Nailfold capillary microscopy in healthy children and in childhood rheumatic diseases: a prospective single blind observational study

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Objectives: To develop an objective method of nailfold capillaroscopy (NFC), applicable to a wide age range of paediatric patients. To compare the morphological characteristics of the nailfold capillaries in different rheumatology patient groups and controls.

Methods: A colour digital video camera attached to a stereomicroscope was used to capture nailfold capillary images. Computerised image processing was used to analyse and store data. Subsequent quantitative and qualitative morphological analysis was performed in the following paediatric patient and control groups: 18 children with connective tissue diseases (CTD: juvenile dermatomyositis, systemic sclerosis, and undifferentiated connective tissue disease), eight with systemic lupus erythematosus, nine with primary Raynaud’s disease, three with primary vasculitis, 15 with juvenile idiopathic arthritis, 17 healthy children and 20 healthy adults. Images were analysed by a single assessor who was unaware of the patient details.

Results: The NFC technique was simple to perform and gave reproducible results, although some intra- and intersubject variation was noted. Capillary density and width was age related, with younger children having fewer and wider capillaries than older children and adults. Linear capillary density was significantly higher in healthy adults (mean [SD] 8.6 (1.6) capillaries/mm) compared with healthy children (HC 6.9 (0.9) capillaries/mm). The group with CTD had the most abnormal findings, with lower linear density (4.9 (1.7) capillaries/mm) and increased capillary loop width (10.7 (7.3) mm) compared with HC (3.5 (1.7) mm). In addition, 11/18 (61%) patients in the CTD group had more than two definitely abnormal capillaries in at least two nailfolds, an abnormality not seen in other subjects. Two qualitative measures, the degree of avascularity and general disarrangement of capillary pattern, were more commonly observed in the CTD group than in HC. The proportion of tortuous capillaries did not differ significantly between study groups.

Conclusions: This study is unique in measuring objective quantitative and qualitative parameters of the nailfold vasculature across a wide spectrum of age and disease. Differences in capillary morphology and frequency in children with CTD compared with other paediatric diseases and healthy controls were demonstrated. In the clinical situation, an assessment of the general degree of disarrangement may offer a fast tool for assessment of the nailfold vasculature which correlates well with NFC data.

Nailfold capillaroscopy (NFC) has become an established investigation for adults with a variety of connective tissue diseases (CTD). Morphological changes of the nailfold capillaries are believed to reflect the microvascular abnormalities present in systemic sclerosis, dermatomyositis, and undifferentiated CTD. Significant changes in capillary morphology (mainly capillary density, avascularity, and abnormal capillaries) are present in >80% of adult patients with scleroderma and related disorders.1–4 The extent of microangiopathy detected by NFC has been shown to correlate with disease severity and prognosis.5–7 Furthermore, the presence of an abnormal capillary pattern in adult patients with Raynaud’s phenomenon is thought to be indicative of the future development of CTD, even in the absence of other disease symptoms.3–8

There are fewer capillaroscopic data available in the paediatric population, and the range and normal findings in the healthy adult population8 may not be applicable to children. Age related differences in various capillaroscopy parameters have been noted, especially for capillary density9 and capillary dimensions10 as well as for some other descriptive parameters such as shape anomalies or subpapillary plexus visibility.11–14 Comparable quantitative capillaroscopic data for healthy children as well as for paediatric rheumatology patients are limited.15–17 Nevertheless, NFC findings have been proposed as a potential marker for more persistent disease in children with dermatomyositis,18 and correlation of the degree of vasculopathy and the clinical course has also been documented.17

In this study we had two aims: (a) to develop a simple, reproducible, and objective method of nailfold capillaroscopy applicable even to young children, and (b) to record the range of capillaroscopic findings in healthy children and children with a variety of rheumatic diagnoses.

