State of the art on nailfold capillaroscopy: a reliable diagnostic tool and putative biomarker in rheumatology?

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Abstract

Capillaroscopy is a non-invasive and safe tool to morphologically study the microcirculation. In rheumatology it has a dual use. First, it has a role in differential diagnosis of patients with RP. Second, it may have a role in the prediction of clinical complications in CTDs. In SSc, pilot studies have shown predictive associations with peripheral vascular and lung involvement hinting at a role of capillaroscopy as putative biomarker. Also and logically, in SSc, microangiopathy, as assessed by capillaroscopy, has been associated with markers of the disease such as angiogenic/static factors and SSc-specific antibodies. Moreover, morphological assessments of the microcirculation (capillaroscopy) seem to correlate with functional assessments (such as laser Doppler). Because of its clinical and research role, eyes are geared in Europe to expand the knowledge of this tool. Both the European League Against Rheumatism (EULAR) and the ACR are stepping forward to this need.

Key words: capillaroscopy, microcirculation, Raynaud’s phenomenon, differential diagnosis, connective tissue diseases, classification criteria, systemic sclerosis, clinical complications, EULAR.

Background

Capillaroscopy is a tool for looking at the microcirculation. The history of capillaroscopy started in 1663, when Johan Christophorous Kolhaus was the first clinician to use a primitive microscope to observe the small blood vessels surrounding the nails. Giovanni Rasori (1776–1873), using a magnifying glass, first reported the close relationship between conjunctival inflammation and the presence of an inextricable knot of capillaries [1].

From the time that Maurice Raynaud (1834–1881) presented his thesis on local ischaemic damage of the hands, feet, nose and tongue, intravital microscopy (or capillaroscopy) became recognized as an important investigation in identifying and analysing microvascular involvement, which is the key feature of RP. The last century was characterized by the optimization of capillaroscopic tools (Fig. 1).

Nowadays the role of capillaroscopy lies in making a differential diagnosis between a primary RP (PRP), not related to any clinical condition, and a secondary RP (SRP), related to a CTD. In addition, a recent meta-analysis showed capillaroscopy to be the best predictor of transition from a PRP to an SRP [2]. Besides this, eyes are geared towards investigation of its possible role as a biomarker in CTDs.

Irreplaceable role of capillaroscopy for early differential diagnosis between PRP and SRP

Microvascular dysfunction and damage seem to constitute an important initial pathological event in several CTDs and in SSc they seem to represent even the origin of progression towards a systemic fibrotic disease [3, 4]. Still, as stated in a recent review, almost 10–12 years ago it could have been justifiably argued that there was relatively limited interest in differentiating between PRP and the very early stages of SRP due to SSc or other CTD [5].

The advent of discriminatory criteria incorporating a central role for capillaroscopy has changed this attitude. In fact, nailfold capillaroscopy is able to distinguish in a reliable manner a PRP from an SRP due to SSc [6].
A patient with a PRP meets, among other criteria (see below), the following criterion: having a normal capillaroscopy (in a normal capillary pattern, capillaries of the distal row of the nailfold have an open hairpin shape, are homogeneously sized and regularly arranged in a parallel fashion and their number ranges in linear mm from 6 to 14 with a mean of 9) [7]. In contrast, the capillaroscopic image of a patient with RP due to SSc is characterized by pathognomonic microvessel structural damage (Fig. 2) consisting of the presence of giant capillaries, capillary loss and other morphologic anomalies such as progressive neoangiogenesis (see later) [8, 10, 11]. These pathognomonic changes can be reliably distinguished from normal images [12].

In addition, patients with merely RP, who are to develop a secondary RP due to SSc in the long run, can nowadays be detected early, before clinically overt disease sets in. In this way, prospective follow-up of a cohort of patients with only RP as clinical presentation has shown that the combination of pathognomonic scleroderma-type changes on capillaroscopy with the presence of SSc-specific antibodies renders progression to definite SSc in 47%, 69% and 79% over 5, 10 and 15 years of follow-up [13].

Interestingly, when using only capillaroscopy (without testing specific SSc antibodies) in the follow-up (29 ± 10 months) of patients with merely RP at baseline, transition from a normal (and non-specific changes) to a pathognomonic abnormal pattern can be seen in ~15% of patients [14].

