Nail-fold capillary abnormalities are associated with anti-centromere antibody and severity of digital ischaemia

Ariane L. Herrick1, Tonia L. Moore2, Andrea K. Murray1, Nina Whidby1, Joanne B. Manning2, Monica Bhushan2 and Andy Vail3

Abstract

Objectives. Advances in nail-fold capillaroscopy allow capillary abnormalities to be quantified. Our aim was to investigate, in patients with SSc, the relationships between the degree of nail-fold capillary abnormality and disease subtypes (lcSSc and dcSSc), duration of RP and the presence of (i) severe digital ischaemia (as defined by previous i.v. vasodilators, debridements or amputations), (ii) a positive ACA, (iii) clinically evident calcinosis, (iv) pulmonary arterial hypertension and (v) telangiectases.

Methods. This was a retrospective study of 176 patients. Six capillary measurements (four semi-automated and two manual) were calculated (automated width, distance between capillaries, tortuosity and derangement, and manual distance and density). Relationships between these measurements and the different clinical features of SSc were examined using multiple linear regressions (adjusted for age, sex and smoking status).

Results. One hundred and forty-two patients had lcSSc and 34 had dcSSc. Sixty-eight (39%) had a history of severe digital ischaemia, 66 (38%) were anti-centromere positive, 53 (30%) had clinically evident calcinosis and 26 (15%) had an estimated pulmonary artery pressure of >30 mmHg. Positive associations were found between both automated and manually measured distance between capillaries and (i) presence of severe digital ischaemia and (ii) positive ACA, and reduced density was also associated with positive anti-centromere. Patients with moderate/severe telangiectases had wider capillaries compared with those with ‘mild’ lesions.

Conclusions. Both severe digital ischaemia and positive ACA are associated with measurable nail-fold capillaroscopic change, which has the potential of being an outcome measure for the structural microvascular disease associated with SSc-spectrum disorders.

Key words: Nail-fold capillaroscopy, Systemic sclerosis, Digital ischaemia, Anti-centromere antibody, Telangiectases.

Introduction

SSc is characterized by vascular abnormalities, excessive fibrosis and immune dysfunction, although how these inter-relate remains unknown. The vascular abnormalities are predominantly microvascular [1] and both structural and functional [2]. At present, there is no good biomarker for SSc-related microvascular disease. Nail-fold capillaroscopy, a technique which is well established in the identification of underlying CTD/structural microvascular disease in the patient presenting with RP [3, 4], allows direct and non-invasive visualization of the microvasculature and therefore has the potential of becoming an outcome measure of microvascular disease. Advances in capillaroscopy over recent years are allowing realization of this potential, as shown in the following:

(i) The high magnification of video microscopy (up to x600), an extension of the widefield technique,
allows detailed visualization of capillary architecture [5]. Single video frames can be stored, allowing subsequent measurement of capillary dimensions and density [6, 7].

(ii) New computerized software [8] allows (a) construction of video microscopy nail-fold ‘mosaics’, thus combining the advantages of a widefield view of the nailfold with those of high magnification, (b) improved image quality by digitizing a series of video frames and (c) tracking of capillaries over time in longitudinal studies [9]. Manual measurement of capillary density and dimensions, however, remains time consuming.

(iii) Preliminary automation of the computerized software allows rapid analysis of mosaics in terms of total capillary width, density, tortuosity and derangement. Automated measurements of width and density correlate well with the corresponding manual measurements [10].

Prospective studies are currently underway, examining whether nail-fold capillaries change over time in a way that can be measured using these new capillaroscopic techniques. Another key issue is whether the degree of nail-fold capillary abnormality correlates with clinical and serological features of the SSc disease process, in particular those related to vascular change. If so, then nail-fold capillaroscopic change might reflect microvascular disease in other vascular beds. Although previous investigators have looked for associations between the degree of nail-fold capillary change and other clinical features of SSc [11, 12], this is a relatively little researched area.

Against this background, our aim was to conduct a retrospective cross-sectional study to investigate the relationships between the degree of nail-fold capillary abnormality and disease subtypes (lcSSc vs dcSSc [13]) and different ‘vascular’ parameters of SSc, specifically: the presence of severe digital ischaemia, of a positive ACA, of clinically evident calcinosis and of pulmonary hypertension; the duration of RP; and the severity of facial telangiectases. ACA is associated with the severity of digital vascular disease [14, 15]. Although the pathogenesis of s.c. calcinosis is not known, this may relate to the degree of ischaemia, and Vayssairat et al. [16] have reported that capillary density is reduced in patients with calcinosis.

