Capillaroscopy in 2016: new perspectives in systemic sclerosis

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ACTA REUMATOL PORT. 2016;41:8-14

ABSTRACT

Systemic sclerosis (SSc) is an autoimmune disorder of unknown etiology characterized by early impairment of the microvascular system. Nailfold microangiopathy and decreased peripheral blood perfusion are typical clinical aspects of SSc. The best method to evaluate vascular injury is nailfold videocapillaroscopy, which detects peripheral capillary morphology, and classifies and scores the abnormalities into different patterns of microangiopathy. Microangiopathy appears to be the best evaluable predictor of the disease development and has been observed to precede the other symptoms by many years. Peripheral blood perfusion is also impaired in SSc, and there are different methods to assess it: laser Doppler and laser speckle techniques, thermography and other emerging techniques.

Keywords: Capillaries; Systemic sclerosis; Raynaud's phenomenon; Scleroderma

INTRODUCTION

Several rheumatic diseases are characterized by structural and functional alterations of microcirculation, with important clinical implications from the beginning up to the progression[1,2]. Microvascular damage and dysfunction represent the earliest morphological and functional markers of systemic sclerosis (SSc). The vascular changes represent an early event in SSc and Raynaud's phenomenon (RP), observed in over 90% of patients, constitutes the clinical expression of altered blood flow regulation due to microvascular damage[1,2]. Microangiopathy can be easily studied by nailfold videocapillaroscopy (NVC), which is the best method to assess the early differentiation between primary and secondary Raynaud phenomenon and to classify the proper pattern of microvascular damage ("Early", "Active", or "Late" pattern), and to calculate the microangiopathy evolution score (MES)[3,4]. Since 2013 the NVC patterns are included in the European League Against Rheumatism and American College of Rheumatology criteria of SSc[5]. In standard conditions NVC, which evaluates capillary morphology, cannot measure blood perfusion[6]. However, the assessment of blood perfusion in SSc may be performed by different laser techniques: laser Doppler flowmetry (LDF), laser doppler imaging (LDI), laser speckle contrast imaging (LSCI) and laser speckle contrast analysis (LASCA)[7-10]. The use of NVC together with laser techniques, all safe and non-invasive tools, allows the morphological and functional assessment of the peripheral microvasculature that is a must for diagnosis, prognosis and therapy in SSc patients.

The aim of this literature review is an overview of the different morphological and functional techniques, that allow to study and follow the microvascular damage.

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NAILFOLD VIDEOCAPILLAROSCOPY

Nailfold videocapillaroscopy (NVC) is a safe and non-invasive technique to assess the morphology of nailfold dermal capillaries using a magnification system (lenses magnification ×200). Videocapillaroscopy consists of the combination of an optical microscope with a digital video camera. The patient undergoing the exam must initially remain seated in an acclimatized room for 15-20 minutes, with a temperature set around 22-

-23° C. For a better visualization of the capillaries and the refractive defect reduction, a drop of immersion (cedar) oil is placed on the cuticle of the fingers to be evaluated. The periungual region of eight out of ten fingers (excluding the thumb, because of a lowest transparency due to the nail width and the absence of distal phalanx) should be examined. In this region, the distal row of capillary loops protrudes into the dermal papillae, allowing a longitudinal view of the capillary segments (afferent, efferent and transition), as arranged in a direction parallel to the skin surface[1,11].

Through NVC qualitative assessment a normal capillary pattern can be distinguished from an abnormal pattern. Important capillaroscopic parameters are: skin transparency, visibility and morphology of subpapillary venous plexus, number of capillaries inside each dermic papilla, disposition of the capillaries in the nailfold bed, capillary morphology (regular hairpin-shaped, tortuousities, dystrophies, ectasias, giant capillaries), occurrence of ramifications, presence of hemorrhages or hemosiderin deposits, and capillary density[11,12].

Capillary density is considered normal if the number of capillaries exceeds 9 per linear millimetre (average range: 9-12 capillaries per mm). The number of capillaries could be slightly decreased in the early phase of SSc, while the considerable and progressive capillary desertification is a diagnostic feature of advanced SSc microangiopathy[2,13].

In their normal disposition capillaries are parallel to each other, in number of one (rarely two) per dermal papilla, and perpendicularly oriented to digital surface. The normal capillaroscopic pattern, by qualitative assessment, is characterized by a homogeneous distribution of hairpin-shaped capillaries as a "comb-like structure".

The capillary is defined as ectasic when it shows a localized dilation ≥ 20 μm (but < 50 μm); if the dilation has an homogeneous aspect (involving both afferent and efferent branches), exceeding 50 μm, the vessel is defined as a giant capillary. In a recent study, our group demonstrated that abnormal dilations of capillary diameter (>30micrometer) at the level of arterial and/or venous branches, were significantly expressed in subjects diagnosed with primary Raynaud phenomenon (PRP) subjects that would later develop secondary Raynaud phenomenon (SRP), associated with SSc. Furthermore, over at least 3.56 years of follow up, the progressive presence of abnormal capillary dilations in PRP subjects, should be considered as a possible very early NVC signal of transition to the "early" scleroderma pattern[14].