Of note, the role of capillaroscopy in CTD other than SSc is not indispensable. Even though structural changes
may be present in other CTDs, they are not a conditio sine qua non and they are moreover non-specific. Consequently, capillaroscopy finds its primary aim in daily practice in detecting those patients with RP who have, or are to have, SSc.

**Place for capillaroscopy in classification criteria of SSc**

For a long time, even though their importance was already well alluded on in 1973, capillaroscopic markers of the scleroderma pattern were not included in the classification criteria of SSc, and only after 2000 were they introduced in published classification criteria [8, 15, 16].

Interestingly, in 2001 the sensitivity of the ACR criteria to identify patients with limited cutaneous disease was found to be significantly improved with addition of nailfold capillary abnormalities (definite enlargement and loss of capillaries) and visible telangiectasias from 34% to 89% in a cohort of 259 patients with a diagnosis of SSC based on expert opinion [17].

In line with this, the year 2001 was also the year in which the hallmark article, definitely establishing the paramount role of capillaroscopy in differentiating a PRP from an SRP due to SSc, was published by LeRoy and Medsger [9]. In this LeRoy stated that a PRP must have a normal capillaroscopic image, absence of antinuclear factors, no sedimentation and no signs reflecting peripheral vascular disease.

In addition, the presence of a scleroderma pattern on capillaroscopy and SSc-specific antibodies were stated to be sufficient to diagnose the patient as having early (preclinical) SSC [8]. These criteria, mainly focusing on the vascular component of the disease, were intended to be more sensitive than the ACR criteria (based on skin involvement, the fibrotic component of the disease). Besides that, the LeRoy criteria have been validated both in a retrospective and in one large prospective study (see above) [13].

In 2011, the following criteria (based on expert opinion, obtained through three Delphi rounds within an international forum of experts in SSC) for the very early diagnosis of SSC (VEDOSS criteria) have been proposed to have high clinical relevance for SSC: RP, puffy swollen fingers turning into sclerodactyly, abnormal capillaroscopy with scleroderma pattern, positive ACAs and positive antitopoiso merase antibodies. These VEDOSS criteria are in line with the LeRoy criteria but differ as to the extent of cutaneous signs of the disease [18].

In addition, in 2012, an international working group (supported by the ACR and EULAR), with the aim of revising the classification criteria for SSC, proposed (based on consensus techniques such as the Delphi and nominal group technique) capillaroscopy as one of the top potential items useful for classification of SSC [19]. Clearly, all these investigations over the past decade have confirmed and enhanced interest in and the need for capillaroscopy, at least in the early diagnosis of SSC.

**Use of qualitatively and quantitatively assessed scleroderma capillaroscopic patterns for the diagnosis and follow-up of SSc**

More than 90% of patients with clinically overt SSc show a scleroderma pattern on capillaroscopy (Fig. 3). This pattern may be recognized on visual inspection (= qualitative assessment) by several types of microscope, at different magnifications [9, 10].

Last century the most frequently used magnification was the widefield technique (low magnification, e.g. magnification ×12) with which a whole nailfold can be evaluated in one capillaroscopic image. With this technique Marico described the hallmark articles attesting a specific pattern to be present in a SSc population vs healthy controls and other CTDs [16, 20, 21].

This century, with the high magnification (e.g. magnification ×200) technique (which better visualizes the capillaries themselves but only one-fourth of the nailfold per capillaroscopic image) Cuto et al. [10] classified these scleroderma patterns into early, active and late scleroderma patterns [10].

Logically, as SSc is characterized by progressive obliteration of the microcirculation, each of these consecutive patterns consists of a higher proportion of capillary loss [10, 22].

Besides that, each of the patterns is also characterized by a different prevalence of characteristic markers of the disease, more specifically giant capillaries, haemorrhages (both present in the early and frequent in the active SSc pattern), SSc and aberrant morphological microvascular shapes (so-called neangiogenic capillaries, present in the active and frequent in the late pattern).

