Nailfold Capillaroscopic Abnormalities in Connective Tissue Disease-associated Pulmonary Arterial Hypertension are Not Specific for Systemic Sclerosis

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ABSTRACT

Objective: Systemic microvascular abnormalities as visualised by nailfold video capillaroscopy are a hallmark of systemic sclerosis (SSc) and are linked with organ complications like pulmonary arterial hypertension (PAH). Data on microvascular abnormalities in other connective tissue disease (CTD) associated PAH are lacking.

Methods: Qualitatively and quantitatively assessed nailfold patterns were compared between four patient groups: 1- non-SSc CTD associated PAH (n=14); 2- SSc associated PAH (n=28), 3-non-SSc CTD without PAH (n=28), and 4- SSc without PAH (n=28). Patient groups were matched for CTD disease duration.

Results: Nailfold capillary patterns (normal, early-, active-, and late-scleroderma) were similar for non-SSc CTD with PAH and SSc with PAH patients. Non-SSc CTD patients with PAH demonstrated a predominance of the active pattern, which differed from the predominance of the normal pattern in non-SSc CTD patients without PAH (p<0.01). SSc patients with PAH and SSc patients without PAH showed a predominance of the active and early pattern, respectively (p<0.01). Quantitative analysis showed no significant differences in capillary density between non-SSc CTD with PAH and SSc with PAH patients (p=0.42). Patients with PAH, Compared to non-SSc CTD and SSc patients without PAH, had significantly reduced capillary densities (p<0.01 for both non-SSc CTD and SSc).
Conclusion: Nailfold abnormalities are similar in all CTD-associated PAH patients, regardless of whether the underlying CTD is SSc and non-SSc. This suggests complication specificity rather than disease specificity of nailfold capillary abnormalities.
INTRODUCTION

Pulmonary arterial hypertension (PAH) is a relatively rare disorder complicating connective tissue disease (CTD) and carries a poor prognosis. Systemic sclerosis (SSc) is the disease most known for its association with PAH, with an estimated lifetime risk of 10-15%\(^1,2\). Other CTD’s associated with PAH are systemic lupus erythematosus (SLE), mixed CTD (MCTD), undifferentiated CTD (UCTD), rheumatoid arthritis (RA), Sjögren disease, and dermatomyositis (DM/PM).

In almost all SSc patients, systemic microvascular abnormalities can be observed with a non-invasive method like nailfold capillaroscopy.\(^3\) The detection of characteristic capillary abnormalities by nailfold video capillaroscopy (NVC) is used in daily practice in patients with Raynaud’s phenomenon. Presently, abnormal capillaroscopy is not only considered a diagnostic criterion for the very early diagnosis of SSc, but may also provide prognostic clinical information as it is linked to important microvascular complications, such as digital ulcers and pulmonary arterial hypertension, in SSc.\(^5-8\)

Capillary abnormalities occur in 9-27% of patients with non SSc CTD, but their clinical significance in such patients is unclear. Whether nailfold capillary abnormalities are also present in PAH complicating non-SSc CTD is unknown, but could be highly relevant for classification of underlying CTD in patients presenting with PAH. If capillary abnormalities would also characterise non-SSc CTD associated PAH, their presence would be much less useful to establish a correct diagnosis with respect to the underlying disease in the absence of other signs and symptoms of CTD.
The aim of the present study therefore was to investigate nailfold capillary characteristics, assessed by nailfold videocapillaroscopy, in non-SSc CTD patients with PAH, diagnosed by right heart catheterisation. These characteristics were compared with those observed in SSc PAH patients, and in SSc and non-SSc CTD patients without PAH who were matched for CTD and disease duration.

