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Original article

Quantitative nailfold video capillaroscopy in patients with idiopathic inflammatory myopathy

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Abstract

Objectives. To quantify nailfold capillary density and dimensions in patients with idiopathic inflammatory myopathy (IIM) and compare them with those in healthy controls; to look for associations with microvascular disease in IIM; and to determine whether nailfold capillary density and dimensions change over time.

Methods. Nailfold video microscopy (×300 magnification) was performed on 24 patients with IIM and 35 healthy controls. Capillary density and dimensions (total width and apical width) were quantified. Patients were clinically assessed and disease activity recorded using the Myositis Disease Activity Assessment Tool. Disease severity and physical function were assessed using the myositis damage index and Stanford HAQ, respectively. Findings were analysed using linear and logistic regression, adjusted for age and sex. In a subgroup of 16 patients with IIM and 27 controls, the process was repeated 6–12 months later and the results were analysed using Student's *t*-test.

Results. Capillary density was lower and dimensions were higher in patients with IIM compared with healthy controls ($P < 0.001$ for all). Anti-Jo-1 antibody was associated with reduced capillary density. In the longitudinal cohort, the mean change in capillary density was −1.4 in patients *vs* −0.4 in controls ($P = 0.07$). Mean change in capillary dimensions did not differ between patients and controls, but some patients demonstrated pronounced changes in capillary morphology over time.

Conclusions. Reduced capillary density and increased dimensions in patients with IIM can be quantified using nailfold capillaroscopy, suggesting that nailfold capillaroscopy may be useful as an outcome measure of microvascular disease in studies of IIM.

Key words: Polymyositis, Dermatomyositis, Nailfold video capillaroscopy.

Introduction

The idiopathic inflammatory myopathies (IIMs) are a heterogeneous group of acquired autoimmune diseases characterized by skeletal muscle inflammation with resultant proximal weakness. The most prevalent IIMs are PM, PM overlapping with another CTD (PM and CTD overlap), DM and IBM. PM, DM and IBM can be separated by their distinct clinical, immunological and pathological characteristics [1, 2]. Though there may be similarities, e.g. $CD8^+$ T cells invade healthy muscle fibres resulting in phagocytosis and necrosis in both PM and DM [3]; in DM microvascular abnormalities also appear to play a key role with reduced muscle capillary density before the onset of inflammatory or structural changes, as detected by immunohistochemistry [4].

Nailfold capillaroscopy is both a clinical and a research tool, which allows the examination of the microvasculature. The major clinical application of nailfold capillaroscopy is in the identification of underlying CTDs through its association with them. For example, in patients with RP, normal capillaroscopy has a negative predictive value as high as 96.7% [5]. The high magnification afforded by video capillaroscopy allows more detailed assessment of capillary architecture than is possible with the wide-field microscope, and thus allows measurement of capillary density and dimensions for research purposes [6, 7].

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CLINICAL SCIENCE

While it is well-recognized that patients with IIM have abnormal nailfold capillaries as viewed by wide-field microscopy with magnification in the order of ×20 [8–10], these abnormalities have not been previously quantified using video capillaroscopy. A Spanish cross-sectional study used high power magnification (×250) to compare capillaroscopic findings from 34 DM patients, 16 PM patients and 3 IBM patients with those from 40 healthy controls, but their analysis was semi-quantitative only [11]. We have developed novel methods for computerized video capillaroscopy that provides enhanced image quality and constructs a panoramic mosaic of an entire nailfold from which capillary density and dimensions can be measured. Moreover, capillary parameters can be tracked, meaning that individual capillaries can be evaluated over time in longitudinal studies [12]. Using this new software, and against this background, the aims of this study were:

(i) to quantify nailfold capillary density and dimensions in IIM patients, and to compare these parameters with those in healthy controls;
(ii) to compare capillary density and dimensions in subgroups of patients with IIM;
(iii) to look for clinical associations of microvascular disease; and
(iv) to determine whether capillary density and dimensions change over time.

Patients and methods

To achieve the objectives, the study incorporated both cross-sectional and longitudinal components.

