Quantitation of microcirculatory abnormalities in patients with primary Raynaud’s phenomenon and systemic sclerosis by video capillaroscopy

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Abstract

Objective. To assess nailfold capillary density and dimensions in patients with primary Raynaud’s phenomenon (PRP), limited cutaneous systemic sclerosis (LSSc) and diffuse cutaneous SSc (DSSc), and healthy control subjects.

Methods. Using the technique of nailfold video capillaroscopy, capillary density and dimensions were averaged from all visible capillaries in a 3 mm length of the nailfold from right and left ring fingers of each subject. Twenty healthy control subjects, 15 patients with PRP, 13 patients with DSSc and 21 patients with LSSc were examined. Intra-observer and inter-observer variability were calculated in 18 and 23 patients, respectively.

Results. There were significant trends for capillary density to fall and for all dimensions to rise across the four groups \( (P < 0.0001 \) for density and all dimensions, order healthy controls, PRP, DSSc and LSSc). Intra- and inter-observer reproducibility studies showed that although there was good correlation between and within observers, the limits of agreement were between \( \pm 25–50\% \) indicating lack of reproducibility.

Conclusions. Microcirculatory abnormalities can be quantified using the technique of video capillaroscopy and were most marked in patients with LSSc.

Key Words: Nailfold video capillaroscopy, Capillary dimensions, Raynaud’s phenomenon, Systemic sclerosis.

The technique of widefield capillary microscopy is now well established in the assessment of patients with Raynaud’s phenomenon and systemic sclerosis (SSc) [1]: capillary dilation and loop drop out are typical of SSc. Indeed, in a patient presenting with Raynaud’s phenomenon these nailfold capillary abnormalities may be the best indicator of underlying connective tissue disease [2]. Video capillaroscopy is an extension of the widefield technique which allows quantitation of these abnormalities: using a video camera and digitizing the system, dimensions of individual capillaries can be measured, thus offering the potential of a non-invasive technique which should allow objective assessment of microvascular disease and its progression. We have previously reported our initial experience of video capillaroscopy, when we measured dimensions of the largest capillary in nine patients with primary Raynaud’s phenomenon (PRP), 10 patients with SSc and 10 healthy control subjects [3]. We found that patients with PRP had increased dimensions compared with controls, and although these abnormalities were not nearly as marked as in the SSc group, nonetheless these did suggest that PRP may not be an entirely vasospastic phenomenon. However, it could be argued that by concentrating on the most abnormal capillary our findings were not truly representative of the nailfold capillary bed. Therefore, the aim of the current study was to assess capillary dimensions as averaged from all visible capillaries in a 3 mm length of the nailfold from both ring fingers, examining patients with PRP and SSc [subdivided into those with limited cutaneous SSc (LSSc) and diffuse cutaneous SSc (DSSc)] and healthy control subjects. In our previous study we did not subdivide SSc patients into two subgroups: a secondary aim of the present study was to test the hypothesis that capillary abnormalities would be more marked in those patients with LSSc as these patients tend to have the most marked digital vascular disease clinically.

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Patients and methods

Patients

The groups of patients/controls were as follows. Clinical characteristics are shown in Table 1:

(1) 20 healthy control subjects;
(2) 15 patients with PRP. All patients with PRP gave a history of typical triphasic colour changes on cold exposure of at least 2 yr duration, and had no clinical or immunological evidence of underlying connective tissue disease. In particular, none had sclerodactyly or digital pitting;
(3) 13 patients with DSSc. These patients had skin involvement extending proximal to elbows and knees;
(4) 21 patients with LSSc. These patients had scleroderma limited to distal to the elbow, knee and neck.

All SSc patients fulfilled the American Rheumatism Association criteria for the disease [4].

Video capillaroscopy

Patients and controls were asked to refrain from smoking and caffeine-containing drinks for 4 h prior to examination. Each patient/control was acclimatized for 20 min at a room temperature of 23°C prior to video capillaroscopy. The ring finger of each hand was examined.

