

Nailfold videocapillaroscopy for the early diagnosis of systemic sclerosis in Raynaud's phenomenon

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Raynaud's phenomenon is characterized by an exaggerated vasospastic response induced by cold or emotional stress. It can be primary (idiopathic) or secondary to a number of different conditions, including predominantly systemic sclerosis (SSc). Currently, the best technique available to study microvascular involvement in Raynaud's phenomenon is nailfold videocapillaroscopy (NVC). The actual and exclusive advantage of early NVC analysis is to distinguish between primary and secondary Raynaud's phenomenon and to allow the early detection of SSc. Recently, microvascular alterations, as detected by NVC in patients with SSc, have been reclassified into three different patterns: early, active and late. These large investigations have confirmed previous observations, indicating enlarged and giant capillaries, together with microhemorrhages and edema, as the earliest NVC finding in SSc (early pattern). The NVC patterns have been correlated with different clinical aspects and manifestations of SSc, as well as treatment results, contributing to the overall study of the disease.

Origin & development of capillaroscopy

Capillaroscopy originated from the magnifying glass observations of an Italian physician, Giovanni Rasori (1766–1837), who described the close relationship between conjunctive inflammation and the presence of an 'inextricable knot of capillary loops' [1]. Following this early description, at the beginning of the 20th century, Raynaud's phenomenon (RP) started to be differentiated into a primary (PRP) as well as a secondary (SRP) phenomenon to different diseases.

After further investigations at the beginning of the 20th century, which addressed interest to the observation of the periungual skin capillaries, Brown and O'Leary used capillaroscopic analysis to show, in detail, the abnormalities that characterize involvement of the microvasculature during RP in systemic sclerosis (SSc) [2]. Subsequently, capillaroscopy was neglected for some decades. However, in the second half of the century, interest was renewed and in 1973 Maricq and colleagues published the first paper in *Arthritis & Rheumatism* describing the specific capillaroscopic patterns in SSc, as well as the modification of capillary blood flow during cold exposure both in PRP and SRP [3].

Recently, Cutolo and colleagues reclassified three major nailfold videocapillaroscopic (NVC) patterns that are presently considered useful in assessing the appearance and progression of sclerodermic microangiopathy [4]. These three patterns include early, active and late characteristic pictures.

Hence, direct observation of the microvasculature with capillaroscopy is useful both for an early diagnosis of connective tissue diseases in the presence of RP (i.e., SSc) and for functional studies, in particular with several technical modifications and implementations achieved throughout the past century.

Raynaud's phenomenon

In 1862, Maurice Raynaud described the phenomenon that now bears his name. His original observations stated that 'local asphyxia of the extremities' was a result of 'increased irritability of the central parts of the cord presiding over vascular innervation' [5]. Following this first observation, Lewis suggested in 1929 that the cause of the phenomenon was not central but peripheral. This was due to 'spasm of the digital arteries' and 'the abnormal element in the reaction to cold is a direct reaction and due to a peculiar condition of the vessel wall locally: it is not the result of a reflex through the vasomotor nerves' [6,7]. However, despite evidence suggesting that peripheral mechanisms are primarily responsible in most patients, it seems likely that central mechanisms also contribute, especially as the phenomenon may also involve internal organs [7].

RP can be primary (idiopathic) or secondary to a number of different conditions, including not only rheumatological diseases but also a variety of other causes, including extrinsic vascular obstruction, as in thoracic outlet syndrome and certain drugs or chemicals (e.g., β -blockers and polyvinyl chloride) [8].

Keywords: capillaroscopy, EULAR, EUSTAR, microvessels, Raynaud's phenomenon, rheumatic diseases, scleroderma pattern, systemic sclerosis, videocapillaroscopy

future
medicine

The pathogenesis and pathophysiology of RP seems to be modulated by these different conditions. However, it has been suggested that the pathogenesis of RP, especially when secondary to SSC, can be explained mainly on the basis of dysregulated neuroendothelial control mechanisms [9]. The key issue is the loss of regular vascular tone with imbalance between vasoconstriction and vasodilation (in favor of vasoconstriction) [9] (Figure 1). Several lines of evidence point to abnormalities in the blood vessel wall (including the endothelium and smooth muscle), in the neural control of vascular tone and in circulating mediators, including those produced as a result of platelet activation and vascular oxidative stress [10]. According to population-based surveys of various ethnic groups, the prevalence of RP is approximately 3–5%, and geographic variations in prevalence reflect differences in climate [11,12].