Cross-sectional study

Patients and controls. Twenty-four patients identified from a computerized myositis patient database at the Rheumatic Diseases Centre, Salford Royal NHS Foundation Trust were recruited. All patients were British Caucasians, and fulfilled the Bohan and Peter criteria for definite PM and DM [13] and in the cross-sectional study were compared with 35 healthy controls with no history of RP. Information regarding their age, gender and smoking status was collected for all patients and controls before capillaroscopy. Salford and Trafford Local Regional Ethics Committee approved this study before commencement, and each subject gave written informed consent.

Clinical assessment. All patients were clinically assessed by the same investigator (L.K.M.). History of RP was documented, and considered to be present if the colour change from white to blue and/or red occurred in one or more digit in response to cold. Patients were examined for muscle weakness. In patients, disease activity was measured using the validated Myositis Disease Activity Assessment Tool (MDAAT) [14]. This collects information on myositis-related clinical features present during the previous 4 weeks. Disease damage, defined as persistent pathological and functional changes present for at least 6 months, was assessed using the validated myositis damage index (MDI) [14]. The Stanford HAQ was used to assess physical function [15]. Patient hospital records were reviewed, and the presence of ANA, anti-Jo-1 antibody, anti-Pm-Scl antibody and anti-RNP antibody was recorded.

Video capillaroscopy. Patients and controls were asked to refrain from smoking and caffeinated drinks for 4 h before assessment. Subjects were acclimatized for 20 min in room temperature controlled at 23°C before video capillaroscopy. The ring finger of the non-dominant hand was examined. Capillaroscopy was performed by the same investigator (T.L.M.).

The optical microscope that was used incorporated a charged coupled devise video camera with ×300 magnification and with a ring of green light-emitting diodes to provide high contrast illumination of the nailfold blood vessels. The output from this video camera was fed via a Snapper video digitizer board (Snapper, Datacell, Finchampstead, UK) interfaced with a computer. A panoramic image of the nail bed capillaries was acquired by initially focusing the microscope on one end of the nailfold. The Snapper board then captured 16 video frames of the image at a rate of 5 Hz, and these frames were automatically adjusted by the computer to compensate for finger movements and then were combined into a single image. The microscope was moved along the nail bed taking further images to build up a panoramic composite of the entire width of the nail bed.

Capillary measurements. Following capillaroscopy, all capillary measurements were made by the same researcher (T.L.M.). Before analysis, capillaroscopy images were anonymized so that the assessor was blinded to the subject's identity. Capillary density was calculated as the number of capillary end loops per millimetre across the entire nailfold. The capillary dimensions that were measured were the total loop width, measured across the widest part of the capillary, and the apex width, measured across the apex of the capillary loop, as shown in Fig. 1. The first five visible capillaries from the right hand side of each nailfold were measured, and the mean total width and mean apex width were calculated. The number of arborized or 'bushy' capillaries and avascular areas in each nail bed were also recorded. An arborized capillary was defined as a capillary with one or more branches. An avascular area was subjectively determined by visual inspection of the image as an area without capillaries.

Longitudinal study

Capillary images from baseline and follow-up for each subject were analysed as a pair, to allow the same capillaries to be tracked and re-measured for each visit. Twenty-three of the 24 patients were recalled 6–12 months after their initial visit. One patient with DM died in the interval. Of the 23 recalled patients 17 attended. One patient with DM overlapping with another CTD was not included in the analysis since there was a computer error that led to the loss of that subject's capillaroscopy data. The 16 remaining patients were: 12 with PM; 3 with DM; and 1 with PM and CTD overlap with SSc.

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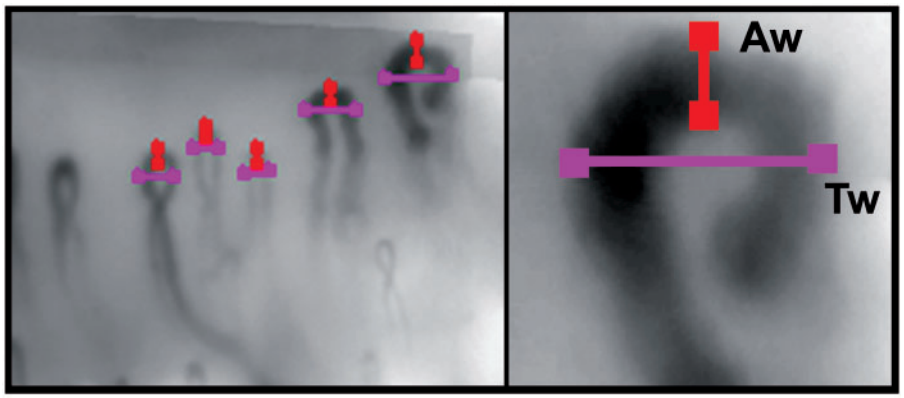
FIG. 1 Mean total width and apex width were calculated from the first five visible capillaries.