MATERIALS AND METHODS

Subjects

Between September 2006 and November 2012 consecutive patients with CTD associated PAH were recruited from the Department of Pulmonology of our hospital. Apart from history taking and physical examination, all patients suspected for P(A)H underwent the following diagnostic procedures: lung function tests; exercise testing, arterial blood gas analysis; measurement of liver enzymes (if abnormal further analysis was performed to evaluate portal hypertension); ANA (if positive, further autoantibody analysis was performed); antiphospholipid antibodies (in patients with SLE, and in patients with signs of pulmonary embolism on pulmonary angiography); HIV test, high resolution CT scan (to exclude parenchymal lung disease); perfusion scintigraphy and/or CT angiography (if pulmonary angiography was abnormal); Doppler echocardiography (to exclude left sided and congenital heart disease); and respiratory polysomnography if there was clinical suspicion of obstructive sleep apnoea syndrome. All patients with positive ANA and/or symptoms and signs suggestive of CTD, or with a CTD diagnosed at another hospital, were evaluated by an experienced rheumatologist (see rheumatologic evaluation below). NVC was not part of the
diagnostic work up. To exclude pulmonary hypertension secondary to interstitial lung disease, all included CTD PAH patients required a total lung capacity (TLC) of >70% of predicted, and a pO₂ of >60 mmHg at rest. Control patients (patients with established CTD, but without symptoms or signs of PAH) in whom NVC was performed were recruited from the Department of Rheumatology of our hospital. CTD patients without PAH were matched in a 2:1 fashion with CTD PAH patients for CTD, CTD duration, sex, and, if possible, for age. Study protocols were approved by the local ethics committee and written informed consent was obtained from all participants.

**Pulmonary arterial hypertension diagnosis**

In all PAH patients, PAH diagnosis was established by RHC. Since the study started in 2006, PAH was diagnosed and classified according to the clinical classification of Venice 2003, with PAH defined as a mean pulmonary arterial pressure (PAP) of >25 mmHg at rest or >30 mmHg during exercise. If baseline mean PAP was less than 25 mmHg, haemodynamic measurements were obtained while cycling as previously described. The wedge pressure had to be ≤15 mmHg.

**Rheumatologic evaluation**

Patients with SSc and SLE had to fulfil the American College of Rheumatology (ACR) criteria. SSc patients were classified into a limited cutaneous SSc (lcSSc) and diffuse cutaneous (dcSSc) group according to LeRoy. A diagnosis of MCTD was made when patients fulfilled the Alarcon-Segovia clinical criteria in the presence of anti-U1-ribonucleoprotein (RNP) antibodies. Polymyositis was diagnosed if the patient met the diagnostic criteria as previously described. Patients were classified as having undifferentiated or unclassified
CTD (UCTD) if they had clinical features as seen in patients with CTD, were ANA positive and APA negative, but did not meet the criteria for a defined CTD. Patients with an overlap syndrome fulfilled the diagnostic criteria of more than one CTD.

**Nailfold videocapillaroscopy (NVC)**

NVC was performed and computerised mosaic images were obtained as previously described. The combination of high magnification of nailfold videocapillaroscopy with the ability to view the whole nailfold (as a mosaic) makes it possible to combine qualitative and quantitative assessments in a reliable way. Both 100x (all digits of both hands) and 300x (most digits of both hands) magnification mosaic images were available. The investigator was blinded to patient diagnosis; all images were coded and subsequently assessed in random order (randomisation by computer). Images of all digits were analysed for the presence of enlarged and giant capillaries (see below), avascular areas (loss of capillaries), haemorrhages, distortion of the capillary architecture, and presence of ramified and bushy capillaries. The nailfold capillary pattern most severely affected was scored as normal, abnormal, or scleroderma pattern. The scleroderma pattern was divided in an ‘early’, ‘active’, and ‘late’ pattern as described by Cutolo. An ‘early’ scleroderma pattern is characterised by fewer than four enlarged/giant capillaries per millimetre, few capillary haemorrhages, relatively well preserved capillary architecture, and no evident loss of capillaries. An ‘active’ scleroderma pattern contains more than six enlarged/giant capillaries, frequent capillary haemorrhages, moderate (20-30%) loss of capillaries, and mild disorganisation of the capillary architecture. Finally, a ‘late’ scleroderma pattern shows irregular enlargement of the capillaries, few or absent giant capillaries and haemorrhages, severe (50-70%) loss of capillaries with large avascular areas, profound disorganisation of the
capillary architecture, and the presence of ramified/bushy capillaries. The ring finger of the left hand was quantitatively assessed. Total loop width was based on the measurements of the five capillaries with the largest total loop width. In case of irregular dilated capillary loops, maximal width was measured. A widened capillary was defined as having a total loop width of >90 µm, but <150 µm.\textsuperscript{20} A giant capillary was defined as having a total loop width of >150 µm.\textsuperscript{20} Capillary density (number of loops per millimetre) was calculated by computer from the manually marked loops in the distal row, where a capillary loop was considered to be a distal loop if the apex of the capillary made an angle of >90° with the apex of its adjacent capillaries.\textsuperscript{19} The randomised assessment of nailfold patterns and quantitative assessment were done with a minimal time interval of two weeks between the first and second assessment.

