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The Abnormalities of Naifold Capillaries in Scleroderma as Assessed by Video Image Analysis and Photomicroscopy

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SUMMARY **Scleroderma is a systemic connective tissue disease in which the diagnosis in supported by morphological changes in nailfold capillary size and density. These changes are open to observer bias. In this paper we describe 2 objective methods that allow quantitative definition of capillary changes, video image analysis (VIA) and photomicroscopy. VIA was used to assess 15 healthy control subjects and 22 patients with scleroderma. Scleroderma patients had a significantly larger capillary diameter (43 µm versus 20 µm, p=0.0001) and capillary density was reduced by a mean factor of 0.5.**

Image stored on computer will facilitate serial assessments of nailfold capillary changes and possibly provide information on disease progression.

Morphological changes in the size, density and form of nailfold capillaries are seen uniquely and almost universally in patients with systemic sclerosis,[1] mixed connective tissue disease and dermatomyositis.[2] These capillary changes are observed early in these disease processes and hence their detection is of diagnostic value and may have prognostic implication.[3] These capillary changes are most easily observed in the nailfold bed which is simply accessed with the aid of a low magnification microscope.[4] There is considerable variation in nailfold capillary morphology in health. Therefore, to detect pathological changes, it is important to have an objective technique free from observer bias. This report describes two methods of quantitative nailfold capillary analysis. Both healthy subjects and patients with scleroderma have been studied. Capillary size and density have been assessed to detect any capillary dilatation and dropout, the hallmark of the microvascular process which characterizes scleroderma and related disorders.

MATERIALS AND METHODS

The control subjects were from two sample populations, young and elderly (over 65 years), the former being healthy medical students at Flinders Medical Centre (n=11) and the latter, patients (n=4) with cardiovascular or respiratory disease but without evidence of collagen vascular disease. The mean ages were 25 and 79 years, respectively.

The scleroderma group consisted of five patients with diffuse scleroderma (positive Scl-70 autoantibody and ACR criteria) and seventeen with limited scleroderma (positive anticentromere antibody [ACA] and ACR criteria).

Capillary diameter

Capillary diameter was measured using a Video Image Analysis technique with a Micro-Orient microscope linked to an NEC multi sync 3 FG computer via a Panasonic WV BL 600 camera. Cold light was provided by a

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Eurome Fibre Optic light source. Video pro software allowed the nailfold image to be digitalized and sorted on disc (Photo 1). A drop of oil was placed on the nailbed to improve resolution. The measurements of capillary diameter were taken from the mid nailfold region of the second and fourth digits of both hands. The diameter measurements were calibrated using the image of a biopsy ruler on screen and measured in 0.5 mm increments (Photo 2). Within each field two or more well defined adjacent capillaries were chosen for measurement. Three capillary diameter measurement were made for each capillary. These measurements were performed at the region of the preloop, the post loop and at the widest point of the loop. The mean diameter was then calculated and the results averaged for each subject. Capillary nailfold diameter was also measured in five healthy subjects at room temperature, after local heating of the hand to 45°C and local cooling to 17°C. A thermocouple was attached to the back of the wrist and middle finger to measure skin temperature. The temperature change was produced by immersing both hands in water baths of 45°C and 17°C, respectively, for 5 minutes prior to assessment by VIA. Finally Nitrobid ointment (2% glyceryl trinitrate and lactose in a lanolin and white petroleum base) was applied to the fingers of the dominant hand. After 20 minutes the capillary diameter was measurement using VIA.

Capillary density

Two methods were used to assess capillary density, viz photomicroscopy and VIA. Photocapillaroscopy was performed using a wide photomicroscope M400 with attached camera. Black and white Kodak TO 135-136 film was used. A KL 1500 electronic fibre optic light provided cold light and a number 40 blue-green filter was used to enhance the contrast between capillaries and nailbed. Oil was placed on the nailfold bed to improve resolution, The second and fourth digits in both dominant and nondominant hands were examined and photographed under 10 x 3.2 magnification. Measurements of density were performed over a 2.00 x 0.74 mm area (see Photo 3). Using the VIA method, the numbers of capillaries per field were also counted to assess density.

Statistical analysis

Unpaired two tailed tests were used on data obtained from photomicroscopy and VIA. p values < 0.05 were regarded as significant.

RESULTS

Healthy controls

The eleven young controls were compared with each other to determine if there were differences in capillary density between the dominant and non-dominant hands and the second and fourth digits. No statistically significant difference in mean density was observed.

Capillary density differences between the young (n=11) and the elderly (n=4) were then assessed. There were nine males and six females in total. No statistically significant difference were seen.

When the capillary diameter in young control subjects was assessed at room temperature, 45°C, 17°C and with Nitrobid paste application, there were no statistically significant differences. However, there appeared to be a trend towards dilatation when the hand was warmed

Scleroderma

Capillary diameter.

