Assessment of capillary density in systemic sclerosis with three different capillaroscopic methods

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ABSTRACT

Objectives. *Capillary abnormalities, such as the enlargement and/or disappearance of capillary loops, occur early in the majority of patients with systemic sclerosis (SSc). The aim of this study was to compare three capillaroscopic methods of determining the capillary density in patients with SSc.*

Methods. *Two of the three methods involved stereo-zoom microscopy at a magnification of 20 times, used either for direct counting, or with a camera and imaging software for determination of the capillary density on coded images. The third method was computerised nailfold video capillaroscopy with 300 x magnification using coded images. The capillary density (loops/mm) was determined on the fourth finger of the non-dominant hand with all three methods in 40 patients, 32 with limited cutaneous SSc (lcSSc) and 8 with diffuse cutaneous SSc (dcSSc), and in 21 healthy control subjects.*

Results. *The median values of capillary density assessed with the three methods were: 4.3, 5.4 and 6.1 loops/mm in lcSSc patients, 4.5, 5.0 and 6.3 loops/mm in dcSSc patients, and 7.0, 7.0 and 6.9 loops/mm in the controls. Capillary density was thus lower in lcSSc and dcSSc patients than in the controls according to all three methods. Agreement between the three methods was good in the controls. In patients, direct counting resulted in lower values than in the two computer-based methods.*

Conclusion. *Assessment of capillary density with three different methods showed good agreement between methods. All methods could differentiate between SSc patients and controls.*

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Introduction

Systemic sclerosis (SSc, scleroderma) is characterised by autoimmunity, microangiopathy and fibrosis in the skin and internal organs. The microangiopathy in SSc is reflected by Raynaud's phenomenon (RP), which occurs in 80–90% of patients, and often precedes other symptoms by several years. Microangiopathy can be studied non-invasively with nailfold capillary microscopy. The "scleroderma pattern" originally described by Hildegard Maricq (1) is characterised by the enlargement and/or disappearance of capillary loops, resulting in decreased capillary density. Capillary abnormalities are known to occur early in the disease, and it has therefore recently been suggested that they should be included in the criteria for the diagnosis of SSc (2).

Nailfold capillary abnormalities can be studied by qualitative, semiquantitative and quantitative methods using instruments such as a handheld ophthalmoscope (3), or a dermatoscope (4), which have 10-20 times magnification, allowing reliable qualitative analysis of dilated and giant capillaries (5). A stereo-zoom microscope (1, 6) with 20–50 times magnification allows quantitative measurements to be made, for example, determination of the capillary density, which is reported to be the best discriminator between primary and secondary RP (7, 8). Capillary microscopy has also been reported to have a prognostic value in SSc (9, 10).

In a pilot study we found good inter- and intra-observer variability in the determination of capillary density using a direct counting method with a stereo-zoom microscope, and we also demonstrated good agreement between the direct counting method and a computer-based analysis of concurrent images in 20x magnification. (6). A video capillaroscope (8, 11), operating at 200–500 times magnification, allows the more detailed quantitative evaluation of several parameters, such as the dimensions of individual loops, which are important in follow-up studies and in research. Standardisation is important when using all methods (12). Lack of guidelines and recommendations represent one of the major problems in capillaroscopy.

Studies have recently been published on reliability with both video capillaroscopy (13) and widefield nailfold capillaroscopy (14). However, there is a lack of studies comparing simple, low-magnification methods, suitable in the clinical setting, with more advanced, video capillaroscopic methods to evaluate the usefulness of the simple methods for patient follow-up. The aim of this study was thus to compare three quantitative methods of determining capillary density, with different resolutions.