From a meta-analysis, it has been calculated that, in patients with presumed PRP, a secondary disorder developed in 12.6%, all but one of whom had a connective tissue disease [13].

Conversely, approximately 15–20% of patients with RP who have autoantibodies, abnormalities of nailfold capillaries or both, and who do not initially meet the criteria for a well defined connective tissue disease, will ultimately develop such a disease within 2 years [13].

As RP is characterized by an exaggerated vasospastic response induced by cold or emotional stress, the digits generally turn white (ischemia), then blue (deoxygenation) and finally red (reperfusion) (Figure 2).

Clinical criteria have been suggested to distinguish between patients with uncomplicated, or PRP, from those with disease-associated SRP [14,15].

Figure 1. Mechanisms contributing to altered vasoconstriction or vasodilation in Raynaud's phenomenon.

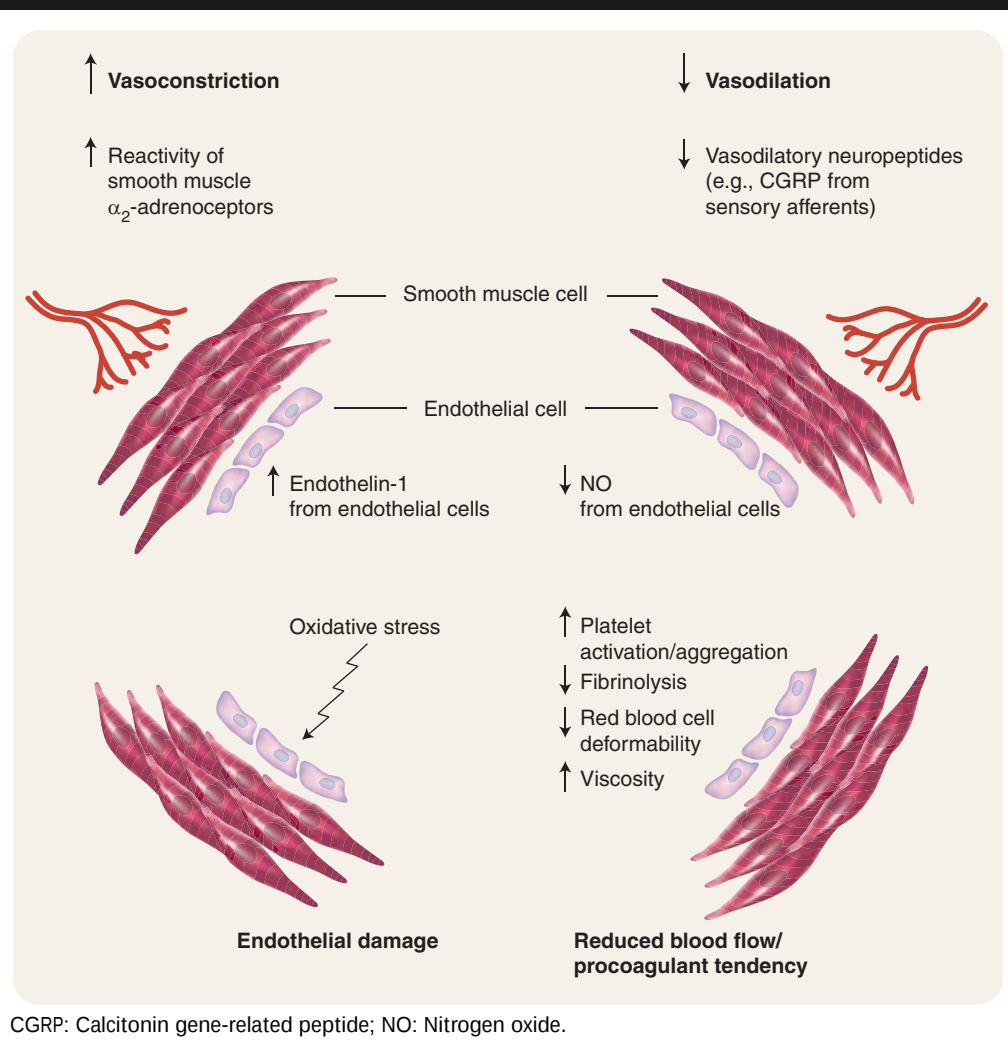


Figure 2. Vasospastic response to Raynaud's phenomenon.