Capillaroscopy equipment. The capillaroscopy equipment includes a dissecting microscope with two interchangeable lenses giving magnifications of ×200 and ×600. A Flexilux 300 long-life fibreoptic light source and filter (Scholly Fibreoptic GmbH, Germany; model D-7819 denglingen) provides cold illumination. The video camera used with the microscope is the Moritex Europa Ltd, UK camera, model MS-500. This allows the image of the nailfold capillaries to be captured and transferred in an analogue format to a Hitachi M930E video cassette recorder. The capillaries are projected from the video cassette recorder onto a 14 inch television screen which is connected to a personal computer. The software used is Capiflow software as developed by the Karolinska Institute for Microelectronics and Diagnostika HB (Stockholm, Sweden). This software enables the image on the television screen to be analysed.

Measurement of capillary dimensions. The dimensions of nailfold capillaries from both right and left ring fingers were measured. A length of 3 mm of the distal capillary row was defined by finding an arbitrary ‘start’ capillary and then measuring 3 mm on the screen by moving the calibration square a distance of 1 mm at a time and reproducing the capillaries by hand on a grid which showed how many capillaries were to be measured. This was done at ×200 magnification.

A magnification of ×600 was then used to find the ‘start’ capillary and to measure the capillary dimensions. For this part of the procedure the snapshot mode of the Capiflow software was used, giving a picture on the video screen of a single capillary. Capillary density was determined by counting the total number of capillaries in the 3 mm length, and a mean result taken from right and left hands. The dimensions measured in each capillary were as follows (Fig. 1): the arterial diameter of the capillary (a), the venous diameter of the capillary (v), the width of the capillary at its apex, or ‘apical loop diameter’ (l) and the total capillary width at its widest point or ‘total loop diameter’ (t).

Although by definition total capillary width could be measured for all capillaries in the 3 mm length (otherwise the arterial, venous and apical loop diameters could not be calculated for each capillary, usually because the capillary was too indistinct or too tortuous. Therefore, for each individual, the number of capillaries in which all dimensions could be measured was documented (Table 2). We felt it important to calculate total capillary width in all capillaries (not only those in which arterial, venous and apical loop dimensions could be measured): otherwise the total capillary width would be falsely low in patients with very distorted capillary architecture (as the most abnormal capillaries would be excluded from analyses).

Therefore, for each individual, the number of capillaries from which the value for total capillary width was derived equalled the number/3 mm, whereas the number from which the arterial, venous and apical loop diameters was derived was usually smaller. For each individual studied a mean value for each of the above dimensions was recorded, by averaging the results from all capillaries in right and left index fingers.

Measurement of inter- and intra-observer variability.

All measurements were originally performed by a vascular flow technician (TM). After this initial analysis the stored videotapes from 18 subjects (spread across all four control/patient groups) were reanalysed to allow calculation of intra-observer variability. To calculate inter-observer variability, a clinician (MB) reanalysed 23 (spread across all groups) of the stored videotapes. For each ‘reanalysis’, the observer attempted to find the same ‘start’ capillary from the sketch of capillary morphology drawn when the capillaries were first measured.

Table 1. Clinical characteristics of patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 20)</th>
<th>PRP (n = 15)</th>
<th>DSSc (n = 13)</th>
<th>LSSc (n = 21)</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>38 ± 13</td>
<td>40 ± 14</td>
<td>50 ± 12</td>
<td>45 ± 13</td>
<td>P = 0.052</td>
</tr>
<tr>
<td>Duration of Raynaud’s phenomenon (yr)</td>
<td>6 [3, 20]</td>
<td>6 [2, 18]</td>
<td>10 [5, 18]</td>
<td>P = 0.57</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>5 (25%)</td>
<td>7 (47%)</td>
<td>4 (31%)</td>
<td>4 (19%)</td>
<td>P = 0.33</td>
</tr>
<tr>
<td>Smokers</td>
<td>5 (25%)</td>
<td>9 (60%)</td>
<td>8 (62%)</td>
<td>14 (67%)</td>
<td>P = 0.04</td>
</tr>
</tbody>
</table>

Ages are mean ± standard deviation (s.d.), durations are median [Q1, Q3].
Fig. 1. Video image (magnification $\times 600$) showing the dimensions measured for one capillary: arterial ($a$), venous ($v$), apical loop ($l$) diameters and total loop width ($t$).