**Statistics**

All numerical data showed a more or less normal distribution and means between two or more categories were analysed using the Student’s t-test or ANOVA respectively. Categorical variables were analysed using the Fisher’s Exact Test. Statistical significance was set at \( p<0.05 \). Results were calculated using computer software (SPSS, version 16.0 for Windows; SPSS; Chicago, IL).

**RESULTS**

Fourteen patients with non-SSc CTD associated PAH (non-SSc with PAH) were included in the study, as were 28 disease duration matched SSc PAH patients (SSc with PAH), 28 non-SSc CTD and disease duration (and also Raynaud’s phenomenon) matched patients without PAH.
(non-SSc without PAH), and 28 disease duration matched SSc patients without PAH (SSc without PAH)(figure 1). The clinical characteristics of the groups are shown in table 1. With the same CTD duration, SSc PAH patients were significantly older compared to the other groups, and had a significantly longer duration of Raynaud's phenomenon than SSc without PAH patients. Of the 4 SLE PAH patients, 1 had Raynaud's phenomenon.

Nailfold capillary patterns, defined as normal, early-, active-, and late scleroderma pattern, were similar between non-SSc and SSc patients with PAH, and differed significantly from all CTD patients without PAH (figure 2). The same trend was observed in the quantitative assessment: non-SSc and SSc patients with PAH showed similar changes in capillary density and total loop width. In SSc patients, those with PAH showed a lower capillary density compared to those without PAH. Similarly, in non-SSc patients, those with PAH showed a lower capillary density and larger total capillary loop width compared to those without PAH (table 2). Twelve out of 42 (28.6%) PAH patients were on, or had a history of PAH-related medical therapy (prostanoids, endothelin receptor antagonists, and/or phosphodiesteras type 5 inhibitors) at the time of nailfold capillaroscopy. Users and non-users of PAH treatment showed no differences in nailfold capillary patterns, capillary density, and total loop width (data not shown).

DISCUSSION

The main finding of our study is that not only in SSc, but also in non-SSc CTD patients, PAH is associated with a more severely affected nailfold capillary pattern than SSc and other CTD patients without PAH who have the same disease duration. Secondly, no differences in the
severity of nailfold patterns could be detected between SSc and non-SSc CTD associated PAH. Thirdly, capillary density reduction was also associated with PAH in both SSc and non-SSc CTD associated PAH patients.