Subjects
A total of 375 nailfold images were captured, of which 300 (80%) from 70 children and 20 adults were suitable for analysis. Unsuitable images resulted from darkly pigmented skin, extensively manicured nailfolds, or thickened, dry nailfold skin. The paediatric age group comprised 26 boys and 44 girls, ranging from 2 to 18 years (mean 10.5). The healthy adults (11

Abbreviations: CTD, connective tissue diseases; HA, healthy adults; HC, healthy children; JDMS, juvenile dermatomyositis; JIA, juvenile idiopathic arthritis; MCTD, mixed connective tissue disease; NFC, nailfold capillary; RS, Raynaud’s syndrome; SLE, systemic lupus erythematosus; SSC, systemic sclerosis; VAS, primary vasculitis

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male, nine female) ranged in age from 21 to 58 years (mean 35). Patients were recruited from paediatric rheumatology outpatient clinics on the basis of clinical diagnoses established by an experienced paediatric rheumatologist (TRS). The subjects were divided into the following groups for the purposes of this study: healthy adults (HA)—20 staff members from the Department of Rheumatology, University of Birmingham; healthy children (HC)—17 children of the staff members or healthy siblings of the patients; juvenile idiopathic arthritis (JIA)—15 children (six oligoarthritis, five polyarthritis, two systemic disease, and two psoriatic arthritis); juvenile systemic lupus erythematosus (SLE)—eight children; CTD—18 children (eight with juvenile dermatomyositis (JDM); three with systemic sclerosis (SSc), four with mixed connective tissue disease (MCTD), three with overlap syndromes; primary Raynaud's Syndrome (RS)—nine children; primary vasculitis (VAS)—three children (two with recurrent Henoch-Schönlein purpura and one with hypocomplementaeic urticarial vasculitis) (table 1). The only patients who were taking vasoactive drugs (nifedipine) at the time of the study was nifedipine in four cases.

Table 1  Group characteristics

<table>
<thead>
<tr>
<th>Group*</th>
<th>Diagnosis†</th>
<th>Patient number (No of images)</th>
<th>Age (years) Mean (range)</th>
<th>Disease duration (years) Mean (range)</th>
<th>Vasoactive drugs‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>Healthy</td>
<td>20 (35)</td>
<td>35 (21–58)</td>
<td>3 [3 m–5 y]</td>
<td>0</td>
</tr>
<tr>
<td>HC</td>
<td>Healthy</td>
<td>17 (60)</td>
<td>7 (2–18)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>JIA</td>
<td>Oligo 6, poly 5, syst 2, PsA 2</td>
<td>15 (55)</td>
<td>9.8 (6–16)</td>
<td>3.6 (5 m–8 y)</td>
<td>0</td>
</tr>
<tr>
<td>SLE</td>
<td>SLE</td>
<td>8 (26)</td>
<td>13.1 (9–17)</td>
<td>5.1 (9 m–11 y)</td>
<td>2 (MCTD, overlap)</td>
</tr>
<tr>
<td>CTD</td>
<td>JDM 8, SSc 3, MCTD 4, other 3</td>
<td>18 (80)</td>
<td>13.3 (5–17)</td>
<td>4.1 (2–7 y)</td>
<td>2</td>
</tr>
<tr>
<td>RS</td>
<td>Primary RS</td>
<td>9 (36)</td>
<td>9.8 (3–14)</td>
<td>10 (6–14)</td>
<td>1.9 (3 m–5 y)</td>
</tr>
<tr>
<td>VAS</td>
<td>HSP 2, urtic 1</td>
<td>3 (8)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*HA, healthy adults; HC, healthy children; JIA, juvenile idiopathic arthritis; SLE, systemic lupus erythematosus; CTD, connective tissue disease; RS, Raynaud’s syndrome; VAS, primary vasculitis.
†Oligo, oligoarthritis; poly, polyarthritis; syst, systemic arthritis; PsA, psoriatic arthritis; JDM, juvenile dermatomyositis; SSc, systemic sclerosis; MCTD, mixed connective tissue disease; other, overlap syndromes; HSP, Henoch-Schönlein purpura; urtic, urticarial hypocomplementemic vasculitis.
‡The only vasoactive drug used at the time of the study was nifedipine in four cases.

