2

Methods & Techniques
NON-INVASIVE TOOLS TO INVESTIGATE MICROVASCULAR STRUCTURE AND FUNCTION

Because of their accessibility, skin and nailfold capillaries are often used to investigate the microcirculation. We choose two non-invasive techniques to study microcirculatory structure and function respectively: nailfold video capillary microscopy, and iontophoresis combined with laser Doppler flowmetry.

SKIN MICROCIRCULATION

Nailfold capillaroscopy and iontophoresis should be considered against the background of the anatomy and physiology of the skin microcirculation. The microvascular bed of the skin consists of nutritive capillaries, a subpapillary plexus and deeper arteriovenous anastomoses (figure 1).

Figure 1. Skin anatomy
The nutritive capillaries are the most superficial ones (10-50 μm from the skin surface). In most areas of the fingers, the nutritional capillary loops run perpendicular to the skin surface and only the apex of the capillary loops can be visualised (figure 2).

Figure 2. In most areas of the finger, only the apex of capillaries can be visualised as dots

In the nailfold area however, the capillary loops run more parallel with the skin surface, and the distal row capillaries can be visualised in their full length (figure 3).

Figure 3. In the nailfold, capillaries can be visualised as loops
In the subpapillary vascular bed (approximately 0.05-2.0 mm from the skin surface) the dominating vessels are venules and, to a smaller extent, arterioles. The deeper arteriovenous anastomoses have a thermoregulatory function. They are especially numerous in the fingertips, ears and nose, whereas the skin of the dorsal finger and forearm are thought to lack arteriovenous anastomoses.\textsuperscript{1} Under conditions of normal environmental temperature (20-25 °C), the majority (>90%) of total skin blood flow passes through the arteriovenous anastomoses. Skin microcirculatory blood flow and pressure are determined by a balance of central and local mechanisms. In addition, microvascular perfusion is influenced by the rheological properties of blood. Central control is achieved by sympathetic adrenergic vasoconstrictor nerves, through the action of norepinephrin on alpha-receptors, which control blood flow through the arteriovenous anastomoses. Capillaries themselves have no nerve supply. Local autoregulatory mechanisms are usually direct responses of vascular smooth muscle to local metabolites, changes in transmural pressure or shear stress. These local autoregulatory mechanisms may in part be endothelium-dependent.\textsuperscript{1,2}

**NAILFOLD CAPILLAROSCOPY**

The technique of nailfold capillaroscopy as a tool to study structural microcirculatory changes in the nailfold has been widely used in the assessment of patients with Raynaud's Phenomenon (RP) and SSc. The nomenclature of structural capillary abnormalities in the literature has not been consistent.\textsuperscript{3} Hildegard Maricq has worked extensively to refine the assessment of the skin capillaries using microscopic and photographic equipment.\textsuperscript{4,5} She distinguished the following characteristics of the nailfold capillary bed, although exact quantification of the capillary dimensions could not be made:
1. Scleroderma capillary pattern. Based on overall estimation of the microvascular abnormalities. Presence of enlarged capillaries of the scleroderma (SD)-type: increased diameter (> 25 µm) of all three portions of the capillary loop (arterial, apical, and venular), often associated with avascular areas: none, slight (0.4-2 mm²), moderate (2-4 mm²) extensive (>4 mm²), and a combination of other morphological features such as capillary haemorrhages, disorganisation of the capillary bed, edema and discoloration of the cuticle.

2. ‘active’ or ‘slow’ capillary pattern, based on the size of the nailfold terminal row capillary loops: normal, definitely enlarged (total width 91-150 µm), or extremely enlarged (>150 µm), and on the extent of the avascular areas. ‘Active’ is defined as moderate to extensive avascular areas without capillary telangiectases (clusters of dilated capillaries) or extremely enlarged loops. ‘Slow’ is defined by extremely enlarged capillaries with no or minimal avascularity with or without capillary telangiectases.

The ‘active’ and ‘slow’ patterns were defined on the basis of an observed correlation between the activity of the microvascular lesions and the clinical progression of the disease in small series of cases.⁵

In 2000, Cutolo et al, classified microvascular changes in SSc patients into 3 distinct patterns⁶:

1. early pattern: few enlarged/giant capillaries, few capillary hemorrhages, no evident loss of capillaries.

2. active pattern: frequent giant capillaries, frequent capillary hemorrhages, mild disorganisation of the capillary network.
3. Late pattern: irregular enlargement of the capillaries, few or absent giant capillaries, hemorrhages, and extensive avascular areas.

These patterns were found to correlate significantly with disease duration, and it was hypothesised that these patterns characterise the evolution of SSc associated microangiopathy, and even predict future organ complications.\textsuperscript{6,7} It should be noted however, that the scleroderma capillary pattern is not entirely specific for SSc: the same pattern can be observed in other CTD.