Pathognomonic in preclinical SSc (presence of merely RP and an SSc-specific antibody, see above) is the presence of giant capillaries (defined when using the widefield technique as definitely enlarged capillaries, with a loop width of 90–150 μm and apical limb diameter of >50 μm and in line with this when using the high magnification technique as homogeneously enlarged capillaries, with apical diameter >50 μm) [13, 23, 24]. The occurrence of clinically overt disease coincides with the occurrence of loss of capillaries, as attested in a large longitudinal follow-up study of patients with RP, with the evolution of capillaroscopic parameters in those who were to develop SSc [13].

Inter-rater reliability in distinguishing scleroderma patterns from normal capillaroscopic images has been confirmed by both low and high magnification techniques [12, 25–27].

Besides visual inspection (qualitative assessment) of the scleroderma patterns, characteristic capillary changes can also be counted (quantitative assessment), usually per linear mm [22, 28, 29].

Of those quantified capillaroscopic parameters for which inter-rater reliability has been established, the count of capillaries, giant capillaries and
haemorrhages diminishes over time and neoangiogenetic capillaries augment over time when evaluated at group level [22].

Given this change over time of capillaroscopic parameters, capillaroscopy has been used as outcome measure in small therapeutic trials with different agents [30–33]. Further large randomized controlled trials are needed to investigate the role of capillaroscopy in quantification of treatment response in terms of improvement in microvascular structure.

Predictive role of capillaroscopy for SSc clinical complications

Next to its role in discerning a primary from a secondary RP due to SSc, focus is on a possible role in informing the clinical researcher of possible future clinical complications. Cross-sectional associations have been made between qualitative and quantitative capillaroscopic parameters and clinical complications of the disease.

In this way, a principal role of loss of capillaries has been attested in associations with pulmonary arterial hypertension, interstitial lung disease, peripheral vascular disease and severity of peripheral vascular disease, heart and lung involvement assessed by the disease severity scale of Medsger [34–37].

Prospective predictive associations, in which baseline capillaroscopy (either qualitatively or quantitatively assessed) is associated with future clinical complications, are scarce, but there is evidence of a putative role of capillaroscopy as biomarker in SSc (Table 1) [38–41].

Links between SSc serum biomarkers and capillaroscopic scleroderma patterns

Two main categories of serum SSc biomarker have been investigated in correlation with the different nailfold videocapillaroscopic (NVC) patterns: angiogenic/static, vasculogenic factors and autoantibodies. Angiogenesis is heavily disturbed in SSc as mirrored by pathognomonic capillaroscopic changes (see above). In addition, despite the hypoxic conditions induced by the progressive SSc fibrosis and capillary number decrease, there is no evidence for sufficient compensatory angiogenesis in SSc.

One culprit may be an imbalance between angiogenic and angiostatic factors [42]. Also vasculogenesis may be impaired. Cross-sectional associations have been described between microangiopathy (as assessed by capillaroscopy) and three angiogenic [vascular endothelial growth factor (VEGF), angiopoietin 2 (Ang-2) and endothelin-1 (ET-1)], one angiostatic factor (endostatin) and one vasculogenetic factor (Table 2) [43–48].

The following associations between antibodies and capillaroscopy have been described in SSc: associations with anti-endothelial cell antibody (AECAs) and with SSc-specific antibodies. AECAs serum levels were recently found to be higher in patients with the late NVC pattern
compared with the early and active ones ($P = 0.04$ and $P < 0.02$). In addition, higher skin scores and cardiovascular involvement were related to the presence of AECA and more severe microvascular changes, suggesting that AECA may have a role in the progression of endothelial damage and SSc disease [49].

Concerning SSc-specific antibodies, studies in populations with established and early SSc have been described. In established SSc, the prevalence of anti-ScI70 antibodies is significantly higher in the active and late vs the early patterns ($P < 0.001$), whereas the prevalence of ACA was highest in patients with the early NVC pattern [50].

In early SSc, prospective follow-up in those patients with RP who are prone to develop SSc has attested a sequence in the occurrence of microcirculatory changes, more specifically, first, appearance of giants, before the onset of clinically overt SSc and second, loss of capillaries, around the same time as occurrence of clinically overt disease. Also a varying time course for occurrence of microcirculatory damage after onset of RP depending on SSc antibody specificity has been described in this early SSc population.