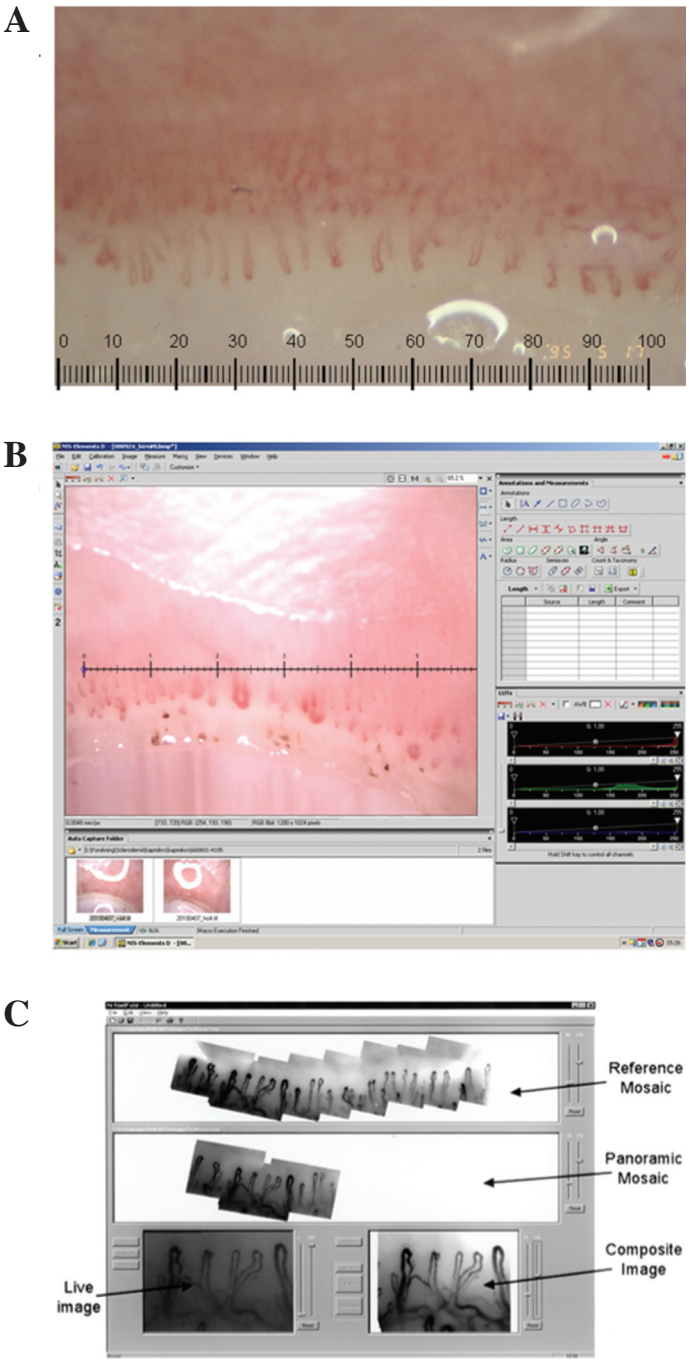

Fig. 1.
(**1A**) Determination of capillary density by direct counting in 20x magnification (600 units = 3mm);
(**1B**) image analysis in 20x magnification;
(**1C**) computerised nailfold video capillaroscopy in 300x magnification

Materials and methods

Patients and controls

Forty-one consecutive patients were included during an 18-month period, and all were investigated with the 3 methods at the same occasion. All three methods showed total disorganisation of the capillary bed in one patient, and the capillary density could thus not be calculated why this patient was omitted. All patients fulfilled the criteria for SSc of the American College of Rheumatology (15). Thirty-two (5 men and 27 women, aged 32–79 years) had limited cutaneous systemic sclerosis (lcSSc), with skin changes restricted to the face and extremities distal to the elbows and knees (16), and 8 (4 men and 4 women, aged 25–82 years) had diffuse cutaneous systemic sclerosis (dcSSc), with skin changes proximal to the elbows and knees. The median duration of the disease from the onset of Raynaud's phenomenon was 7.0 years (range 0.5–40 years) in the lcSSc patients and 1.0 years (range 0.5–4 years) in the patients with dcSSc. The regional ethics committee approved the study and informed consent was obtained from all patients. Twenty-one healthy controls (6 men and 15 women, aged 29-78 years) were included in the study.

Nailfold capillary microscopy

All three capillaroscopic methods were performed by one investigator (MW), who had no information regarding the clinical status of the individual patients. The first method used was direct counting (DC) of the capillaries along 3 mm in the centre of the nailfold using a stereo-zoom microscope (Olympus SZ-Pt, Japan) set at 20 times magnification, and equipped with a ruler in one of the eyepieces (17) (Fig. 1A). The second method involved the use of the stereo-zoom microscope equipped with a DeltaPix camera (DP 200, DeltaPix, Denmark) for determination of the capillary density on coded images using the Nikon imaging software NIS elements for image analysis (IA) (Fig. 1B). The third method was computerised nailfold video capillaroscopy (CNVC) (KK Technologies, Honiton, Devon, UK) using a CCD video camera with 300 times magnification (8) to analyse coded images (Fig. 1C). The capillary density in the distal row (expressed in terms of loops/mm) was determined on the fourth finger of the non-dominant hand using all 3 methods. A loop was considered to be distal when the angle between the apex of the capillary and the apex of adjacent capillaries was ≥90° (Fig. 2). The time required to measure the capillary density in one finger using the three methods, DC, IA and CNVC, is 1, 2 and 8 minutes, respectively.