Capillaroscopy

After waiting for 20 minutes in a room at a standardised temperature of 24°C, the patient was seated at the capillaroscopy equipment, either alone or on the parent’s lap as appropriate. The equipment was explained to the subject and a drop of immersion oil was applied to the nailfold to maximise the translucency of the keratin layer. The finger to be examined was gently held in position on the microscope base plate, by the parent if necessary. Direct capillaroscopy was performed using an Olympus SZ-40 stereomicroscope and fibre optic illumination. The nailfolds of all fingers except thumbs were examined in children and fourth fingers only in healthy adults.

The whole nailfold was initially examined under low magnification to determine the distribution of any obvious abnormalities. Usually the abnormalities were evenly distributed, so a 3 mm section of the middle portion of the nailfold was magnified 66 times (3 mm of nailfold was equivalent to 19.8 cm on the computer screen) and photographed using a colour digital video camera TK-1280; a hard copy was made with a polaroid printer. In the minority of cases where the abnormalities were unevenly distributed, a 3 mm section of the most abnormal area was photographed. Images were subsequently captured, coded, and stored through NIH Image software (version 1.59) on a Macintosh IIX computer. The total examination for one patient, including capturing images from all eight nailfolds, took 15–20 minutes. Image analysis was performed at a later time by an observer (PD) who was unaware of the patient details. Each analysis took about five minutes. Only images with clearly distinguishable capillary loops were analysed (about 80% of the total images). A standardised image of a transparent ruler was also captured to enable calibration of screen millimetres to actual micrometres (1 mm screen = 15.15 µm, fig 1).

Capillary image analysis

Six parameters of each image were measured: linear capillary density, capillary width, capillary tortuosity, avascular areas, capillary disarrangement, and the number of abnormal vessels.

Figure 1  Nailfold capillary morphology: (A) normal capillaries, healthy adult; (B) normal capillaries, healthy 2 year old child; (C) abnormal capillaries, 15 year old boy with JDM. a, Capillary width in a giant capillary; b, tortuous loop, c, avascular area.
Capillary density and capillary width

Capillaries in the end row were counted manually. The linear capillary density was obtained by counting the number of clearly visible end row capillaries over a standard screen distance (either 19.8 screen cm or 13.2 screen cm) depending on the sharpness of the image, which was equivalent to actual nailfold distances of 3 mm or 2 mm, respectively. The end row capillary number was then divided by the end row length, and expressed as capillaries/mm. In calculating the mean capillary width, the maximum diameter of the three widest capillaries was measured on the computer screen (maximum loop width in mm).

Capillary morphology and arrangement

The number of tortuous and definitely abnormal capillaries, the presence of avascular areas, and the degree of general capillary disarrangement were also recorded. In defining capillary morphology, we modified previously published data.11 20–22 Tortuous loop morphological patterns, including curled, crossed, or meandering capillaries but not widened capillary limbs, were noted. The proportion of tortuous loops compared with the total number of capillaries in the area was expressed as a percentage, to give an index of tortuosity.

Capillaries were considered definitely abnormal if they were either extremely enlarged (giant capillaries or megacapillaries, aneurysmal loops) or had striking shape abnormalities (bushy and bizarre loops) (fig 1C). These formed the abnormal capillary count. The presence of abnormal capillaries was defined as a minimum of two definitely enlarged capillaries for each observation site on at least two fingers.23 Avascular (deletion) areas were defined as a loss of more than two consecutive capillaries.24 The extent of avascularity was graded as 0 (no avascular areas), 1 (moderate capillary loss of up to two avascular areas), or 2 (severe capillary loss, multiple or confluent avascular areas).

General capillary disarrangement was defined as distortion of the normal regular capillary pattern. This descriptive parameter was quantified by the investigator's global assessment of the degree of change and graded as 0 (regular pattern), 1 (slight irregularity), 2 (obvious disarray of vasculature), or 3 (extremely disarranged pattern).

Reproducibility

An assessment of the reproducibility of each of the six parameters (capillary density, width, tortuosity, avascularity, disarrangement, and number of abnormal vessels) was undertaken by the observer (PD) using 20 randomly selected nailfold capillary images. The images were assessed on two occasions several days apart. To ensure an unbiased assessment of reproducibility, each image was coded and randomly reordered between the two observation periods. The six parameters were calculated for each image during both the initial and the repeat observation periods. A coefficient of variability was estimated for the continuous variables (capillary density, width, and number of tortuous loops) by determining the standard deviation of the differences between the first and second observations for each parameter. It was expressed as a percentage of the mean value for each parameter. For the non-continuous parameters (avascularity, disarrangement, and number of abnormal vessels), the percentage of values in perfect agreement and within ±1 unit was calculated.