Twenty-seven of the original control group underwent repeat capillaroscopy.

Statistical analysis

Cross-sectional study. The baseline characteristics of patients and controls were compared using descriptive statistics. The primary outcome measure used to compare patients and controls was capillary density. Secondary measures included capillary dimensions (mean total width and mean apex width) and the presence of arborized capillaries and avascular areas. Capillary density and capillary dimensions were analysed using linear regression, adjusted for age and sex. Arborized capillaries and avascular areas were categorized as being either present or absent and binary logistic regression was used to compare patients and controls, adjusted for age and sex.

A subgroup analysis excluding overlap patients was performed. Statistical analysis comparing PM, DM and overlap subgroups was not performed, as the numbers in each IIM subgroup were too few for formal statistical analysis.

The possibility that associations were present between capillary density and individual disease features was analysed in patients using multifactorial linear regression, adjusted for age and sex. These possible associations were the categorical variables of RP and anti-Jo-1 and the continuous variables disease activity (MDAAT), disease damage (MDI) and physical function (HAQ).

Longitudinal study. Changes in capillary density and dimensions between baseline and follow-up were calculated for each subject. Linear regression was used to compare mean change in patients and controls. Due to the small numbers, adjustment for age and sex was not made. A subgroup analysis excluding overlap patients was performed. The level of statistical significance was set at 5% and SPSS (SPSS Inc, IBM, Chicago, Illinois) 15.0 for Windows was used throughout.

Results

Of the 24 patients recruited, 14 (58%) were females, and the mean age at assessment was 55 years (S.D. 12). Fourteen had PM; six DM; and four DM/PM/ CTD overlap. Two had PM overlapping with SSc, one had DM, SSc and inflammatory arthritis, and one had DM and SS. The median disease duration was 45 months (range 1–299 months). Thirty-five controls were recruited, 20 (57%) women, mean age 46 (12). The baseline characteristics of patients with IIM and controls are shown in Table 1. Patients with IIM had relatively mild, stable disease with low levels of disease activity and damage and moderately impaired physical function. Overlap patients had the most severe disease, with a tendency to higher disease activity and damage and reduced physical function compared with those with PM or DM alone. Five of the DM patients had active skin lesions and none had digital ulcers. Fifteen patients were ANA positive, eight had anti-Jo-1 antibodies (five with PM, three with DM), one patient with IIM and SSc overlap had anti-PM-Scl antibodies and another IIM and SSc overlap patient had anti-RNP antibodies.

Cross-sectional study

Patients vs controls. Capillary density was lower and capillary dimensions were higher in patients with IIM compared with healthy controls ($P < 0.001$ for all comparisons). Arborized capillaries were present more frequently in patients with IIM than in controls (OR = 20.6; 95% CI 4.4, 97.5; $P < 0.001$), as were avascular areas (OR = 38.2; 95% CI 3.9, 371.2; $P < 0.001$) (Table 2).

Subgroup analysis. Capillaries appeared more abnormal in DM compared with PM. In DM, lower capillary density and higher dimensions were noted. Avascular areas were also seen more frequently in DM than PM. The overlap group possessed the most abnormal nailfolds, with very low capillary density, enlarged capillaries and avascularity (Table 2). Omission of the overlap group from statistical analysis did not materially alter the findings.