**Patients and methods**

**Patients**

Patients were all under review at Salford Royal Hospital and all signed informed consent. The study was approved by the Salford and Trafford Local Research Ethics Committee. Demographic details, autoantibody status (including anti-centromere), duration of RP and smoking habit were recorded for all patients. Other clinical data to correlate with the nail-fold capillary measurements were collected or defined as follows.

(i) Disease subtype was based on the degree of skin involvement as defined by LeRoy et al. [13]. In lcSSc, skin involvement is confined to distal to the elbows, knees and neck. In dcSSc, proximal limb and/or trunk are involved.

(ii) Severe digital ischaemia was defined as a history of previous admission for i.v. prostanooids, or digital ischaemia and/or ulceration requiring surgical debridement, or amputation.

(iii) Clinically evident calcinosis, as defined by either calcinotic lumps on examination or a good history from the patient of extrusion of calcinotic deposits. Clinically evident rather than radiographic calcinosis was documented, as not all patients had hand radiographs, and the clinical relevance of minor degrees of radiographic calcinosis is not known.

(iv) Pulmonary hypertension was defined in two ways. First, by an estimated pulmonary artery systolic pressure of >30 mmHg plus right atrial pressure on the most recent echocardiogram. Secondly, by the finding of a raised mean pulmonary artery pressure of >25 mmHg at rest or >30 mmHg on exercise, at right heart catheterization.

(v) Telangiectases. At the outpatient clinic, patients were asked if they would be agreeable to having facial photographs (frontal, left and right oblique) taken to document the degree of their telangiectases. Telangiectases were scored by consensus by two observers (one consultant rheumatologist and one consultant dermatologist) as follows: 0 = no telangiectases, 1 = mild, 2 = moderate and 3 = severe. Data on telangiectases were available on a subgroup of patients only.

All patients included in the analysis underwent nail-fold capillaroscopy as described below.

**Nail-fold video capillaroscopy**

Patients were acclimatized for 20 min at a room temperature of 23°C. The ring finger of the patient’s non-dominant hand was examined. A panoramic, high-magnification image of this nail-fold was obtained as previously described [8]. The image was cropped to 3 mm, such that all images to be analysed represented the same length of nailbed. Images were manually marked at each capillary apex.

Measurements were made using an automated analysis programme [10] to give automated measures (in arbitrary units) of capillary width, intercapillary distance, tortuosity (curliness) and derangement (variation in dominant direction of capillaries). In addition, a ‘manual’ measure (in micrometres) of intercapillary distance was calculated (based upon the manual mark-up of apices), and also manual density (number of capillaries/3 mm). All subjects were asked to refrain from smoking and caffeine-containing drinks for 4 h before examination.

**Statistical analysis**

Analysis was carried out using STATA 9.2 (Statacorp LP, College Station, TX, USA) statistical package. Multiple linear regression was performed on each of the capillary measurements to assess relationships with the clinical
factors. Pearson’s correlation analysis was performed to assess relationships between the nail-fold capillary measurements themselves. We omitted automated values that were clearly in error. The values omitted represented a very small proportion of capillaries that were of very distorted shape and much larger than expected. Therefore, the program was unable to process these as capillaries and thus gave invalid data.

Separate analysis was performed on the sample of 47 patients with telangiectases. The relationships of severity of telangiectases with capillary measurements were assessed by linear regression, controlling for age, sex and smoking status. Due to the small sample size, no other additional variables were entered into the model.

Results

One hundred and seventy-six patients (142 with lcSSc and 34 with dcSSc) were included in the study. Their clinical features are summarized in Table 1. Sixty-eight (39%) had severe digital ischaemia (58 had admissions for i.v. vasodilators, 26 had one or more digital debridements and 10 had one or more digital amputations), 66 (38%) were ACA positive and 53 (30%) had clinically evident calcinosis. Twenty-six patients had an estimated pulmonary artery pressure of $>30$ mmHg and 17 had confirmed pulmonary arterial hypertension on right heart catheterization. The median duration of RP was 16.4 years. A subgroup of 47 patients (39 lcSSc and 8 dcSSc) had facial photographs (Table 1) and was, therefore, included in the telangiectases analysis.