Statistical analysis

Individual medians for each parameter were calculated from the values of all nailfolds analysed in each subject. Kruskal-Wallis non-parametric analysis of variance tests and Dunn's multiple comparison tests were used to detect differences between groups of patients.

RESULTS

Reproducibility

In replicate measurements of 20 nailfold capillary images, the coefficient of variability for linear capillary density was 10%, for capillary width 20%, and for the number of tortuous capillaries 23%. For the non-continuous parameter of abnormal capillary counts there was perfect agreement between replicates in 80% of cases, and in 95% of cases agreement between replicates was within ±1 unit. Avascularity and disarrangement values showed perfect agreement in 90% and 85% of cases, respectively, and were within ±1 unit in 100% of cases.

Controls

The median linear capillary density in healthy childhood controls was 6.7 capillaries/mm (range 5.3–9.3) in children, significantly fewer than that seen in adults (median 8.9 capillaries/mm, range 5.7–12.0, p<0.01) (fig 2). Capillary density varied directly with age (p<0.01), with younger children having fewer capillaries (fig 3). Median capillary width was 3.1 screen mm (range 2.2–9.4) in healthy children.
massive bushy and giant capillaries in almost all of the examined nailfolds. Similar morphological characteristics were seen in patients with MCTD and SSc. The only other subject who met this criterion was a healthy 2 year old boy who had a total of 11 abnormal capillaries in two nailfolds, but with total absence of avascularity or disarrangement.

Avascularity of grade 2 in at least one capillary image was present in the nailfolds of 10/18 (56%) patients with CTD and corresponded with the presence of abnormal capillaries. This feature was not seen in the other groups.

Definite capillary disarrangement (grades 2 or 3) was present in nailfolds of patients with 14/18 (78%) subjects with CTD, 1/8 (13%) patients with SLE, and 2/15 (13%) patients with JIA (table 3). The only abnormality seen in the remaining groups was slight irregularity (grade 1). A significant difference was found between CTD and HC (p<0.01) and between JIA and HA (p<0.001). Children with juvenile arthritis, including two patients with psoriatic arthritis who did not have nail involvement, did not differ significantly from control groups in any of the observed parameters.

**DISCUSSION**

Our study is unique in measuring parameters of the nailfold vasculature across a wide spectrum of age and disease. The modifications of capillaroscopic methodology outlined in this paper have combined the advantages of classical wide field microscopy with computer image processing.

Several methods of nailfold capillaroscopy have been reported during the past two decades, including direct in vivo capillaroscopy, wide field (panoramic) photomicrography, or videocapillaroscopy and computer based image analysis. In most methods, the time taken for capillary counting (direct in vivo capillaroscopy) or image capture (wide field photomicrography) precludes their use in young children, and lack of recorded images prevents serial studies. The advantages of our method were in rapid nailfold scanning and fast image capture of the most abnormal areas. The digitisation process resulted in clearly distinguishable black and white capillary images where quantitative as well as qualitative parameters could be assessed easily on the computer screen. We demonstrated good reproducibility and correlations with disease.

**Linear capillary density**

This is the first time that linear capillary density has been objectively evaluated in healthy subjects ranging in age from 2 to 58 years in a single study. The tendency of capillary density to increase with age (fig 3) may be a part of the maturation process, and has been observed by other authors.

In older children and adolescents this tendency is less remarkable. The linear capillary density we demonstrated in the healthy adult and paediatric controls (table 2).

**Connective tissue disease**

The group with CTD had markedly fewer capillaries (median linear density of 4.8 capillaries/mm (range 2.1–7.8) than both normal children and adults (p<0.001). Within the CTD group, patients with JDMS and SSc had the lowest density values (table 2). Children in the CTD group had also the widest capillaries with median value of 8.1 screen mm (range 2.6–27.0), differing significantly from healthy children (p<0.05). The widest capillaries were seen in patients with SSc and JDMS (table 2). Significant differences were seen between CTD and healthy adults (p<0.001), healthy children and JIA (p<0.001) and SLE (p<0.01). The SLE group appeared to have narrower capillaries than the other groups, but this was not statistically significant (fig 4).