In this way, giant capillaries occur earliest in patients with anti-RNAP III antibodies, latest in those with anti-CENP-B antibodies, whereas time of occurrence was intermediate with anti-Th/To ($P = 0.002$). A similar temporal development occurred for capillary loss in relationship to these three antibodies ($P = 0.021$) [13]. Further research, based on these associations, will clarify the role of the immune response in the pathogenesis and progression of SSc.

**Table 1** Role of videocapillaroscopy as putative biomarker

<table>
<thead>
<tr>
<th>Author</th>
<th>Capillaroscopic assessment</th>
<th>Prospective association</th>
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<tbody>
<tr>
<td>Sebastiani et al. [38]</td>
<td>—</td>
<td>Specificity of 85.9% and sensitivity of 94.3% to predict development of new digital ulcers within 3 months after the capillaroscopic visit.</td>
</tr>
<tr>
<td>Smith et al. [39]</td>
<td>—</td>
<td>Specificity of 69.77% and sensitivity of 70.00% in predicting development of digital trophic lesions within 6 or 12 months after the capillaroscopic visit.</td>
</tr>
<tr>
<td>Sebastiani et al. [40]</td>
<td>—</td>
<td>Specificity of 81.4% and sensitivity of 92.98% in predicting development of new digital ulcers within 3 months after the capillaroscopic visit.</td>
</tr>
<tr>
<td>Smith et al. [41]</td>
<td>Early, active and late scleroderma patterns according to Cutolo et al. [10]</td>
<td>Odds that rise through early/active/late of developing severe peripheral vascular or lung disease within 18 or 24 months after the capillaroscopy visit.</td>
</tr>
</tbody>
</table>

Prospective associations between individual capillaroscopic quantified characteristics (quantitative assessment) or between qualitative scleroderma patterns (qualitative assessment [10]) and future clinical complications in SSc.

**Table 2** Association between angiogenic/angiostatic factors and qualitative scleroderma/quantitative patterns according to Cutolo et al. [10]

<table>
<thead>
<tr>
<th>Author</th>
<th>Comments</th>
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<tr>
<td>VEGF</td>
<td>Higher levels in scleroderma patterns vs healthy controls ($P &lt; 0.001$).</td>
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<tr>
<td>Distler et al. [46]</td>
<td>No association within scleroderma patterns ($P = 0.32$).</td>
</tr>
<tr>
<td>Avouac et al. [44]</td>
<td>Association between higher VEGF and late scleroderma pattern ($P = 0.0008$) within an SSc population.</td>
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<tr>
<td>Ang-2</td>
<td>Association with late vs early/active pattern ($P = 0.05$) within an SSc population.</td>
</tr>
<tr>
<td>Riccierei et al. [45]</td>
<td>Higher levels in the active and late scleroderma pattern vs healthy controls ($P = 0.003$).</td>
</tr>
<tr>
<td>ET-1</td>
<td>Association with capillary dimension ($P &lt; 0.05$) within an SSc population.</td>
</tr>
<tr>
<td>Sulli et al. [48]</td>
<td>Inverse association with presence of giants ($P \leq 0.02$) within an SSc population.</td>
</tr>
<tr>
<td>Kirm et al. [47]</td>
<td>Inverse association with the late scleroderma pattern ($P = 0.007$) within an SSc population.</td>
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<tr>
<td>Endostatin$^a$</td>
<td>Inverse association with presence of giants ($P \leq 0.02$) within an SSc population.</td>
</tr>
<tr>
<td>Distler et al. [46]</td>
<td>Inverse association with the late scleroderma pattern ($P = 0.007$) within an SSc population.</td>
</tr>
<tr>
<td>EPC$^b$ Avouac et al. [44]</td>
<td>Inverse association with the late scleroderma pattern ($P = 0.007$) within an SSc population.</td>
</tr>
</tbody>
</table>

EPC: endothelial progenitor cell. $^a$Angiostatic factors; $^b$impaired vasculogenesis.
Correlations between morphological analysis of microcirculation (capillaroscopy) and functional analysis of microcirculation

While NVC measures capillary morphology, methods such as laser Doppler imaging and thermography can be used to measure cutaneous blood vessel function, e.g. blood flow [11]. Notably, the signal arising from the aforementioned functional measurements of blood flow is from more than just the capillary bed. In this way thermography reports skin temperature, representative of underlying blood flow, with both muscle and skin perfusion believed to contribute to the signal. Laser Doppler measures not only superficial capillary blood flow but also the arterial and venous vessels of the superficial and mid-dermis [51].