Raynaud's phenomenon is characterized by an exaggerated vasospastic response induced by cold or emotional stress. Generally, the digits turn white (ischemia), then blue (deoxygenation) and finally red (reperfusion) (personal images).

Suggested criteria for PRP include:

- Symmetric attacks
- The absence of tissue necrosis
- Ulceration or gangrene
- The absence of a secondary cause (based on medical history and physical examination of the patient)
- A negative test for antinuclear antibodies
- A normal erythrocyte sedimentation rate
- The presence of normal nailfold capillaries

The mean age of onset of PRP is 14 years, with 27% of cases beginning at the age of 40 years or later [16]. However, SRP is characterized by different findings including [17]:

- Age at onset of greater than 30 years
- Episodes that appear intense, symmetric, painful and/or associated with ischemic skin lesions
- Clinical aspects suggestive of a connective tissue disease
- Presence of specific autoantibodies
- Evidence of microvascular alterations as assessed by microscopy of nailfold capillaries

The best technique currently available to study microvascular involvement in RP is still nailfold capillaroscopy (NC) [18].

Figure 3. Videocapillaroscopic analysis of the nailfold.



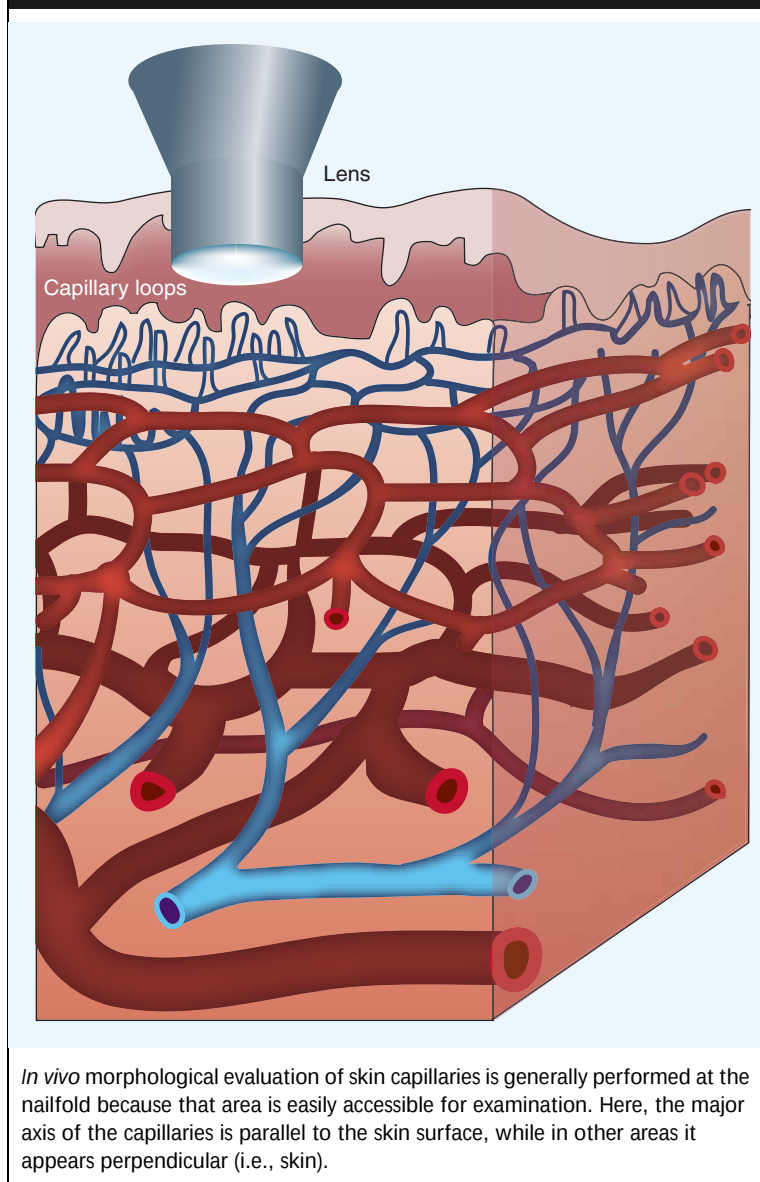
The videocapillaroscopic analysis of the nailfold is, at the present time, considered to be the most sophisticated method and might also detect the blood flow at the level of the microvessels. In addition, nailfold videocapillaroscopy is connected to efficient software that allows the computerized processing of the images and the storage of the data (Capillaroscopic Unit, University of Genova).