Relationship between capillary measurements and disease subtype

No associations were found between any of the capillaroscopic parameters and disease subtype (Table 2). Capillary measurements by subtype of SSc are presented in box and whisker plots (Fig. 1) and were very similar for both disease subtypes. For example, median and interquartile range (IQR) for width was 13.4 (10.6–16.0) AU for lcSSc and 14.4 (12.3–16.4) AU for dcSSc.

Relationship between capillary measurements and digital ischaemia, ACA, calcinosis, pulmonary hypertension and duration of RP

The results of the regressions are presented in Table 2. All factors entered into the regression were controlled for age, sex and smoking. Positive associations were found between both automated and manually measured distance between capillaries and (i) presence of severe digital ischaemia and (ii) positive ACA status. Reduced density was also associated with positive anti-centromere status. There were no associations between any other capillaroscopic and clinical features: specifically, none of the capillaroscopic parameters was associated with clinical calcinosis, a raised pulmonary artery pressure or with the duration of RP. Table 2 shows results of pulmonary artery pressure as defined by an estimated pressure on echocardiography of $>30$ or $<30$ mmHg on echocardiogram. The analyses were repeated defining pulmonary artery hypertension as confirmed on right heart catheterization, but conclusions were unaffected (data not shown).

Table 1: Clinical and demographic characteristics of patients with SSc

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>LcSSc (n = 142)</th>
<th>DcSSc (n = 34)</th>
<th>Total (n = 176)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (min, max), years</td>
<td>58.8 (18, 87)</td>
<td>57.5 (27, 82)</td>
<td>58 (18, 87)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>124 (87)</td>
<td>26 (76)</td>
<td>150 (85)</td>
</tr>
<tr>
<td>Non-smokers, n (%)</td>
<td>113 (80)</td>
<td>23 (68)</td>
<td>136 (77)</td>
</tr>
<tr>
<td>Ex-smokers, n (%)</td>
<td>14 (10)</td>
<td>6 (18)</td>
<td>20 (11)</td>
</tr>
<tr>
<td>Current smokers, n (%)</td>
<td>15 (11)</td>
<td>5 (15)</td>
<td>20 (11)</td>
</tr>
<tr>
<td>Duration of RP, median (min, max), years</td>
<td>18.6 (0, 66)</td>
<td>8.6 (0, 35)</td>
<td>16.4 (0, 66)</td>
</tr>
<tr>
<td>Duration from first non-RP clinical feature of disease, median (min, max), years</td>
<td>10.7 (0, 35)</td>
<td>9.0 (1, 21)</td>
<td>10.6 (0, 35)</td>
</tr>
<tr>
<td>Admission for i.v. vasodilators, n (%)</td>
<td>42 (30)</td>
<td>16 (47)</td>
<td>58 (33)</td>
</tr>
<tr>
<td>Digital debridement, n (%)</td>
<td>22 (16)</td>
<td>4 (12)</td>
<td>26 (15)</td>
</tr>
<tr>
<td>Digital amputation, n (%)</td>
<td>51 (36)</td>
<td>17 (50)</td>
<td>68 (39)</td>
</tr>
<tr>
<td>Anti-centromere positive, n (%)</td>
<td>64 (45)</td>
<td>2 (6)</td>
<td>66 (38)</td>
</tr>
<tr>
<td>Calcinosis (clinical), n (%)</td>
<td>48 (34)</td>
<td>5 (15)</td>
<td>53 (30)</td>
</tr>
<tr>
<td>Pulmonary artery pressure $&gt;30$ mmHg, n (%)</td>
<td>19 (13)</td>
<td>7 (21)</td>
<td>26 (15)</td>
</tr>
<tr>
<td>Pulmonary artery hypertension on right heart catheterization, n (%)</td>
<td>13 (9)</td>
<td>4 (12)</td>
<td>17 (10)</td>
</tr>
<tr>
<td>Telangiectases, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>5 (13)</td>
<td>1 (13)</td>
<td>6 (13)</td>
</tr>
<tr>
<td>Moderate</td>
<td>7 (18)</td>
<td>1 (13)</td>
<td>8 (17)</td>
</tr>
<tr>
<td>Mild</td>
<td>27 (69)</td>
<td>6 (75)</td>
<td>33 (70)</td>
</tr>
</tbody>
</table>
TABLE 2 Multiple linear regression: outcome variables-capillary measurements