Table 2. Capillary density and dimensions—between-group comparisons

<table>
<thead>
<tr>
<th></th>
<th>Control ($n = 20$)</th>
<th>PRP ($n = 15$)</th>
<th>DSSc ($n = 13$)</th>
<th>LSSc ($n = 21$)</th>
<th>PRP vs control</th>
<th>PRP vs SSc</th>
<th>LSSc vs DSSc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number/mm ($a$, $v$, $l$)</td>
<td>16.5 $\pm$ 3.6</td>
<td>18.9 $\pm$ 4.7</td>
<td>9.0 $\pm$ 5.7</td>
<td>8.3 $\pm$ 4.4</td>
<td>$P = 0.49$</td>
<td>$P &lt; 0.0001$</td>
<td>$P = 0.14$</td>
</tr>
<tr>
<td>Arterial diameter ($\mu$m)</td>
<td>13.0 [21%]</td>
<td>15.7 [31%]</td>
<td>18.7 [30%]</td>
<td>27.4 [70%]</td>
<td>$P = 0.08$</td>
<td>$P = 0.004$</td>
<td>$P = 0.0007$</td>
</tr>
<tr>
<td>Venous diameter ($\mu$m)</td>
<td>15.6 [21%]</td>
<td>19.3 [30%]</td>
<td>22.5 [24%]</td>
<td>34.7 [74%]</td>
<td>$P = 0.07$</td>
<td>$P = 0.004$</td>
<td>$P = 0.0003$</td>
</tr>
<tr>
<td>Apical loop diameter ($\mu$m)</td>
<td>17.2 [24%]</td>
<td>20.6 [31%]</td>
<td>23.3 [33%]</td>
<td>34.4 [76%]</td>
<td>$P = 0.11$</td>
<td>$P = 0.01$</td>
<td>$P = 0.001$</td>
</tr>
<tr>
<td>Number/mm ($t$)</td>
<td>19.6 $\pm$ 3.6</td>
<td>22.1 $\pm$ 3.7</td>
<td>13.3 $\pm$ 5.5</td>
<td>12.7 $\pm$ 4.4</td>
<td>$P = 0.38$</td>
<td>$P &lt; 0.0001$</td>
<td>$P = 0.21$</td>
</tr>
<tr>
<td>Total loop diameter ($\mu$m)</td>
<td>42.7 [19%]</td>
<td>45.6 [26%]</td>
<td>58.2 [28%]</td>
<td>85.4 [54%]</td>
<td>$P = 0.34$</td>
<td>$P &lt; 0.0001$</td>
<td>$P = 0.0002$</td>
</tr>
</tbody>
</table>

Values are mean $\pm$ s.d. for number per 3 mm and geometric mean [CV] for measurements. Comparisons are adjusted for age. Number/mm ($a$, $v$, $l$) relates to the number of capillaries adequately visualized to allow measurement of arterial diameter ($a$), venous diameter ($v$) and apical loop diameter ($l$). Number/mm ($t$) is capillary density—the number of capillary loops/3 mm. In all of these capillaries, total loop diameter ($t$) could be measured.

However, sometimes it was difficult to be certain that the same ‘start’ capillary had been identified on each occasion.

Statistical analysis

Demographic data were compared across the groups using analysis of variance (age), the chi-squared test (sex, smoking) or Kruskal–Wallis non-parametric analysis of variance. Density and dimensions were compared across groups using analysis of covariance, adjusted for age, sex and smoking status. Three pre-planned comparisons were carried out between the four groups (PRP vs controls, PRP vs SSc, LSSc vs DSSc): these were based on the pooled variance from the analysis of covariance. All dimensions were log transformed prior to analysis to achieve normality and the results are reported as geometric mean with coefficient of variation (CV).

Intra- and inter-observer reproducibility were assessed using Pearson’s correlation, and bias with limits of agreement [5].