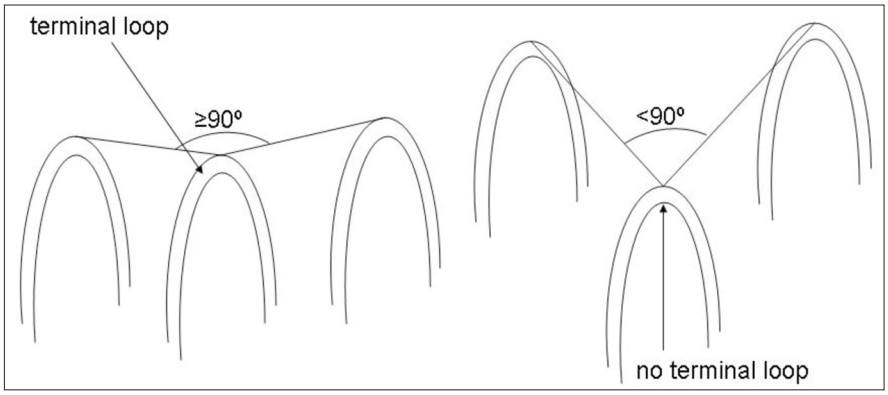

Fig. 2. Definition of a distal loop; the angle between the apex of the capillary and adjacent capillaries is ≥90°.

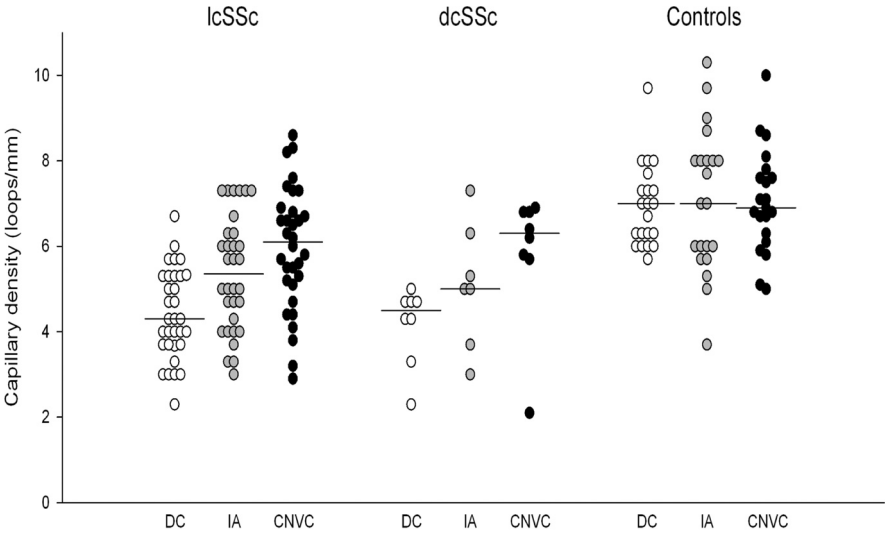

Fig. 3. Capillary density (loops/mm) in 40 SSc patients divided according to skin involvement, and in 21 controls, assessed with 3 different methods: DC (direct counting at 20 times magnification, open symbols), IA (blind image analysis at 20 times magnification, shaded symbols) and CNVC (blind computerised nailfold video capillaroscopy at 300 times magnification, filled symbols). The median values are indicated by horizontal lines.

Table I. Systematic and random errors in the measurement of capillary density in 40 patients and in 21 controls. The Systematic error is presented as the mean difference between two methods and random error as standard deviation of the difference between the two methods.

	Systematic	Random	Systematic	Random
DC *vs*. IA	-0.96	0.68	-0.14	1.35
DC *vs*. CNVC	-1.59	1.05	-0.11	1.22
IA *vs*. CNVC	-0.63	1.37	-0.03	1.88
	SSc (n=40)		Controls (n=21)	

Statistics

Levels of significance of the differences between two groups were calculated with the Mann-Whitney U-test for unpaired observations, and the Wilcoxon test was used for pairwise comparisons between the three methods. The systematic error is presented as the mean difference between methods, and the random error as the standard deviation of the difference between methods.