Associations with microvascular disease. Eleven (46%) of the 24 patients reported RP. The mean capillary density in those patients was 6.3/mm (2.9/mm) compared with 6.0/mm (3.6/mm) in those without RP, indicating no evidence of a relationship between capillary density and the presence of RP ($P = 0.65$). Eight (33%) of the 24 patients

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Table 1 Baseline characteristics of patients and controls

	Healthy controls ($n = 35$)	Patients ($n = 24$)	PM ($n = 14$)	DM ($n = 6$)	Overlap ($n = 4$)
Age, mean (S.D.), years	46 (12)	55 (12)	55 (14)	55 (8)	52 (12)
Male/female, *n* (% of females)	15/20 (57)	10/14 (58)	7/7 (50)	1/5 (83)	2/2 (50)
Current smokers, *n* (%)	5 (14)	9 (38)	7 (50)	1 (17)	1 (25)
Months since diagnosis, median (range)	–	44.5 (1–299)	42.5 (2–299)	89 (20–124)	12.5 (1–44)
RP present, *n* (%)	–	11 (46)	6 (43)	1 (17)	4 (100)
ANA present, *n* (%)	–	15 (63)	8 (57)	4 (67)	3 (75)
Anti-Jo-1 present, *n* (%)	–	8 (33)	5 (36)	3 (50)	0 (0)
MDAAT, median (range)	–	0.06 (0–0.59)	0.05 (0–0.15)	0.10 (0–0.55)	0.34 (0.1–0.59)
MDI, median (range)	–	0.10 (0–0.50)	0.10 (0–0.35)	0.09 (0–0.4)	0.27 (0.1–0.5)
HAQ, median (range)	–	0.88 (0–2.75)	0.75 (0–2.25)	0.63 (0–2.38)	2.44 (1.63–2.75)

Table 2 Baseline capillary density and dimensions in patients and controls

	Healthy controls ($n = 35$)	Patients ($n = 24$)	PM ($n = 14$)	DM ($n = 6$)	Overlap ($n = 4$)	Controls *vs* patients (*P*-value)
Capillary density, mean (S.D.), per mm	10.4 (1.5)	6.1 (3.2)	7.2 (3.2)	4.7 (3.4)	4.7 (2.0)	<0.001*
Total width, mean (S.D.), μm	35.3 (4.9)	57.4 (28.1)	48.8 (21.6)	62.8 (36.2)	79.3 (28.0)	<0.001*
Apex width, mean (S.D.), μm	12.8 (2.3)	22.0 (10.4)	19.1 (7.3)	23.6 (15.9)	29.8 (7.6)	<0.001*
Persons with one or more arborized capillary, *n* (%)	6 (17)	19 (79)	11 (79)	5 (83)	3 (75)	<0.001**
Persons with one or more avascular area, *n* (%)	1 (3)	12 (50)	4 (29)	4 (67)	4 (100)	<0.002**

*Linear regression. **Binary logistic regression.

possessed anti-Jo-1 antibodies. There was lower capillary density in anti-Jo-1-positive patients [4.8/mm (2.2/mm)] compared with anti-Jo-1 negative-patients [6.8/mm (3.5); $P = 0.02$]. The MDAAT score at baseline [median 0.06 (range 0–0.59)], MDI [0.10 (0–0.50)] and HAQ [0.875 (0–2.750)] did not demonstrate association with capillary density ($P = 0.11$, $P = 0.21$ and $P = 0.51$, respectively).

Longitudinal study

There was no statistically significant change in the capillary density or dimensions of the control group over the study period. Capillary density reduced in the patient group but did not reach statistical significance (Fig. 2) (Table 3). However, some individuals did demonstrate marked changes in capillary morphology over time (Fig. 3). Omission of the overlap group from statistical analysis did not materially alter the findings (Table 3).

Discussion

This study of IIM patients has shown that reduced capillary density and increased dimensions may be quantified using computerized nailfold capillaroscopy. This is the first nailfold capillary study of IIM to quantify abnormalities and track changes over time. We found nailfold capillaries to be more abnormal, and of lower density, in DM than in PM, consistent with previous findings and reflecting the likely importance of the vasculature in the pathophysiology of DM [9, 16, 17].