Results

Between-group comparisons

The results are shown in Table 2 and Fig. 2. There were a surprisingly large number of smokers in the
three patient groups (Table 1), but the results were adjusted for smoking as well as for age and sex. There were significant trends for capillary dimensions to rise across groups, healthy controls having the smallest dimensions and patients with LSSc having the largest ($P < 0.0001$ for all dimensions). Capillary density was significantly lower, and dimensions larger, in patients with SSc compared with patients with PRP. Although dimensions were larger in patients with PRP than in healthy controls, differences were not statistically significant, and capillary density did not differ between patients with PRP and controls. Dimensions were significantly larger in patients with LSSc than in patients with DSSc, although capillary density was similar.

Gender had no significant effect on the results ($P > 0.75$ for all measurements).

These dimensions are smaller than those previously published in abstract form [6, 7]. This is because of a previously unrecognized fault in the computing of the nailfold dimensions, suggesting a magnification of $\times 450$ instead of $\times 600$. This fault also affected the analysis of the results in our previously published paper [3], but because the fault was consistent the analysis of compari-
sons between groups is not affected, only the absolute measurements.

Reproducibility— intra- and inter-observer

This is shown in Table 3. Repeated measurements by the same observer (TM2) and a different observer (MB) were highly correlated with the original measurements (TM1), but the limits of agreement were between ± 25–50%, indicating lack of reproducibility. The actual measurements showed small, but often statistically significant, bias both within and between observers.

Discussion

Perhaps the most interesting finding from this study is that although capillary dimensions are significantly increased in both subsets of patients with SSc compared with control subjects and patients with PRP, they are most abnormal in patients with LSSc. This finding has not been previously reported, although Scheja et al. found that the capillary density and loop area were more abnormal in patients with LSSc than in DSSc [8]. This lends further support to the increasing body of clinical evidence which suggests that patients with LSSc have the more severe vascular disease: these patients often have severe digital ischaemia, which is associated with anti-centromere antibodies [9], and can go on to develop late-stage ‘primary-type’ pulmonary hypertension.

Also, patients with the LSSc subtype have more marked microcirculatory abnormalities on laser Doppler imaging than patients with DSSc [10].

While we have confirmed our previous finding that capillary dimensions are larger in patients with PRP compared with healthy controls, this was only a trend that was not statistically significant and differences between the two groups were small. Nonetheless these findings lend some further support to our previous suggestion that PRP is not an entirely benign vasospastic phenomenon, but may be associated with subtle microcirculatory changes. In the present study we averaged dimensions across all measurable capillaries in two 3 mm lengths of nailfold—one from the left and one from the right ring finger. This would seem to be a more objective method of evaluation than in our previous study, in which we chose the largest capillary of the distal row.

The technique of video capillaroscopy facilitates the quantitation of the nailfold capillary abnormalities which have been long recognized in patients with connective tissue disease [1]. Because images are recorded on video, images can be stored for analysis and reanalysis (as in this study). So far only a small number of studies have applied this technique in patients with primary and secondary Raynaud’s. Kabasaki et al. used the technique to define a set of abnormal findings including enlarged capillaries, giant capillaries, haemorrhage, and avascularity and compared frequencies of these abnormal findings (and also capillary density) between patients with connective tissue disease and healthy controls [11], but did not compare capillary dimensions between groups. Patients with SSc demonstrated a reduced capillary density compared with patients with systemic lupus erythematosus, undifferentiated connective tissue disease or healthy controls, and patients with SSc had the highest frequency of severe avascularity, giant capillaries and haemorrhage [11]. Studer et al. used the technique of ‘quantitative television microscopy’ (capillary microscopy connected to a television camera) to measure a number of parameters in patients with localized scleroderma, SSc, cutaneous and systemic lupus erythematosus, and healthy control subjects: the visibility of the venous plexus and the number of capillary haemorrhages were measured in addition to capillary dimensions and density [12]. Dimensions were greatest in patients with systemic connective tissue disease, especially in those with SSc [12].

There is also the potential to extend the technique of video capillaroscopy to examine red blood cell velocity in response to a dynamic challenge such as cooling [13–15] and to examine capillary permeability [16, 17]. The study by Studer et al. included measurement of the ‘stop-flow duration’ [12].