A total of two or more capillaries with abnormal morphology on at least two fingers were present in nailfold images of 11/18 (61%) patients in the CTD group (table 2). One patient with primary vasculitis had three abnormal capillaries in a single nailfold field. Five of eight patients with JDMS had the most striking morphological abnormalities characterised by

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**Table 2**

<table>
<thead>
<tr>
<th>Diagnosis (n)</th>
<th>Linear capillary density</th>
<th>Capillary width</th>
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<tbody>
<tr>
<td></td>
<td>capillaries/mm</td>
<td>Median (range)</td>
</tr>
<tr>
<td>JDMS (8)</td>
<td>4.5 (2.0–7.6)</td>
<td>12.0 (2.7–27.7)</td>
</tr>
<tr>
<td>SSc (3)</td>
<td>3.7 (2.5–7.6)</td>
<td>14.9 (6.5–23.3)</td>
</tr>
<tr>
<td>MCTD (4)</td>
<td>5.0 (2.5–11.0)</td>
<td>3.1 (2.2–9.4)</td>
</tr>
</tbody>
</table>

JDMS, juvenile dermatomyositis; SSc, systemic sclerosis; MCTD, mixed connective tissue disease; HC, healthy children.

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**Table 3**

<table>
<thead>
<tr>
<th>Group</th>
<th>Tortuosity % of total capillary count</th>
<th>Abnormal capillaries % of patients</th>
<th>Significant avascularity % of patients</th>
<th>Definite disarrangement % of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>27 (0–71)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HC</td>
<td>29 (5–49)</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>JIA</td>
<td>16 (0–51)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SLE</td>
<td>19 (0–27)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CTD</td>
<td>34 (0–86)</td>
<td>61</td>
<td>56</td>
<td>78</td>
</tr>
<tr>
<td>RS</td>
<td>27 (9–57)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VAS</td>
<td>60 (40–80)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
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</table>

HA, healthy adults; HC, healthy children; JIA, juvenile idiopathic arthritis; SLE, systemic lupus erythematosus; CTD, connective tissue diseases; RS, primary Raynaud’s syndrome; VAS, primary vasculitis.
capillaries in the nailfold may lie in different planes, and in our technique usually only one plane is sufficiently focused under the microscope to allow counting and measurement. Secondly, the end row of nailfold capillaries is not always clearly distinguishable from capillaries in adjacent dermal papillae. Nailfold capillary density appears to be similar in healthy children across Europe. French authors reported a mean capillary density of 9 capillaries/mm in children of up to 10 years of age, compared with 10 capillaries/mm in older ones. An Italian group showed slightly higher capillary counts ranging from 9 capillaries/mm in infants to 14 capillaries/mm in adolescents. In the largest study reported, Terreri and coworkers found that the mean capillary density was in the range of 6.8–7.3 capillaries/mm in 329 children aged 2.1–16.7 years. Using a digitised method of capillary image analysis, British authors found capillary density in the range of 5–7.3 capillaries/mm in children aged 6–15 years, similar to our findings. We also found relatively high variability between different nailfolds of one subject as well as between subjects within specific disease groups, suggesting a wide range of normality for this parameter. Wildt et al found good correlation between density results obtained by direct capillaroscopy and computer based analysis of nailfold photographs, but there was a tendency towards slightly higher values from the direct capillaroscopy method. Precision focusing during the examination might enable better visualisation of capillaries lying in adjacent planes. Our finding of lower capillary density in children with connective tissue diseases (JDMS, SSc, MCTD) was in agreement with similar studies in children as well as in adults. Variability within this group (fig 1, table 2) was probably caused by differences in disease activity and duration where children with long disease remission as well as those with active disease were included. Capillary density in paediatric disease control groups (JIA, RS, SLE) did not differ significantly from that of healthy children. In adults, quantitative data are available for rheumatoid arthritis and SLE and they similarly do not show any significant difference in capillary density in comparison with healthy adults.

