

[a] p values were referred to the comparison between SSc-PAH patients with and no-PAH.

[b] the adjusted ORs were calculated on the following multivariate model (sex, age, disease duration, serological status, skin subset, use of prostanoids, endothelin receptor antagonists, phosphodiesterase 5 inhibitors, and presence of digital ulcers).

** significant p values for multivariable analysis

/=Not Calculated

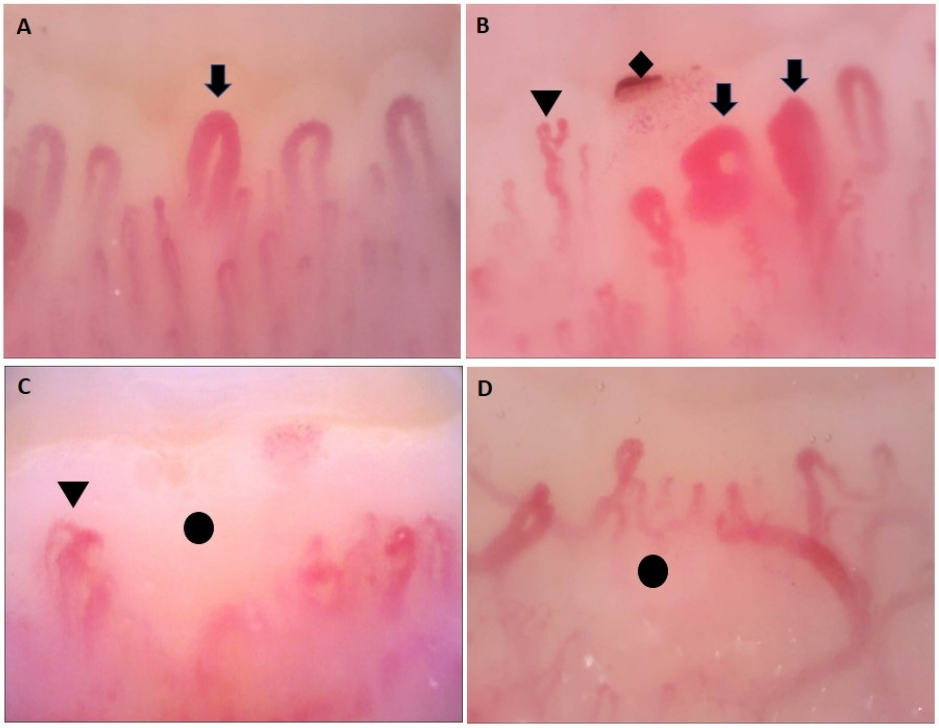
Table 3. Diagnostic performance of nailfold capillaroscopic findings in SSc patients with (n=55) and without (n=55) pulmonary arterial hypertension