Capillaroscopic analysis of the nailfold vascular bed

NC has an impressive cost-effectiveness ratio: it is simple, noninvasive and relatively inexpensive [19]. In clinical practice, the skin capillaries are generally observed just through an incident light microscope. However, NC can be performed using a series of instruments, including the ophthalmoscope, the stereomicroscope, photomacrography and more recently, videocapillaroscopic systems.

Videocapillaroscopic analysis of the nailfold (NVC) is considered the most sophisticated of these methods and might also enable the detection of the blood flow at the microvessel level. In addition, NVC is connected to efficient software that allows the computerized processing of the images and data storage (Figure 3) [20–23]. Recently however, handheld dermatoscope analysis of the nailfold showed interesting results that were comparable with those described by other, more sophisticated instruments [24].

In vivo morphological evaluation of skin capillaries is generally performed at the nailfold as that area is easily accessible for examination

Figure 4. *In vivo* morphological evaluation of skin capillaries.

and the major axis of the capillaries is parallel to the skin surface, while in other areas it appears in a perpendicular state (Figures 4 and 5). Other areas, such as the labial mucosa, have also been examined in SSc [25]. The subject under analysis must be kept inside the room for a minimum of 15 mins, before the nailfold analysis can be performed in order to adapt to a room temperature of 20–22 °C. A drop of immersion oil is placed on the nailfold bed to improve the lens contact and image resolution (Figure 6). The nailfolds of all ten fingers should be examined every time in each patient (Figure 7). Image magnifications of 20–1000x are available.

Fingers affected by recent local trauma are not analyzed since false positives for microvascular abnormalities might arise. Preferably, the operator should perform the NVC examination in a blinded fashion, (i.e., without knowledge of the patient's clinical diagnosis and/or disease severity).

As a general rule, all fingers should be evaluated; however, the most accurate morphological assessments are commonly performed at the fourth and fifth fingers, owing to the greater transparency of the skin at these levels. The following major abnormal vascular parameters are considered according to previous classifications (Figure 8) [1]:

- Presence of enlarged and giant capillaries
- Hemorrhages
- Edema
- Loss of capillaries
- Disorganization of the vascular array

NVC parameters to distinguish primary from secondary RP

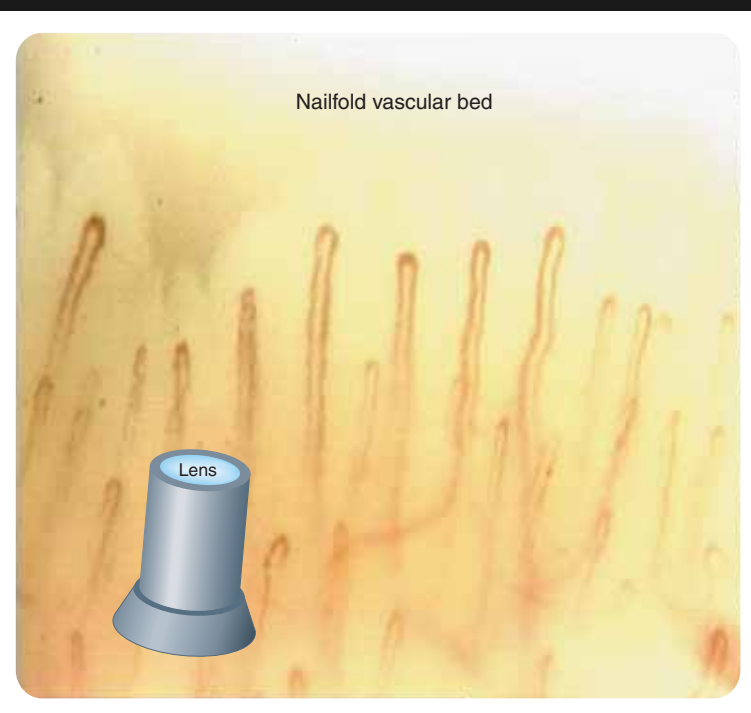
The actual and exclusive advantage of the early NVC analysis is to distinguish between PRP and SRP, and to allow the early detection of SSc.

In normal conditions, or in PRP (excluding during the cold exposure test), the normal nailfold capillaroscopic pattern shows a regular disposition of the capillary loops along the nailbed (Figure 5). However, in subjects suffering from SRP, one or more of the following capillaroscopic findings should alert the physician to the possibility of a connective tissue disease not yet detected.