A larger number of studies quantified nailfold capillary abnormalities using primarily the widefield technique without continuous recording. Different methods of quantifying capillary abnormalities have included the following: the number of capillaries in the distal row

<table>
<thead>
<tr>
<th>Table 3. Intra- and inter-observer variability in measurement of capillary density and dimensions</th>
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<tbody>
<tr>
<td><strong>Correlation</strong></td>
</tr>
<tr>
<td><strong>TM1 vs TM2</strong></td>
</tr>
<tr>
<td><strong>Number/3 mm (a, v, l)</strong></td>
</tr>
<tr>
<td><strong>Arterial diameter (μm)</strong></td>
</tr>
<tr>
<td><strong>Venous diameter (μm)</strong></td>
</tr>
<tr>
<td><strong>Apical loop diameter (μm)</strong></td>
</tr>
<tr>
<td><strong>Number/3 mm (t)</strong></td>
</tr>
<tr>
<td><strong>Total loop diameter (μm)</strong></td>
</tr>
</tbody>
</table>

Values are bias (limits of agreement); for the measurements a, v, l and t these are expressed as a percentage, whereas for the numbers per 3 mm, these are absolute numbers. Number/3 mm (a, v, l) relates to the number of capillaries adequately visualized to allow measurement of arterial diameter (a), venous diameter (v) and apical loop diameter (l). Number/3 mm (t) is capillary density—the number of capillary loops/3 mm. In all of these capillaries, total loop diameter (t) could be measured.
measured over 5 mm [18]; an index derived from capillary apex width and maximum limb width [19]; afferent and efferent luminal diameters [20]; the mean diameter of the capillary loops, averaged across the whole nailfold, and capillary density [21]; mean capillary surface area [22]; computer-based analysis of capillary density and median capillary loop area [8]. In a study of 800 healthy individuals and using a widefield technique, Andrade et al. proposed a method of objectively assessing nailfold capillaries which included assessment of devascularization, morphology of the distal loops, endothelial damage (microhaemorrhage) and plexus visibility [23].

Although the technique of video microscopy clearly has the potential for allowing us to quantify microcirculatory abnormalities in patients with Raynaud’s phenomenon and connective tissue disease, the current method of quantification has its limitations. Calculating mean dimensions using our current software is extremely laborious and time consuming. Some capillaries are difficult to visualize, and there is therefore a subjective element in deciding which capillaries to include in the analysis, which capillaries belong to the distal row (some capillary loops fall short of others) and, especially for tortuous capillaries, to which point to measure dimensions. To gain insight into this subjective element of the analysis, we studied both intra-observer and inter-observer variability. The results from this analysis showed that the usefulness of video microscopy is somewhat limited by its lack of reproducibility as evidenced by the substantial intra- and inter-observer variability, even though both observers had considerable experience in the technique. Although the between-group differences seen in this study are much larger than could be accounted for by measurement error alone, our present technique of quantifying abnormalities could not be used to measure change within an individual over time or in response to treatment, as small differences would not be reliably detectable. In the future more sophisticated image analysis programmes [24] may partially obviate the above problems and improve upon intra- and inter-observer variability. At present, measurement of capillary dimensions by nailfold video microscopy is purely a research tool, as opposed to widefield microscopy which is useful in clinical practice.

As a final point, the high number of smokers among the three patient groups is of interest. We have previously reported that patients with Raynaud’s phenomenon who smoked cigarettes had lower plasma concentrations of ascorbic acid than did non-smokers, and suggested that cigarette smoking may be a risk factor [25]. There is therefore a need for studies addressing the specific issue as to whether cigarette smoking is an independent risk factor for developing severe Raynaud’s phenomenon.

In conclusion, video microscopy allows capillary abnormalities to be quantified, and in this study has demonstrated how these abnormalities in patients with SSC are most pronounced in the LSSc subtype of disease. Further studies examining capillary density and dimensions in different patient groups prospectively, using improved image analysis programmes, are now required to elucidate whether video capillaroscopy might be a useful tool in the assessment of disease progression.

References