The capillaries of twenty-two patients with scleroderma and eight control subjects were assessed by VIA. The mean diameter in the control group was 20 μm with a standard deviation of ± 10 μm. In the scleroderma group, the mean diameter was 43 μm (SD±17). The range was 15-120 μm. Using the two tailed unpaired t test, the difference in diameter between these two groups was significant ($p = 0.0001$; Fig. 1).

Capillary density

Using photomicroscopy, the relationship of mean capillary density between fifteen controls and five scleroderma patients was calculated and the average determined. The mean capillary density in the control group was 36 capillaries per field (SD±15) and for scleroderma patients the mean was 20 capillaries per field (SD± 10); $p = 0.0002$.

When capillary density was assessed by VIA, again, a statistically significant difference in density was seen between patients and control (Fig. 2; $p<0.0001$). Scleroderma patients were found to have half the capillary density of controls by both methods.

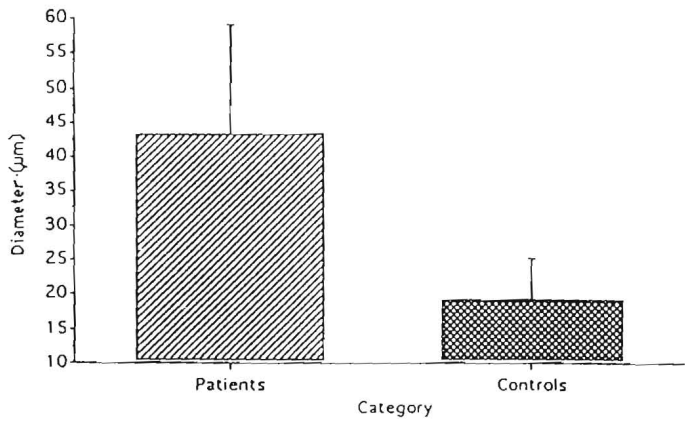

Fig. 1 Diameter of nailfold capillaries as measured by VIA in scleroderma patients (n=22) and controls(n=8).

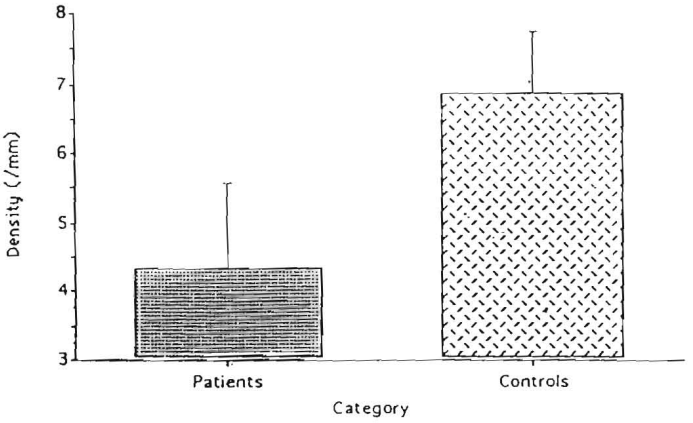

Fig. 2 Capillary density as measured by VIA in scleroderma patients and controls.

DISCUSSION

Capillary dilation and drop out are well recognized morphological changes in patients with scleroderma.[6] Nailfold capillary microscopy has proven itself a useful noninvasive procedure to observe these microvascular changes. However, most previous studies have used subjective techniques which are open to observer bias.

In this study, using two objective measurable techniques, we have shown that the capillary density in normal young patients does not vary between the dominant and non-dominant hand nor between the 2nd and 4th digits. We chose to study these variable to assess if capillary density correlated with the extent of hand usage, assuming that the dominant hand is used more than the non-dominant. The 4th and 5th digits have been shown previously to have the longest capillaries and the most prominent sub papillary venous plexus,[7] whereas the 2nd digits has the shortest capillary loops and the least visible sub papillary venous plexus.[7] We therefore chose to study one digit from each category. No differences were seen between the 2nd and 4th digits in the health. Changes in diameter were also assessed in the controls, who were subjected to temperature change and Nitrobid paste. These changes of local heat, cold or use of a vasodilator did not substantially effect capillary diameter.

The second part of this study looked at the extent of capillary density according to age and gender. This was performed in order to exclude the possibility that aging may lead to a reduced number of capillaries. We found, however, no significant differences in capillary density between the groups. Neither was there a significant effect of gender on capillary density. We recognize, however, that a type two statistical error can not be ruled out since we only studied 4 elderly patients and 11 young individuals.