This functional analysis has been used to discriminate PRP from SRP and has been linked to morphological analysis (capillaroscopy) within SSc populations.

In patients with RP, a study comparing various non-invasive methods for distinguishing patients with SRP due to SSc from those with PRP and healthy controls reported that laser Doppler imaging and thermography yielded correct classification rates of 72% and 74%, respectively, on the basis of blood vessel function [52].

However, NVC was found to be the superior discriminator with a rate of correct disease classification of 89% [52]. A combination of all three techniques increased the rate of correct classification from 89% to 94%, but did not improve the sensitivity.

Interestingly, also other, less common combinations, such as the combination of laser Doppler and photoplethysmography (an optical measurement technique), both assessing flow in the microvascular bed, may be useful in the characterization of PRP from SRP due to SSc [53].

In patients with SSc, a correlation between morphological evaluation and functional evaluation (at baseline and after application of a stimulus) has been established. In this way correlations between microangiopathy (as assessed by capillaroscopy) and blood flow (as assessed by laser Doppler, measuring blood flow over a certain point) both at baseline temperature and after heat stimulus \( R = \pm 0.5 \) and \( -0.7 \) have been described [54].

Moreover, SSc patients showing the late NVC pattern of microangiopathy have been shown to display significantly lower blood flow on laser Doppler flowmetry at baseline temperature and after heat stimulus than patients with active and early NVC patterns \( P = 0.05/0.03 \) and \( P = 0.07/0.05 \) [54]. This association has also been shown using a new laser device, laser speckle contrast imaging (LASCA), which measures perfusion distribution over an area [55, 56]. The topic deserves further study.

Actual status and progressive diffusion of capillaroscopy in rheumatology

For several decades, capillaroscopy had difficulties in becoming an accepted and widely used tool. There is recent growing interest for NVC as witnessed by the results of a recent survey in 32 EULAR countries in which the 170 participants selected capillaroscopy as the second most interesting tool (out of 14 tools) for their future learning [57, 58]. Training opportunities in learning NVC are stepping forward to this growing interest [59].

Up-to-date, NVC full-immersion training courses (held in Italy in 2004, 2006, 2008, 2010, 2011, 2012) have recently been provided by international faculties and supported by EULAR. In addition, from 2010, a study group with yearly meeting and dedicated to capillaroscopy and rheumatic diseases was started at the ACR. Furthermore, a new Atlas/Textbook on capillaroscopy has been published under the aegis of EULAR and the authorship of the most prominent world experts (http://www.eular.org/, search education/bookshelf).

Interestingly, in line with the growing interest, an expansion in articles on capillaroscopy can be seen throughout the years. A Medline search performed using the term capillaroscopy (retrieving a total of 831 articles available from 1950 to date) revealed only 53 articles published between 1950 and 1979 (30 years), 557 articles between 1980 and 2007 (28 years) and 221 articles between 2008 and 2012 (almost 4 years). Further, among 230 articles that focused on capillaroscopy and SSc from 1980 to 2012 (32 years), almost 94 articles (40%) were published between 2008 and 2012 (4 years). Further progression and optimization of the potentialities of NVC, linked to widespread training and education on its use, may reveal further applications in clinics and research. In conclusion, nailfold capillaroscopy may be considered today as a reliable diagnostic tool and putative biomarker in rheumatology.

Rheumatology key messages

- Capillaroscopy is paramount in the differential diagnosis between a PRP and an SRP.
- Capillaroscopy may be used to predict future clinical complications in SSc.
- Capillary changes that characterize the scleroderma pattern can be counted and quantified.

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References

2 Spencer-Green G. Outcomes in primary Raynaud phenomenon: a meta-analysis of the frequency, rates, and