<table>
<thead>
<tr>
<th>Factor</th>
<th>Level</th>
<th>Width, Coef. (95% CI)</th>
<th>Automated distance (× 10), Coef. (95% CI)</th>
<th>Tortuosity, Coef. (95% CI)</th>
<th>Derangement, Coef. (95% CI)</th>
<th>Manual distance, µm, Coef. (95% CI)</th>
<th>Density, Coef. (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subtype of SSc</td>
<td>DcSSc vs lcSSc</td>
<td>0.83 (–0.88, 2.53)</td>
<td>–4.25 (–16.45, 7.94)</td>
<td>0.04 (–0.04, 0.13)</td>
<td>–2.08 (–5.50, 1.39)</td>
<td>15.93 (–11.88, 43.74)</td>
<td>–1.10 (–3.60, 1.40)</td>
</tr>
<tr>
<td>Severe digital ischemia</td>
<td>Yes vs no</td>
<td>0.13 (–1.25, 1.51)</td>
<td>10.24 (0.38, 20.10)</td>
<td>0.03 (–0.10, 0.04)</td>
<td>–0.06 (–2.83, 2.71)</td>
<td>30.81 (8.74, 52.87)</td>
<td>–1.95 (–3.94, 0.03)</td>
</tr>
<tr>
<td>ACA status</td>
<td>Positive vs negative</td>
<td>–0.56 (–2.01, 0.89)</td>
<td>11.58 (1.20, 21.95)</td>
<td>0.03 (–0.05, 0.10)</td>
<td>–1.33 (–4.24, 1.58)</td>
<td>25.47 (2.13, 48.82)</td>
<td>–2.45 (–4.55, –0.35)</td>
</tr>
<tr>
<td>Clinical calcinosis</td>
<td>Yes vs no</td>
<td>1.21 (–0.30, 2.72)</td>
<td>–7.58 (–18.40, 3.24)</td>
<td>0.03 (–0.05, 0.11)</td>
<td>1.88 (–1.16, 4.91)</td>
<td>0.97 (–23.38, 25.33)</td>
<td>–0.35 (–2.54, 1.84)</td>
</tr>
<tr>
<td>Pulmonary artery pressure</td>
<td>PAP &gt; 30 mmHg</td>
<td>1.24 (–0.58, 3.07)</td>
<td>12.28 (–0.77, 25.34)</td>
<td>–0.05 (–0.15, 0.04)</td>
<td>2.84 (–0.82, 6.51)</td>
<td>–14.03 (–44.01, 15.96)</td>
<td>0.15 (–2.55, 2.84)</td>
</tr>
<tr>
<td>Duration of RP</td>
<td>Per 10 years</td>
<td>–0.23 (–0.73, 0.26)</td>
<td>0.83 (–2.70, 4.36)</td>
<td>0.00 (–0.02, 0.03)</td>
<td>–0.41 (–1.40, 0.58)</td>
<td>6.50 (–1.51, 14.52)</td>
<td>–0.23 (–0.95, 0.49)</td>
</tr>
</tbody>
</table>

Width, automated distance, tortuosity and derangement measured in arbitrary units. Each capillaroscopic variable was used in turn as a dependent (outcome) variable with the explanatory factors (rows) as multiple independent variables. PAP: pulmonary artery systolic pressure.