Previous studies have shown an association between the severity of PAH in SSc patients and a reduction of nailfold capillary density\(^5,21\), and severity of capillary pattern abnormalities.\(^21\)

In 2000, Cutolo et al, classified microvascular changes in SSc patients into 3 distinct patterns: an early pattern (few enlarged/giant capillaries, few capillary hemorrhages, no evident loss of capillaries), an active pattern (frequent giant capillaries, frequent capillary hemorrhages, mild disorganization of the capillary network), and a late pattern (irregular enlargement of the capillaries, few or absent giant capillaries, hemorrhages, and extensive avascular areas).\(^22\) These patterns were found to correlate significantly with disease duration, and it was hypothesised that these patterns characterise the evolution of SSc associated microangiopathy, and even predict future organ complications.\(^22\) In the present study, we show that different scleroderma patterns can be detected, even though groups where matched for disease duration. It was predominately the ‘active’ pattern (and to a lesser extent the ‘late’ pattern) that was associated with PAH. A recent small pilot study of SSc patients demonstrated an association between baseline nailfold videocapillaroscopy patterns and future peripheral vascular and lung involvement, with higher risk according to worsening scleroderma patterns (from early to active to late).\(^7\) It could be hypothesised that structural changes in the microcirculation, as shown by NVC, may be related to those vascular abnormalities presenting in pulmonary circulation. The common loss of capillaries at nailfold and pulmonary bed may share a similar pathway and could identity those CTD patients at risk of developing PAH. Of interest, SSc patients with PAH treated with a dual
endothelin receptor antagonist, one of the treatment modalities in SSc associated PAH, showed improvement of scleroderma patterns during treatment. Therefore, it seems worthwhile to investigate whether nailfold capillaroscopy could serve as a biomarker of organ involvement like PAH not only in SSc, but also in non-SSc CTD patients.

Of note, 3 out of 4 SLE patients with PAH had no Raynaud’s phenomenon. Data on nailfold capillary abnormalities in SLE are scarce, but studies suggest that in approximately 35% of SLE patients with Raynaud’s phenomenon scleroderma pattern abnormalities can be observed, whereas SLE patients without Raynaud’s phenomenon predominantly demonstrate a normal capillary pattern. Two studies showed that mean PAP, as determined by cardiac ultrasonography, was higher in SLE patients with Raynaud’s phenomenon than in patients without Raynaud’s phenomenon, and a recent study showed a higher prevalence of Raynaud’s phenomenon in SLE PAH patients than in SLE patients without PAH. The link between Raynaud’s phenomenon and PAH on the one hand, and Raynaud’s phenomenon and nailfold capillary abnormalities on the other, may point to microvascular damage as a common denominator explaining the link between Raynaud’s phenomenon and PAH in SLE-patients. Our current data, however, do not corroborate this hypothesis.

This study has several limitations. Firstly, the group of non-SSc patients with PAH was quite small, and no subgroup analysis of separate CTD’s could be performed. Although the inclusion of PAH patients was based on right heart catheterisation, the exclusion of PAH in CTD patients was mainly based on the absence of symptoms like shortness of breath or decrease in exercise tolerance (although in some patients heart ultrasound and/or exercise
testing were performed). Secondly, the design of the study was cross-sectional, and therefore can only be hypothesis generating rather than providing a proof of the hypothesis generated.

In conclusion, this study shows that severe scleroderma-pattern nailfold capillary abnormalities are a hallmark of CTD-associated PAH, regardless of whether the underlying CTD is SSc and non-SSc. These abnormalities differ significantly from disease duration matched CTD patients without PAH. These data suggest complication specificity rather than disease specificity of nailfold capillary abnormalities. Prospective studies are necessary to substantiate this claim.
In conclusion, this study shows that severe scleroderma-pattern nailfold capillary abnormalities are a hallmark of CTD-associated PAH, regardless of whether the underlying CTD is SSc and non-SSc. These abnormalities differ significantly from disease duration matched CTD patients without PAH. These data suggest complication specificity rather than disease specificity of nailfold capillary abnormalities. Prospective studies are necessary to substantiate this claim.

