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

M. Wildt, D.M. Wuttge, R. Hesselstrand, A. Scheja

Department of Clinical Science, Division of Rheumatology, Lund University, Lund, Sweden.
Marie Wildt, MSc
Dirk M Wuttge, MD, PhD
Roger Hesselstrand, MD, PhD
Agneta Scheja, MD, PhD

Please address correspondence to: Agneta Scheja, MD, PhD, Department of Clinical Science, Lund Section of Rheumatology, Lund University, SE-221 00 Lund, Sweden. E-mail: agneta.scheja@med.lu.se

Received on September 8, 2011; accepted in revised form on December 21, 2011.

© Copyright CLINICAL AND EXPERIMENTAL RHEUMATOLOGY 2012.

Key words: capillary microscopy, capillaroscopy, systemic sclerosis, capillary density

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.

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 capillaroscopy. 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 capillaroscopic 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 stereozoom 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). Capillaroscopic 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 stereozoom microscope, and we also demonstrated good agreement between the direct counting method and a computerbased 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.

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 (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.
Capillary density in systemic sclerosis / M. Wildt et al.

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), \( p < 0.001 \) (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 it is to separate patients with SSC from patients with primary Raynaud’s phenomenon a bedside examination with 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-
Capillary density in systemic sclerosis / M. Wildt et al.

The use of video capillaroscopy, defined as the 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 a 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 sufficient.

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 videocapillaroscopic equipment.

Acknowledgements

The software for video capillaroscopy was received as a license agreement from the Department of Imaging Science and Biomedical Engineering, University of Manchester (UK). We would like to thank A.L. Herrick and the Manchester Group for their help. We would also like to thank Jan-Åke Nilsson for help with the statistics.

References