We have demonstrated that both severe digital ischemia and anti-centromere positivity are associated with structural microvascular disease: those patients with severe digital ischaemia or with a positive ACA had a greater derangement distance between capillaries (this was true for both automated and manual distances and also for digital ischaemia or with a positive ACA had a greater derangement distance between capillaries (this was true for both automated and manual distances and also for digital ischaemia and anti-centromere positivity). Therefore, the closer the capillaries (and the more difficult it will be for the patient to maintain digital perfusion (and the more structurally abnormal the capillaries and the more difficult it will be for the patient to maintain digital perfusion), the more likely he/she is to develop severe digital ischaemia). It could be argued that this result is intuitive: the greater the distance between the capillaries the lower the capillary density, the more structurally abnormal the capillaries and the more difficult it will be for the patient to maintain digital perfusion (and the more structurally abnormal the capillaries and the more difficult it will be for the patient to maintain digital perfusion). We have demonstrated that both severe digital ischemia and anti-centromere positivity are associated with structural microvascular disease: those patients with severe digital ischaemia or with a positive ACA had a greater derangement distance between capillaries (this was true for both automated and manual distances and also for digital ischaemia and anti-centromere positivity). The closer the capillaries, the more likely the capillaries and the more difficult it will be for the patient to maintain digital perfusion (and the more structurally abnormal the capillaries and the more difficult it will be for the patient to maintain digital perfusion), the more likely he/she is to develop severe digital ischaemia. It could be argued that this result is intuitive: the greater the distance between the capillaries the lower the capillary density, the more structurally abnormal the capillaries and the more difficult it will be for the patient to maintain digital perfusion (and the more structurally abnormal the capillaries and the more difficult it will be for the patient to maintain digital perfusion).
However, none of these parameters—disease subtype, calcinosis, pulmonary hypertension or duration of RP—is as direct a reflector of digital vascular disease as the need for i.v. vasodilators, debridements and amputations. Although we have previously suggested that microvascular disease is more marked in lcSSc than in dcSSc [7, 20], digital ulcers (a key feature of digital vascular disease), including multiple ulcers, occur in either subtype [21]. We did not confirm the findings of Vayssairat et al. [16] that capillary density is reduced in patients with calcinosis. Nor did we confirm the findings of Hofstee et al. [12] that capillary density is reduced in those patients with SSc and pulmonary arterial hypertension compared with those without. However, that fact that ours was a retrospective study in which presence/absence of pulmonary arterial hypertension were less strictly defined than by Hofstee et al. [12] could have contributed to these conflicting results.

Our study concentrated on measurement of capillary abnormalities, our long-term aim being to establish capillaroscopy as a biomarker of microvascular disease for use in cross-sectional and longitudinal studies. Cutolo et al. [22] examined the relationship between pattern of capillary abnormality (early, active and late) and disease subtype, duration and autoantibody subtype. Our quantitative analysis is likely to be complementary to the already existing semi-quantitative scoring systems incorporating giant capillaries, capillary ramification and haemorrhages in the assessment of nail-fold microvascular structural change. However, for capillaroscopy to serve as a biomarker some quantitative assessment will be essential. De Angelis et al. [23] reported that expertise in the technique of video capillaroscopy can be rapidly acquired. Thus, the combining of qualitative and quantitative assessments is a realistic possibility.

In our study, only the non-dominant ring finger was examined. Ideally, we would have examined nailfolds from all 10 digits, but this was not possible within the context of the current study due to time constraints. The ring finger of the non-dominant hand was chosen for examination, because it has been previously suggested that capillaries are easiest to visualize in the ring finger, and that the ring finger best discriminates between primary and secondary RP [24]. Nonetheless, because it is not currently known how representative the non-dominant ring finger is of all nailfolds, then the fact that not all 10 digits were examined is a limitation of our study.

A further limitation of the study is the lack of evidence of reliability of the measurements. Reproducibility of different measurements is the subject of ongoing research. Initial studies of reproducibility [25] are not directly applicable to the current study, because these were in a small cohort of individuals who were either healthy or had primary RP. If there is lack of consistency between measurements, then this would introduce measurement error into the outcome, which would also result in attenuation bias and
again underestimate the strength of the associations identified. However, our results need to be interpreted cautiously, given the current lack of reliability data and their relating to one nailbed only.

In conclusion, our findings suggest that quantitative nail-fold capillaroscopy, specifically the intercapillary distance, holds promise as a biomarker for severity of digital vascular disease. The fact that this was true for automated as well as manual measures has significant implications for clinical researchers because automated measures are fast and operator independent, meaning that in the context of multicentre studies, images could be rapidly analysed without the requirement for clinical/technician training. The next step is to undertake prospective studies (currently in progress), to determine whether quantitative capillaroscopy predicts clinical outcome.

**Rheumatology key messages**

- Severe digital ischaemia and ACA positivity are independently associated with measurable nail-fold capillary change.
- Computerized nail-fold capillaroscopy has potential as an outcome measure for SSc-related microvascular disease.

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