The first large capillaroscopy series of patients with myositis that looked for differences between subtypes of IIM included 19 patients with PM and 16 with DM [9]. In this study, enlarged loops were seen in 21% of patients with PM and 56% with DM, and bushy (arborized) capillaries in 5% of patients with PM and 25% with DM (compared with the higher frequencies of 79 and 83% in our study) [9]. However, the way in which the capillaries were analysed differed considerably between our study and the previous study. Ganczarczyk *et al*. [9] used ×20 magnification, whereas our study used ×300 magnification and computerized software to enhance image quality. It is possible that the higher magnification used here has increased sensitivity to detect bushy capillaries. As a general point, the grossly distorted capillary architecture seen in some patients with IIM makes assessment difficult and there is an inevitable degree of subjectivity, particularly when determining avascular areas and arborized capillaries. In our study, an arborized capillary was defined as having one or more branches and counted as one capillary for the purpose of measuring density. Other investigators may have counted each branch separately, as it is not clear in the published literature how other groups have dealt with this issue. Such subjectivity has to be borne in mind when comparing the results of studies, and it highlights the importance of using objective measures.

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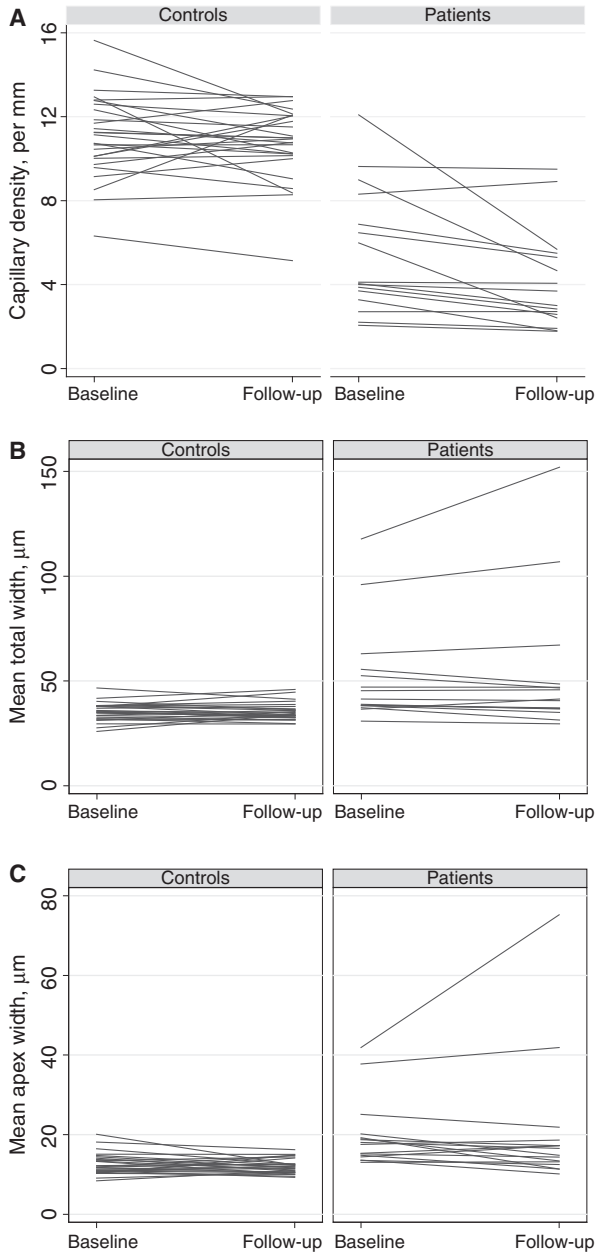
FIG. 2 Capillary density (**A**), mean total width (**B**) and mean apex width (**C**) in 17 patients and 27 controls at baseline and follow-up.



The largest capillaroscopy series in patients with IIM to date was reported by Selva-O'Callaghan *et al*. [11]. The study compared 53 adults with PM, DM or IBM and 40 healthy controls. A semi-quantitative rating scale was used to score a variety of capillaroscopy abnormalities: Group 0 (no change); Group 1 (few changes, <4/mm); and Group 2 (frequent changes, $\geqslant 4$/mm). For statistical analysis, Group 0 and 1 changes were compared with Group 2 changes. Fifteen patients (28%) [13 (38%) with DM; 2 (13%) with PM; and none with IBM] had a capillaroscopy score of 2 compared with none of the controls. Capillary enlargement and microhaemorrhage were seen more frequently in the patients with DM than in those with PM. No associations were found between abnormal nailfold microscopy and presence of cancer, presence of anti-synthetase or PM-Scl antibodies (unlike in our study, in which anti-Jo-1 antibody-positive patients exhibited lower capillary density than those who were anti-Jo-1 negative) or disease duration [11]. Mean disease activity and damage scores were higher in patients with Group 2 capillaroscopy changes (both $P < 0.05$), although the between-group differences of mean disease activity (0.2) and damage (0.1) are difficult to interpret clinically. RP was associated with Group 2 capillary changes ($P < 0.05$) [11]. In contrast, in our study, no associations were found between capillaroscopic findings and disease activity, severity or RP.