Giant capillaries

The presence of homogeneously and/or irregularly enlarged microvascular loops represents one of the earliest and most striking features of SRP (Figure 8). These enlargements show a peculiar shape that differentiates them with respect to those observed in other pathological conditions, such as diabetes mellitus and acrocyanosis. Capillaries with a normal shape and diameter might coexist, in most instances, together with giant loops. Even the detection of a single loop over ten fingers, with a circumscribed or homogeneous diameter greater than 50 micron, should be considered as a potential marker of microangiopathy related to an early scleroderma spectrum disorder. It has been suggested that microvascular dilation (giant capillaries) represents a local autoregulatory

Figure 5. Picture of a normal nailfold vascular bed as analyzed by nailfold videocapillaroscopy (magnification 200x).



response to tissue hypoxia [26]. In a recent study, giant capillaries have been found in 100% of SSc patients [7].

Local microhemorrhages

Local microhemorrhages are also associated with the early vascular involvement in SRP and

represent a clear sign of microvascular damage. Local trauma at the fingers must be excluded as a possible cause of microhemorrhages (Figure 8).

Edema

The presence of edema at the level of the dermal papillae is observed frequently in SSc patterns (mainly in the active NVC pattern).

Angiogenesis

Various morphological features of capillary neoformation may be observed in patients with SRP [27,29]. Highly tortuous and arborized capillary loop clusters, often surrounded by dropout of normal capillary loops, are a characteristic feature of angiogenesis [24,29].

The main morphological hallmark of angiogenesis is the clustering of tortuous capillaries with pronounced shape heterogeneity, including thin or large meandering and bushy capillaries (ramified capillaries). Moreover, coiled capillaries are the morphological hallmark of angiogenesis in the elongated papillae of psoriatic plaque [30].

Loss of capillaries and/or avascular areas

A decreased number of loops (< 30 over 5 mm in the distal row of the nailfold) should be considered highly specific for SRP, especially in the advanced phases [31]. Loss of capillaries may result in critical tissue hypoxia. In fact, it has been estimated that the number of normal capillaries might be reduced to only 20% in SSc patients [32]. The loss of capillaries, when extensive, may generate large avascular areas with a 'desert-like' appearance of the nailbed. Even in patients with recent onset of RP, the appearance of rapidly progressive capillary loss might represent the first dramatic capillaroscopic evidence of severe SSc. Progressive loss of capillaries has been associated with more extensive skin involvement and with a poor prognosis [33].

Architectural derangement of the nailfold microvascular network

Generally, architectural arrangement of the skin in the microvascular bed is remarkably regular in healthy subjects, even with intersubject differences (Figure 5). However, the last row of nailfold capillaries are characterized by a uniform distribution, shape and diameter and most of them show a hairpin or U-shaped aspect. An evident modification of the normal architectural arrangement represents an early morphological feature in SSc as a consequence of the previously discussed mechanisms

Figure 6. Subject analysis prior to nailfold videocapillaroscopy.



A drop of immersion oil is placed on the nailfold bed to improve the lens contact and image resolution. The patient is at room temperature for at least 15 min prior to nailfold videocapillaroscopy.

Figure 7. Nailfold videocapillaroscopy examination.

Preferably, the operator should perform the nailfold videocapillaroscopy examination in a blind manner (i.e., without knowledge of the patient's clinical diagnosis and/or disease severity). As a general rule, all fingers should be evaluated, however, the most accurate morphological assessments are commonly performed at the fourth and fifth fingers because of the greater transparency of the skin at these levels.

(Figure 8). However, in patients with recent and aggressive SRP onset, these changes may be expressed on some fingers, unilaterally or on a single finger.

Architectural disorganization, enlarged loops, loss of capillaries, hemorrhages, angiogenesis and avascular areas characterize more than 95% of patients with overt SSc. Therefore, the term 'scleroderma pattern' includes all of these sequential capillaroscopic changes typical of the microvascular involvement in SSc, and represents an important diagnostic criterion pattern [34].

The scleroderma pattern: the reference pattern

The detection of scleroderma patterns, in particular the early scleroderma pattern, is essential for an early differential diagnosis between PRP and SRP (i.e., SSc).

In fact, peripheral microvascular damage in SSc is characterized mainly by increasing structural alterations of the capillaries with progressive decrease of their density. Blood flow is also altered, with flow slowing on average and increased periods of stasis [35,36].

Early in the disease, the peripheral microangiopathy may well be effectively recognized and studied with NVC, a safe technique that is

well reported to have both diagnostic and prognostic value in the presence of RP [37–40].