Video capillary microscopy is a further development which allows quantification of the nailfold abnormalities: using high magnification (figure 4) combined with a video camera and digitising system, dimensions of individual capillaries can be measured.

![Nailfold capillaroscopy](image)

**Figure 4.** Nailfold capillaroscopy

Recently, newly developed software to analyze digitised images makes it possible to create a panoramic mosaic of the nailfold (figure 5).\textsuperscript{8}
Applying this technique to a control group and a group of patients with Primary RP and SSc, a clear distinction between the SSc group versus the control and PRP group, became clear. The most powerful discriminator between groups was the number of loops per mm in the terminal row of the nailfold, and the mean total width of the capillaries. The technique of digitised panoramic mosaic images was used in this thesis.

THE ROLE OF NAILFOLD CAPILLAROSCOPY IN CLINICAL PRACTICE

Nowadays, nailfold capillaroscopy is used for the differentiation between primary and secondary (CTD associated) RP and the early diagnosis of SSc. In patients with RP, a normal nailfold capillary pattern combined with negative or low antinuclear antibodies (ANA <160, or <++) has a negative predictive value of CTD development of 98% (prior likelihood of CTD : 13%). For patients with RP, the positive predictive value of scleroderma specific antibodies or abnormal capillaroscopy for a scleroderma spectrum disorder within 5 years (SSc, MCTD, poly/dermatomyositis) is 47% in a group with an prior likelihood of 13%. If both specific antibodies and nailfold abnormalities are present, the positive predictive value increases to
SSc is a CTD characterised by auto-immunity, fibrosis and vasculopathy with substantial morbidity and mortality. The diagnosis of SSc is based on the criteria of the American College of Rheumatology (ACR). However, by the time SSc is diagnosed, many (organ) complications may already be present. New criteria have been proposed for the early identification of SSc, based on a combination of RP, specific scleroderma antibodies, and nailfold capillary abnormalities. SSc is diagnosed according American College of Rheumatology (ACR) preliminary classification for definite SSc. However, the ACR classification criteria for SSc were not developed for diagnostic purposes, but rather with the intent to “establish a standard for definite disease in order to permit comparison of groups of patients from different centres”. One study evaluated a group of 259 ‘definite’ SSc patients, who were diagnosed by expert clinicians. The investigators wanted to evaluate the sensitivity of the ACR criteria and to determine whether addition of nailfold capillaroscopy could increase their sensitivity in this group. The study showed an increase in the sensitivity of the ACR criteria for SSc from 34% to 89% when nailfold capillaroscopy was added. As expected, most patients with limited SSc (scleroderma skin changes below the elbows) were excluded by the ACR criteria and identified by detection of characteristic nailfold capillary changes by nailfold capillaroscopy. The same procedure was followed in another cohort of 101 patients, showing an increase from 67% to 99% in the sensitivity of SSc diagnosis when nailfold capillaroscopy was added to the ACR criteria. Whether the early diagnosis of patients with SSc improves prognosis in terms of morbidity (development of organ complications) and mortality remains to be seen.
IONTOPHORESIS AND LASER DOPPLER FLOWMETRY

Iontophoresis is a non-invasive method of drug application that allows the local transfer of charged substances across the skin by use of a small electric current. The principle is based on the fact that, when an electrical voltage difference is applied to a solution, solute ions will migrate towards an electrode of opposite charge. Thus, positively charged drug ions can be introduced through the skin under a positively charged electrode (anodal iontophoresis) and vice versa (cathodal iontophoresis)(figure 6).

![Figure 6. Iontophoresis of the skin](image)

To investigate endothelial function, skin microvascular responses to iontophoresis of acetylcholine, an endothelium-dependent vasodilator and sodium nitroprusside, an endothelium independent vasodilator can be studied using a laser Doppler flowmetry. Laser Doppler flowmetry is a noninvasive method to measure skin perfusion. A laser beam penetrates the skin and a fraction of the light is backscattered by moving blood particles, undergoing a frequency shift according to the Doppler principle. From the frequency shift, tissue perfusion can be derived in arbitrary units. After refraining from eating, smoking and beverages for at least 4 h and acclimatisation for 20 minutes at 23 °C, iontophoresis combined with laser Doppler flowmetry was performed. Acetylcholine (1%) was delivered using anodal current, and sodium nitroprusside (0.01%) was delivered with a cathodal current. Laser Doppler flux was measured on the middle phalanx of the left and right third finger with the Periflux 4000 system (Perimed) and expressed as arbitrary perfusion units.
Day-to-day reproducibility was assessed previously in our institute and was 15.9%±8.4% for acetylcholine and 13.9%±9.0% for nitroprusside, as determined in 5 subjects on 2 occasions.¹⁵
REFERENCES