The differences between our study findings and those of Selva-O'Callaghan *et al*. [11] could relate to the relatively small numbers of patients in each study (particularly relevant in such a heterogeneous group of diseases as IIM) and methodological differences between the studies. Selva-O'Callaghan *et al*.'s [11] scoring system was semi-quantitative, involving multiple variables most of which were subjective, and only a 1-mm section of the capillary bed was examined, although each finger was examined (in contrast to our study in which only the non-dominant ring was assessed). Since capillary architecture can vary substantially across a nail bed, it would be interesting to know how the 1-mm length was selected, for instance, was only the most abnormal area chosen to report from?

Other studies have reported the presence of the 'scleroderma pattern' [8] with low capillary density, capillary enlargement and areas of avascularity in IIM [16, 18, 19]. The proportion of patients reported to have abnormal capillaries varied considerably from 27% in 26 patients with PM and DM [18] to 100% in 15 patients with DM [19]. The results from these studies are not, however, directly comparable with the current findings due to differences in the ways the capillaries were measured. A further study reported megacapillaries in 33% of patients with PM and 86% with DM [17]. Again, it is difficult to directly compare those observations with those of the current study, especially due to the different magnifications used at $\times$125 *vs* $\times$300, respectively.

Our study is the first to look for interval changes in nailfold capillaries of adults with IIM. We found no significant change in the capillaries of patients with IIM and healthy controls over 6–12 months of this study. However, in certain patients marked changes in the morphology of individual capillaries were noted over time that were easily trackable using the computerized system (Fig. 3). The aetiopathological significance of this observation and its relevance to the IIM diseases overall, is unknown. The fact that most patients had stable muscle disease could have contributed to lack of interval change overall (data not shown), with the result that it was not possible to correlate changes in disease activity and in capillaroscopy findings. In children with DM, changes in

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FIG. 3 Baseline and follow-up capillaroscopy (×300 magnification) in (**A**) a control demonstrating normal capillaries with no change between baseline and follow-up after 12 months and (**B**) a patient with DM demonstrating reduced capillary density, arborized and enlarged capillaries with marked changes at follow-up after 6 months.