Older studies have partially graded the morphological aspects of the vascular damage in patients with SSc, as assessed by nailfold capillaroscopy. Two major patterns within the term scleroderma pattern have been recognized from the beginning: namely the active and slow patterns [37]. More recently, attempts have been made to rate the capillary abnormalities in relation to selected characteristics of disease progression, in order to improve the diagnostic and prognostic power of the capillaroscopic analysis [41,42].

In a more recent study microvascular alterations, as detected by NVC in patients with SSc, have been reclassified in three different patterns [4]. The patterns identified within the 'scleroderma pattern' include (Figure 9):

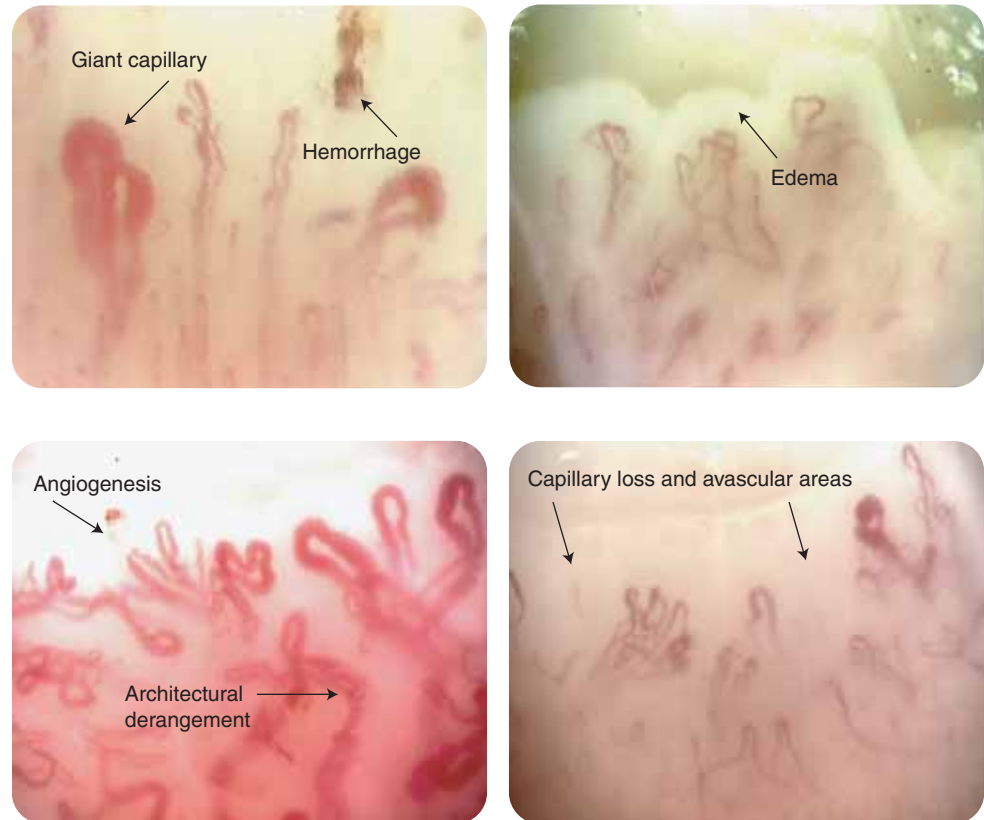
- Early NVC pattern: few giant capillaries (at least one), few capillary hemorrhages, relatively well preserved capillary distribution, no evident loss of capillaries
- Active NVC pattern: frequent giant capillaries, frequent capillary hemorrhages and edema, moderate loss of capillaries, mild disorganization of the capillary architecture, absent or mild ramified capillaries
- Late NVC pattern: irregular enlargement of the capillaries, few or absent giant capillaries and hemorrhages, severe loss of capillaries with extensive avascular areas, disorganization of the normal capillary array, ramified/bushy capillaries

This study confirmed previous observations indicating enlarged and giant capillaries, together with hemorrhages, as the earliest NVC findings in SSc [32,36]. In the late stage of the disease these abnormalities become rare.

However, as already reported by other authors, the early stage is also characterized by microvessels with a normal diameter coexisting with few enlarged capillaries. These must be investigated carefully on all fingers by considering the limited number of these nailfold changes during the early phases of the disease.