A marked decrease in capillary density was demonstrated when scleroderma patients were compared to healthy controls, as has previously

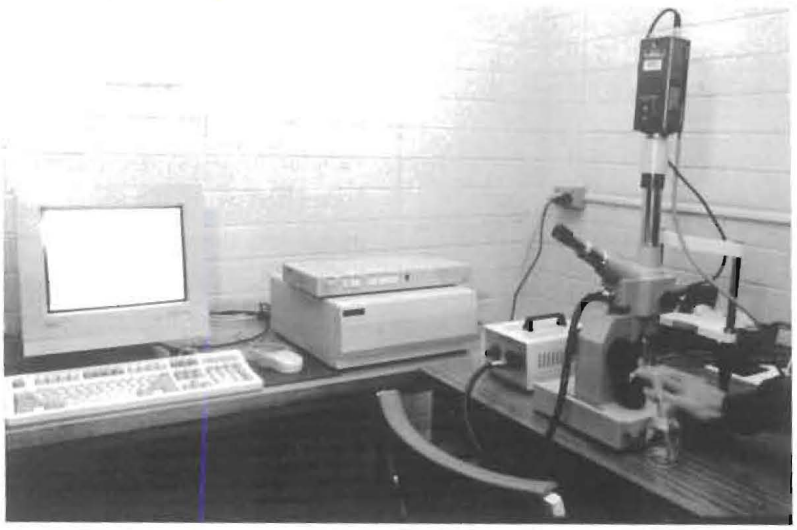

Photo 1 Video image analysis set with microscope, camera lens, relay system and hardware.

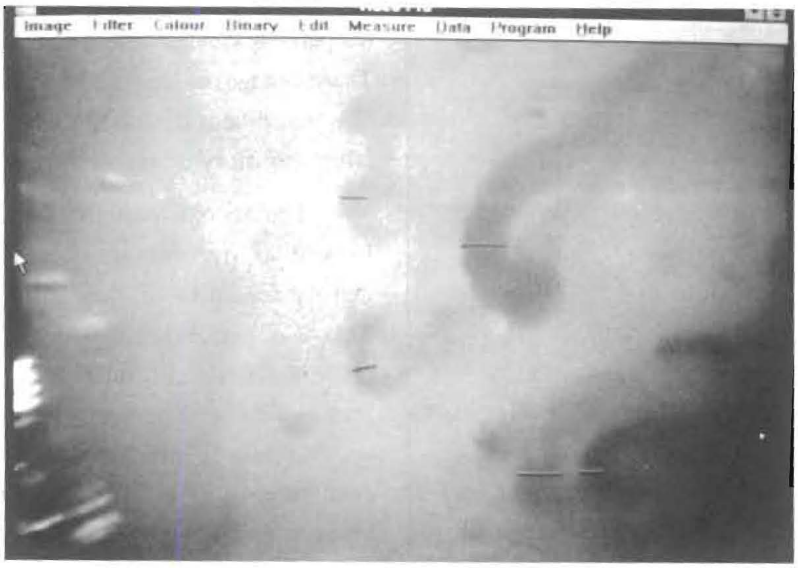

Photo 2 Close up of VIA image of nailfold capillaries in scleroderma (with diameter annotated at widest point of loop).

been reported.[8,9] However, the main focus of this study was to provide a quantitative assessment of capillary dilatation. In scleroderma nailfold capillaries become dilated by a factor of 2-3 folds over normal. As the images of dilated capillaries are stored in computer, this technique will facilitate sequential follow up of patients so that progressive changes in capillary diameter can be quantitated. Changes in capillary morphology over time may provide insight into the yet unanswered question of the correlation between capillary abnormalities and extent of internal organ involvement.

A group of patients without scleroderma or Raynauds phenomena, but with positive ACA was also assessed (unpublished). These patients had capillaries of normal diameter, suggesting that the centromere autoantibody itself is not pathogenic for the vascular injury, and that other mechanisms must be responsible. We have also observed a single patient with Scl-70 but without evidence of scleroderma who also had normal capillary findings.

Further uses of the techniques described in this paper include the collection of a database on our scleroderma patients, divided into the subsets of diffuse disease, limited disease and an overlap group. It is possible to assess their naifold capillaries at diagnosis, after treatment (for example with d Penicillamine) and changes over time, and correlate this with disease progression seen elsewhere in the body, e.g. lung or skin. This may provide definitive information regarding the prognostic value of changes in nailfold capillary morphology in patients with different subsets of scleroderma.

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Photo 3 Photomicroscopy of nailfold capillaries in a healthy subject.

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GENERAL INFORMATION

DATE	:	8-11 December 1998
VENUE	:	Philippine International Convention Center Manila, Philippines
LANGUAGE	:	English
OFFICIAL HEADQUATER HOTEL	:	Westin Philippine Plaza Hotel
PRELIMINARY TOPICS	:	Respiratory allergies Atopic dermatitis, contact dermatitis, urticaria, skin allergies Occupational allergies Pediatric allergies Molecular biology, allergic inflammation Pharmacotherapy Autoimmune diseases, connective tissue disorders Immunotherapy Immunology of tropical disorders (malaria, dengue, TB, hepatitis, AIDS)
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