Results

The capillary density assessed by the three methods, DC, IA and CNVC, in lcSSc patients was: median (range) 4.3 (2.3–6.7), 5.4 (3.0–7.3) and 6.1 (2.9-8.6) loops/mm, and in the dcSSc patients 4.5 (2.3–5.0), 5.0 (3.0–7.3) and 6.3 (2.1–6.9) loops/mm. These values can be compared with those obtained for the controls: 7.0 (5.7–9.7), 7.0 (3.7–10.3) and 6.9 (5.0–10.0) loops/mm (Fig. 3). The significance of the difference in capillary density between patients and controls was $p<0.001$ (DC), <0.001 (IA) and $p=0.01$ (CNVC) for lcSSc patients, and $p<0.001$ (DC), $p=0.01$ (IA) and $p=0.05$ (CNVC) for dcSSc patients.

Comparison between the three methods is shown in Table I as systematic and random error. The systematic error was greater in patients than in controls, while the random error was comparable in the controls and patients. The capillary density in lcSSc patients assessed by DC (20 times magnification) was lower than that obtained with IA (also 20 times magnification) ($p<0.001$), and also lower than that obtained with CNVC (300 times magnification) ($p<0.001$). The second method, IA, resulted in only slightly lower values than CNVC ($p<0.05$). The results were similar in the smaller group of dcSSc patients, but with lower degrees of significance: $p<0.05$ for DC *vs*. IA, and $p<0.05$ for DC *vs*. CNVC, while the difference between IA and CNVC was not significant. No difference was found between the values obtained for the controls with any of the methods.

Discussion

Nailfold capillary microscopy is an important non-invasive tool for clinicians and researchers studying microvascular abnormalities in SSc. For diagnostic purpose ie to separate patients with SSc from patients with primary Raynauds's phenomenon a bedside examinationwith dermatoscope or stereomicroscope showing definite scleroderma pattern with enlarged capillaries and capillary drop outs resulting in a markedly decreased capillary density is reliable and feasible. Qualitative measurements of capillary morphology are useful to study the evolution of the vascular injury of SSc patients whereas a quantitative assessment of capillary density is reported to be the best dis-

criminator between primary and secondary RP (7, 8) and to be reproducible within and between investigators (8, 14). In the present study, all three quantitative methods of assessing capillary density differentiated between patient and control groups.

In the patient groups, the capillary density assessed by DC was lower than that assessed by IA, despite the same magnification, which may be explained by the enlargement of the image on the screen. The third method CNVC besides having higher magnification, also includes 16-frame video registration, making it possible to see empty capillaries, not containing any red blood cells (*i.e.* ghost capillaries) (8). We speculate that such capillaries may be missed when using the other two methods. Empty capillaries are probably more frequent in SSc patients, known to have a compromised microcirculation, than in controls. Another possibility is that capillaries that have already begun to be destroyed may be visible using CNVC, but not in the other, lower-magnification methods. The capillary density in the controls determined with all three methods was lower than reported by others (7, 8). One explanation of this could be the cut-off level used to define the capillaries in the distal row. We considered a loop to be distal only when the angle between the apex of the capillary and adjacent capillaries was ≥90°.

The direct counting method is cheap, simple and suitable in clinical practice to separate primary from secondary Raynaud's phenomenon. In a pilot study we found the inter- and intra-observer variability to be reasonably good, even with moderately experienced assessors (6). In a recent larger study of 214 consecutive patients we could confirm a good intra-observer variability between 8 fingers with variation coefficient of 9% for controls and 14% for patients (15). The second method, image analysis, is slightly more expensive than the first, but offers the possibility of saving the data. Measurements can be performed immediately, or later and blindly. Neither method is particularly time-consuming. The third method, computerised nailfold video capillaroscopy, is the most sophisticated method used in the present study. The advantages of this method are its high magnification, the possibility of blind analysis of the data, and the possibility of measuring the dimensions of individual capillaries and loops. This method in contrast of most videocapillaroscopic methods also offers the opportunity to follow individual capillaries in the patients by identifying the same region as was investigated on an earlier occasion (8), which is a great advantage in longitudinal studies and in research. The disadvantages are the cost and time required for each measurement (8 minutes/finger), making this method more suitable for research than for clinical use at small rheumatological units. Interestingly this method showed slightly wider range within groups and lower degree of significance between patients and controls. This paper focuses on three different methods for determination of capillary density. The overlap between patients and controls found by us and others underscores that an evaluation of capillary morphology is needed for a complete capillaroscopic assessment. Particularly in the evaluation of disease state, an assessment of capillary morphology and of the development from early to active and late pattern as described by Cutolo (18) is needed. For such purposes sensitive methods like CNVC are preferable and the direct counting would probably not be insufficient.

In conclusion, assessment of capillary density with three different methods showed good agreement between methods. All methods could differentiate between SSc patients and controls. The direct counting method can be performed also by rheumatologists with only moderate experience and with no access to videocapillaroscopy equipment.

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