On the contrary, these changes are strongly increased in SSc patients with an active pattern. Loss of capillaries, together with vascular architectural disorganization and ramified capillaries, were found to be rare in the early stages of SSc, whereas they seem to increase with the progression of the fibrotic phase of the disease (active and late patterns).

Figure 8. Major abnormal vascular parameters.



The following major abnormal vascular parameters are considered during nailfold videocapillaroscopy (NVC) analysis: presence of enlarged and giant capillaries, hemorrhages, edema, loss of capillaries and /or avascular areas, disorganization of the vascular array (magnification 200x).

A significant and gradual increase of these latter vascular abnormalities is observed during SSc progression, and the three NVC patterns have been found to correlate with both RP and SSc duration, autoantibody expression and serum markers of inflammation, reflecting at least the possible evolution of the disease process [43,44]. An increased vascular permeability and a reduced blood flow are observed in all NVC groups, confirming previous studies [45].

Furthermore, NVC allows, particularly in SSc, the observation of the prolonged phases of reduced or ceased capillary perfusion as a result of cold-induced peripheral vasospasm.

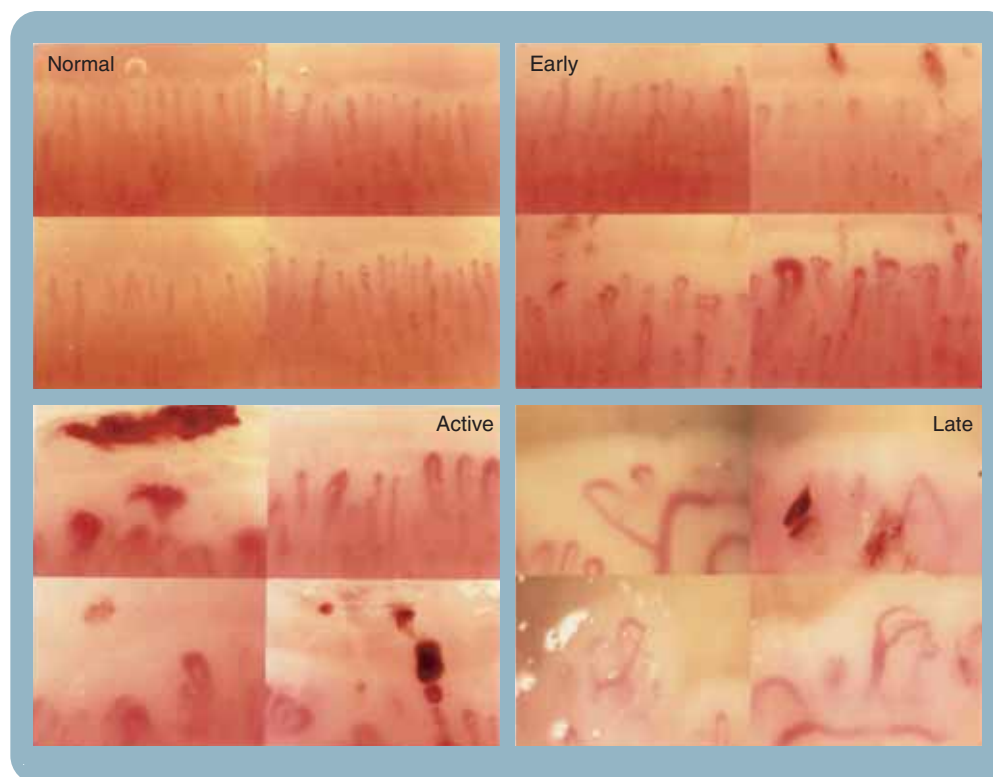
The NVC patterns have been correlated with different clinical aspects and manifestations of SSc, and with the effects of treatment, contributing to the overall study of the disease [46–50]. Finally, a quantitation (computerized) of the microvascular damage is now also available during NVC analysis [4,51].

Conclusions

Today NVC represents the best tool for the early diagnosis of scleroderma and for the micromorphological follow-up of the disease. The early NVC pattern is helping in the early differentiation between PRP and SRP. Correlations between the NVC patterns and biological markers of the disease (i.e., plasma endothelin levels) will help in better understanding the pathophysiology of scleroderma, as well as the recognition of its severity [52].

Future perspective

RP is one the most common and significant clinical conditions suggesting immediate capillaroscopic analysis. Since the actual and select advantage of the early NVC analysis is to distinguish between PRP and SRP and to allow the early detection of SSc, the most correct therapeutic approach should be started as soon as possible.