	Sensitivity (95%CI)	Specificity (95%CI)	Accuracy (95%CI)	AUC (95%CI)
NVC pattern				
Non-specific, n (%)	1.8 (0.1-9.7)	100 (93.5-100)	50.9 (41.2-60.6)	0.80 (0.73-0.88)
Early, n (%)	9.1 (3.0-20.0)	69.1 (55.2-80.9)	39.1 (29.9-48.9)	
Active, n (%)	34.6 (22.2-48.6)	38.2 (25.4-52.3)	36.4 (27.4-46.1)	
Late, n (%)	55.6 (40.6-68.0)	92.7 (82.4-98.0)	73.6 (64.4-81.6)	
Disorganization of the vascular bed				
0	1.8 (0.1-9.7)	89.1 (77.8-95.9)	45.5 (35.9-55.2)	0.57 (0.47-0.67)
1	10.9 (4.1-22.3)	83.6 (71.2-92.2)	47.3 (37.7-57.0)	
2	44.6 (32.3-57.5)	58.2 (44.1-71.4)	50.8 (41.6-60.1)	
3	34.6 (22.2-48.6)	69.1 (55.2-80.9)	51.8 (42.1-61.5)	
Non-specific capillary abnormalities				
0	0 (0-6.5)	98.2 (90.3-100)	49.1 (39.4-58.8)	0.71 (0.62-0.80)
1	10.9 (4.1-22.3)	61.8 (47.7-74.6)	36.4 (27.4-46.1)	
2	40.0 (27.0-54.1)	58.2 (44.1-71.4)	49.1 (39.4-58.8)	
3	49.1 (35.4-62.9)	81.8 (69.1-90.9)	65.5 (55.8-74.3)	
Giant capillaries				
0	12.7 (5.3-24.5)	83.6 (71.2-92.2)	48.2 (38.6-57.9)	0.59 (0.49-0.69)
1	23.6 (13.2-37.0)	61.8 (47.7-74.6)	42.7 (33.3-52.5)	
2	56.4 (42.3-69.7)	58.2 (44.1-71.4)	57.3 (47.5-66.7)	
3	7.3 (2.0-17.6)	96.4 (87.5-99.6)	51.8 (42.1-61.5)	
Capillary density				
0	1.8 (0.1-9.7)	87.3 (75.5-94.7)	44.6 (35.1-54.3)	0.72 (0.64-0.80)
1	5.5 (1.1-15.1)	76.4 (63.0-86.8)	40.9 (31.6-50.7)	
2	56.4 (42.3-69.7)	45.5 (32.0-59.5)	50.9 (41.2-60.6)	
3	36.4 (23.8-50.4)	90.9 (80.1-97.0)	63.6 (53.9-72.6)	
Avascular areas				
0	9.1 (3.0-20.0)	65.5 (51.4-77.8)	37.3 (28.2-47.0)	0.70 (0.61-0.80)
1	18.2 (9.1-30.9)	67.3 (53.3-79.3)	42.7 (33.3-52.5)	
2	40.0 (27.0-54.1)	85.5 (73.3-93.5)	62.7 (53.0-71.8)	
3	32.7 (20.7-46.7)	81.8 (69.1-90.9)	57.3 (47.5-66.7)	
Microhaemorrhages				
0	32.7 (20.7-46.7)	76.4 (63.0-86.8)	54.6 (44.8-64.1)	0.40 (0.30-0.50)
1	47.3 (33.7-61.2)	60.0 (45.9-73.0)	53.6 (43.9-63.2)	
2	18.2 (9.1-30.9)	72.7 (59.0-83.9)	45.5 (35.9-55.2)	
3	1.8 (0.1-9.7)	90.9 (80.1-97.0)	46.4 (36.8-56.1)	
Subpapillary plexus visibility				
0	16.4 (7.8-28.8)	81.8 (69.1-90.9)	49.1 (39.4-58.8)	0.59 (0.49-0.69)
1	20.0 (10.4-33.0)	69.1 (55.2-80.9)	44.6 (35.1-54.3)	
2	47.3 (33.7-61.2)	50.9 (37.1-64.7)	49.1 (39.4-58.8)	
3	16.4 (7.8-28.8)	98.2 (90.3-100.0)	57.3 (47.5-66.7)	
Capillary flow				
0	3.6 (0.4-12.5)	100 (93.5-100)	51.8 (42.1-61.5)	0.60 (0.50-0.70)
1	12.7 (5.3-24.5)	61.8 (47.7-74.6)	37.3 (28.2-47.0)	
2	54.6 (40.6-68.0)	60.0 (45.9-73.0)	57.4 (47.5-66.7)	
3	29.1 (17.6-42.9)	78.2 (65.0-88.2)	53.6 (43.9-63.2)	
Mean NVC score				
0	0 (0-6.5)	100 (93.5-100)	50.0 (40.3-59.7)	0.74 (0.66-0.82)
1	5.5 (1.1-15.1)	72.7 (59.0-83.9)	39.1 (29.9-48.9)	
2	47.3 (33.7-61.2)	36.4 (23.8-50.4)	41.8 (32.5-51.6)	
3	47.3 (33.7-61.2)	90.9 (80.1-97.0)	69.1 (59.6-77.6)	

AUC: Area Under the Curve NVC: Nailfold Videocapillaroscopy

Table 4. Predictive ability of different models in estimating the risk of PAH.

	AUC	95%CI	P value (using clinical model as reference)
Clinical model	0.87	0.80-0.94	
NVC model	0.88	0.81-0.94	0.92
NVC+clinical model	0.99	0.93-0.99	<0.01
Clinical model: sex, age, disease duration, serological status (anti-Scl70 vs anti-centromere vs other anti-ENA antibodies), skin subset (diffuse vs limited), use of prostanoids, ERAs, PDE5Is, telangiectasias, and DUs. ***NVC model:*** NVC pattern, non-specific abnormalities, capillary density, avascular areas, NVC score			



Nailfold videocapillaroscopy x 200 magnification. A) “early pattern”; B) “active” pattern; C-D) “late” pattern. Arrows: giant capillaries. Arrowheads: neoangiogenetic loops (non-specific abnormalities). Rhombus: micro-hemorrhage. Circle: avascular area.

254x190mm (96 x 96 DPI)