**Figure 1.** Characteristics of patient groups included in the study. Groups were matched for disease duration.

- **CTD with PAH**:
  - SLE n=4
  - UCTD n=4
  - MCTD n=2
  - PM n=2
  - Overlap n=2

- **CTD without PAH**:
  - SLE n=8
  - UCTD n=8
  - MCTD n=4
  - PM n=4
  - Overlap n=4

- **Non-SSc CTD**:
  - SSc n=28

- **SSc CTD**:
  - SSc n=28

CTD=Connective Tissue Disease; PAH=Pulmonary Arterial Hypertension; SLE=Systemic Lupus Erythematosus; UCTD=Undifferentiated CTD; MCTD=Mixed CTD; PM=Polymyositis; SSc=Systemic Sclerosis
Table 1. Clinical characteristics of CTD patients, matched for disease duration

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<tr>
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<th>CTD with PAH</th>
<th>CTD without PAH</th>
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<tr>
<td></td>
<td>Non-SSc n=14</td>
<td>SSc n=28</td>
</tr>
<tr>
<td></td>
<td>Non-SSc n=28</td>
<td>SSc n=28</td>
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<tr>
<td>Median duration CTD, yr (range)</td>
<td>5.0 (0-23)</td>
<td>5.0 (0-17)</td>
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<tr>
<td>Age, yr (range)</td>
<td>55.4 (27-79)</td>
<td>64.2 (37-79)</td>
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<tr>
<td>Female sex (%)</td>
<td>14 (100)</td>
<td>23 (82.1)</td>
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<tr>
<td>Raynaud’s phenomenon (%)</td>
<td>10 (71.4)</td>
<td>28 (100)</td>
</tr>
<tr>
<td>Median duration of Raynaud’s phenomenon, yr (range, median)</td>
<td>6.5 (1-15)</td>
<td>13.5 (1-51, 13.5)</td>
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<tr>
<td>Mean PAP, mmHg (SD)</td>
<td>42.5 (10.2)</td>
<td>41.4 (11.1)‡</td>
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</table>

* differences significant (p<0.05) between SSc CTD with PAH and Non-SSc CTD with PAH, Non-SSc CTD without PAH, and SSc CTD without PAH
†difference significant (p<0.05) between SSc CTD PAH and Non-SSc CTD PAH
‡excluding 5 SSc patients with PAH during exercise
CTD=Connective Tissue Disease; PAH= Pulmonary Arterial Hypertension; += Present; -= Absent; SSc= Systemic Sclerosis; PAP= Pulmonary Arterial Pressure; NA= Not Applicable
Figure 2. Nailfold patterns in CTD patients, matched for CTD, and CTD duration

CTD=Connective Tissue Disease; SLE= Systemic Lupus Erythematosus; SSc=Systemic Sclerosis; PAH= Pulmonary Arterial Hypertension
Table 2. Quantitative analysis of nailfolds in CTD patients, matched for disease duration.

<table>
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<tr>
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<th>Non-SSc CTD with PAH</th>
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<th>SSc CTD without PAH</th>
<th>Non-SSc CTD with PAH vs. SSc CTD with PAH</th>
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<td>n=28</td>
<td>n=28</td>
<td>n=28</td>
<td>p value</td>
<td>p value</td>
<td>p value</td>
</tr>
<tr>
<td>Density, loops/mm (SD)</td>
<td>5.1 (1.4)</td>
<td>4.6 (1.7)</td>
<td>7.0 (2.0)</td>
<td>6.0 (2.0)</td>
<td>0.42</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total loop width, µm (SD)</td>
<td>97.9 (39.9)</td>
<td>103.9 (40.7)</td>
<td>71.8 (28.8)</td>
<td>100.5 (42.0)</td>
<td>0.63</td>
<td>0.04</td>
<td>0.74</td>
</tr>
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CTD=Connective Tissue Disease; PAH= Pulmonary Arterial Hypertension; SSc=Systemic Sclerosis
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**p value**

- Non-SSc CTD with PAH vs. SSc CTD with PAH: 0.42
- Non-SSc CTD with PAH vs. Non-SSc CTD without PAH: <0.01
- SSc CTD with PAH vs. SSc CTD without PAH: <0.01

**CTD=Connective Tissue Disease; PAH= Pulmonary Arterial Hypertension; SSc=Systemic Sclerosis**

### REFERENCE LIST