Figure 9. Nailfold videocapillaroscopy patterns within the scleroderma pattern.

The nailfold videocapillaroscopy (NVC) patterns identified within the scleroderma pattern include:

1. Early NVC pattern: few enlarged/giant capillaries, few capillary hemorrhages, relatively well preserved capillary distribution, no evident loss of capillaries; **2. Active NVC pattern:** frequent giant capillaries, frequent capillary hemorrhages and edema, moderate loss of capillaries, mild disorganization of the capillary architecture, absent or mild ramified capillaries; **3. Late NVC pattern:** irregular enlargement of the capillaries, few or absent giant capillaries and hemorrhages, severe loss of capillaries with extensive avascular areas, disorganization of the normal capillary array, ramified/bushy capillaries (magnification 200x).

By considering the reliability of the scleroderma patterns, NVC analysis must be introduced among the diagnostic criteria of SSc. Architectural disorganization, hemorrhages, enlarged loops, loss of capillaries, angiogenesis and avascular areas characterize more than 95% of patients with SSc.

The three NCV patterns (early, active, late) need further computerized quantitation of their expression in order to be as reliable as possible during the follow-up of the patient. The NVC patterns have been correlated with different clinical aspects and manifestations of SSc, thus further relationships should be investigated. Incoming practical instruments integrating the NVC and the Doppler analysis will allow the simultaneous evaluation of both the microvessel structure and blood flow. The noninvasiveness, simplicity and cheapness

of NVC must support its diffuse use at least to all rheumatological units. The EULAR study group EUSTAR is working actively in such a direction.

In September 2004, the First European Course on Capillaroscopy and Rheumatic Diseases was organized in Genova, supported by EULAR. The next course is planned in 2006. During the last three EULAR meetings (Lisbon 2003, Berlin 2004, Vienna 2005), six fully attended workshops have presented the basic and applied aspects of capillaroscopy in rheumatology. Two further workshops are planned in EULAR (Amsterdam 2006).

Acknowledgements

The authors would like to thank Carmen Pizzorni, Maria Elena Secchi, Alessandro Calvia and Alberto Sulli for their contribution during the clinical studies.

Executive summary

Raynaud's phenomenon

- Raynaud's phenomenon (RP) is caused primarily by peripheral mechanisms. However, it seems likely that central mechanisms also contribute, especially as the phenomenon may also involve internal organs.
- RP can be primary (idiopathic) or secondary to a number of different conditions, including not only rheumatological diseases but also a variety of other causes, including extrinsic vascular obstruction.
- As RP is characterized by an exaggerated vasospastic response induced by cold or emotional stress, the digits generally turn white (ischemia), then blue (deoxygenation) and finally red (reperfusion).

Capillaroscopic analysis of the nailfold vascular bed

- Videocapillaroscopic analysis of the nailfold (NVC) is considered the most sophisticated of the nailfold capillaroscopy methods, and might also enable the detection of the blood flow at the microvessel level.
- *In vivo* morphological evaluation of skin capillaries is generally performed at the nailfold, as that area is easily accessible for examination and the major axis of the capillaries is parallel to the skin surface, while in other areas it appears in a perpendicular state.
- All fingers should be evaluated; however, the most accurate morphological assessments are commonly performed at the fourth and fifth fingers, owing to the greater transparency of the skin at these levels.

Nailfold videocapillaroscopic parameters to distinguish primary from secondary RP

- In normal conditions, the nailfold capillaroscopic pattern shows a regular disposition of the capillary loops along the nailbed.
- In subjects suffering from secondary RP, one or more of the following capillaroscopic findings should alert the physician to the possibility of a connective tissue disease not yet detected: giant capillaries; local microhemorrhages; edema; angiogenesis; loss of capillaries and/or avascular areas; architectural derangement of the nailfold microvascular network.

The scleroderma pattern: the reference pattern

- Peripheral microvascular damage in systemic sclerosis (SSc) is characterized mainly by increasing structural alterations of the capillaries with progressive decrease of their density. Blood flow is also altered, with flow slowing on average and increased periods of stasis.
- Microvascular alterations, as detected by NVC in patients with SSc, have been reclassified in three different patterns: early, active and late.

Conclusions

- NVC represents the best tool for the early diagnosis of scleroderma and for the micromorphological follow-up of the disease. Correlations between the NVC patterns and biological markers of the disease will help in understanding the pathophysiology of scleroderma better, as well as the recognition of its severity.

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