Capillary width
Capillary width is a more controversial parameter for which there is no universally agreed scoring technique. We based our rating method on Maricq's work, in which capillary enlargement was defined as a four- to 10-fold increase in capillary size. Definitely enlarged capillaries have a loop width of at least 90 to 150 µm. Other authors designate capillaries of at least 50 µm as enlarged or express capillary size in terms of individual limb widths or capillary loop area. The upper limit of normal capillary width in adults ranges from 25 to 50 µm. The median widest capillary width we observed in the control groups corresponded with published data. Not surprisingly, capillary width was enlarged in the CTD group by a factor of 2.3. Computer based quantitative capillaroscopy has been used to demonstrate significant differences in capillary loop area between healthy children and those with JDMS. Unfortunately, the numbers of patients in individual CTD subgroups were insufficient to allow statistical analysis. Semiquantitative and qualitative data on tortuosity, abnormal capillaries, avascularity, and disarray are more difficult to interpret objectively and compare with published data as rating techniques are not unified. Definition of the shape anomalies of nailfold capillaries remains a subjective measure.

Capillary tortuosity
Tortuosity appeared to be the least reliable of the parameters we assessed. We found high interindividual variability within the disease groups and healthy controls. Our data did not correspond well with previous studies, where percentages of tortuous loops of >20% were considered abnormal. This discrepancy may be due to differences in interpretation of "hair-pin" versus "tortuous" capillaries.

Abnormal capillaries and avascularity
The presence of abnormal capillaries has been widely reported and its association with connective tissue disease well established. The proportion of patients in the CTD group who had significant numbers of abnormal capillaries was similar to that reported by Kabasakal and coworkers. Others have also shown that an abnormal nailfold capillary pattern in children with Raynaud's syndrome suggests that it may be secondary to an underlying CTD such as systemic sclerosis. We did not see abnormal capillaries in any healthy adults, in contrast with others who reported that between 15 and 34% of healthy adults have abnormal capillaries. In the largest paediatric series, Terreri et al reported the presence (without quantification) of bushy capillaries in 6% and bizarre capillaries in 27% of healthy children, considering them as a distinct feature of children's capillary network. Abnormal yet regular capillaries were found in one healthy young child in this study, probably reflecting the maturational changes outlined elsewhere.

In this study, avascularity was a semiquantitative parameter assessed by a simplified three point scale. Only the presence of multiple or large avascular areas (grade 2) was considered important, as the relatively narrow depth of field of the capillaroscope sometimes reduces the number of end row capillaries in focus. Additionally, it was not always feasible to distinguish end row capillaries from those in the adjacent dermal papilla. Avascularity was present only in patients with CTD, as we have previously reported. It is possible that the published definition of avascular areas in adult nailfolds is inappropriate for children, leading to reduced specificity. Using a more detailed scoring system, other authors reported the presence of avascular areas in adult patients with SLE, in up to 7% of healthy adults and in 2% of healthy children. Avascularity may reflect either ischaemia or local trauma, and it is important to assess both its extent and any associated abnormal capillaries. Bushy loops surrounding an avascular area are a sign of neovascularisation typically present in JDMS. Therefore we believe that qualitative analysis of nailfold capillary image may enhance objective quantitative data.

Vascular disarray
This study was the first to quantify the degree of nailfold vascular disarray as a highly reproducible global scoring parameter. A definitely disarranged pattern was virtually absent in the control population studied, irrespective of age. Neither was this degree of disarray seen in SLE and JIA disease controls. In keeping with other published information, vascular disarray was present in the majority of patients with CTD and corresponded closely with the presence of abnormal capillaries and avascularity. In summary, nailfold capillaroscopy is a clinically useful technique which can result in objective, reproducible, quantitative data to discriminate between control and disease populations for diseases such as JDMS and SSc. It may provide a window on the process of angio genesis, which may be disordered in chronic multisystem inflammatory disease. It is still unclear whether NFC will be sufficiently sensitive to change to be useful for monitoring one aspect of the chronic inflammatory process.

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