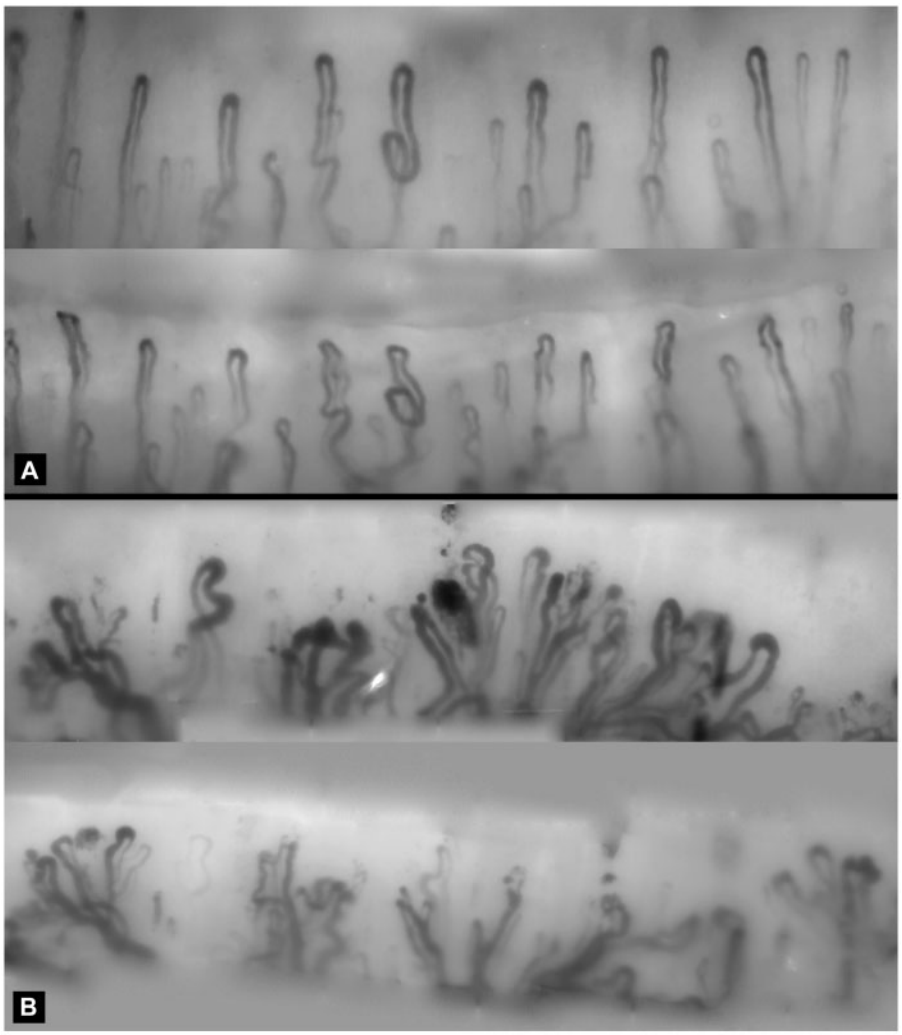

TABLE 3 Change in capillaroscopy parameters over time in patients with IIM and controls

	Capillary density, mean (S.D.), per mm	Total width, mean (S.D.), μm	Apex width, mean (S.D.), μm
Controls ($n = 27$)			
Baseline	11.1 (2.0)	35.2 (4.3)	12.6 (2.7)
Follow-up	10.7 (1.7)	35.2 (4.1)	12.0 (1.9)
Change	−0.4 (1.6)	0.0 (3.5)	−0.6 (2.6)
Baseline *vs* follow-up, *P*-value	0.46	0.98	0.39
Patients ($n = 16$)			
Baseline	5.5 (3.0)	51.8 (23.4)	19.8 (8.4)
Follow-up	4.1 (2.4)	53.2 (32.1)	20.4 (16.4)
Change	−1.4 (1.9)	1.7 (10.1)	0.6 (9.3)
Baseline *vs* follow-up, *P*-value	0.16	0.9	0.9
Controls *vs* patients			
Change, *P*-value	0.07	0.55	0.44

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nailfold capillaries have been shown to be associated with change in disease activity [20, 21].

The size of our study group was limited by the fact that the IIMs are a heterogeneous group of rare diseases. The small numbers of patients in each subgroup, in particular the DM group, made meaningful statistical analysis within these groups difficult. Nonetheless, in our samples capillaries were more abnormal in DM than PM, consistent with previous observations. SSc is known to be associated with characteristic nailfold capillary abnormailities, but omission of this subgroup from analysis did not materially influence the findings in either the cross-sectional or longitudinal study. The limited sample size and relatively short study duration, coupled with the fact that the patients in this study had well-treated, stable disease with low levels of disease activity and damage, meant that any potential capillary changes over time would be difficult to detect. Ideally, in future studies, capillaroscopy should be performed at the time of diagnosis and repeated at intervals over the disease course, and correlated with disease activity and damage. There was a higher proportion of smokers in the patients than the control group. The effect of smoking on nailfold capillary morphology is unclear and ideally would be adjusted for in a larger study.

We believe that a key finding from our study was the ability to quantify abnormalities in patients with IIM and to track capillaries over time. This lends weight to the use of capillaroscopy in assessment of idiopathic inflammatory myopathy.

Rheumatology key messages

- Reduced capillary density and increased dimensions in patients with IIM can be quantified using nailfold capillaroscopy.
- In patients with IIM, capillary abnormalities can be tracked over time.

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Disclosure statement: The authors have declared no conflicts of interest.

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