Hemosiderin deposits (microhemorrhages) are associated with early vascular damage, as well as their presence represents a transition phase between giant capillaries and the subsequent loss of capillaries. On the other hand, the modification of the normal architectural arrangement represents an early morphological feature in SSc and other connective tissue diseases of the scleroderma-spectrum disorders[2,11,12]. Although typical of microvasculopathy, the microhemorrhages, are not specific of the scleroderma spectrum diseases and can be found in other connective tissue diseases, such as SLE or antiphospholipid syndrome[1,11].

Either in normal conditions or in PRP the nailfold capillaroscopic pattern shows regular disposition of capillary loops along the nailfold bed and no abnormal enlargements or capillary loss[1,12]. In patients with RP, however, one or more abnormal capillaroscopic findings should alert the physician to the possibility of SRP, owing to the presence of a previously undetected connective autoimmune disease[1,11].

In healthy subjects the NVC pattern is characterized by: normal skin transparency, morphology of the capillary to "U" or "hairpin shape", morphological/ /structural homogeneity, 10-12 capillaries/mm, one capillary/dermal papilla, diameters of capillary branches <20 μm and lack of morphological atypia. Furthermore, in healthy subjects three different capillary morphologies may be observed. The first category is stereotype hairpin shape which is present in 50% of healthy subjects; another category is called "nonspecific variations" which are tortuosities that occur in 40% of controls; the third category represents crossing capillaries which are present in 8% of control. This variations could be influenced by job, trauma, age, onychophagy, manicures, exposure to chemical substances and individual physiological variability[1,2,11,12,15].

In PRP nailfold capillaries are frequently normal, but it is possible to observe capillaries with efferent branch enlargement or tortuous capillaries.

Conversely, SRP is characterized by the morphological signs that represent microvascular damage, these include giant capillaries, microhaemorrhages, capillary loss, the presence of avascular areas and angiogenesis. These sequential capillaroscopic changes are typical of the microvascular involvement observed in more than 95% of SSc patients and described by the term 'scleroderma-pattern[1,2,11].

NVC technique identifies morphological patterns

specific to various stages of SSc ('Early', 'Active' and 'Late' patterns)[3,4,11]. The 'Early' SSc pattern is characterized by few enlarged and giant capillaries, few capillary microhaemorrhages, no evident capillary loss and a relatively well preserved capillary distribution. The 'Active' SSc pattern, a marker of disease progression, is characterized by frequent giant capillaries, frequent (more than 6 per millimetre) capillary microhaemorrhages, moderate (20-30%) capillary loss, absent or mild ramified capillaries and a mild disorganization of the capillary architecture. In the 'Late' SSc pattern, although giant capillaries and microhaemorrhages are almost absent, there is irregular enlargement of the capillaries, severe (>50%) capillary loss with evident extensive avascular areas, ramified or bushy capillaries and a severe disorganization of the capillary array[3,4,11] (Figure 1).

The 'scleroderma-like' pattern is defined as a capillary pattern showing mixed microvascular markers of the scleroderma capillary patterns, but not fully fitting the definition for the single 'Early', 'Active' and 'Late' scleroderma pattern[3,4,11]. It may be found in those clinical conditions included into the scleroderma-spectrum diseases category, which is defined as having either an 'Early', 'Active' and 'Late' scleroderma pattern or a "scleroderma-like" pattern of nailfold microangiopathy[3,4,11]. The clinical conditions other than SSc that may present the scleroderma pattern of nailfold microangiopathy are mainly mixed connective tissue disease and dermatomyositis; however, undifferentiated connective tissue diseases and lupus erythematosus may display a such pattern in limited cases[1,2,11]. As matter of fact, specific capillaroscopic patterns are present only in systemic sclerosis.

NVC is also used to make a quantitative assessment (i.e. quantify certain characteristics and make semi-quantitative scoring) of the microvascular damage. The usual capillaroscopic parameters can been evaluated by a semi-quantitative scale, consisting of diagnostic parameters (irregularly enlarged capillaries, giant capillaries, micro-haemorrhages) and progression parameters (reduced capillary number, capillary ramifications and capillary architectural disorganization). Score 0-3 is adopted for all these parameters (score 0: no changes; score 1: <33% of capillary changes; score 2: 33% to 66% of capillary changes; score 3: >66% of capillary changes). The mean score value for each capillaroscopic parameter is calculated from the analysis of at least two linear millimetres in the middle area of the nailfold bed, in each finger; the score values from the eight fingers are added together, and the final value divided for eight fingers. The resulting value represents the score for each capillaroscopic parameter analyzed. An abnormal capillaroscopic finding should be

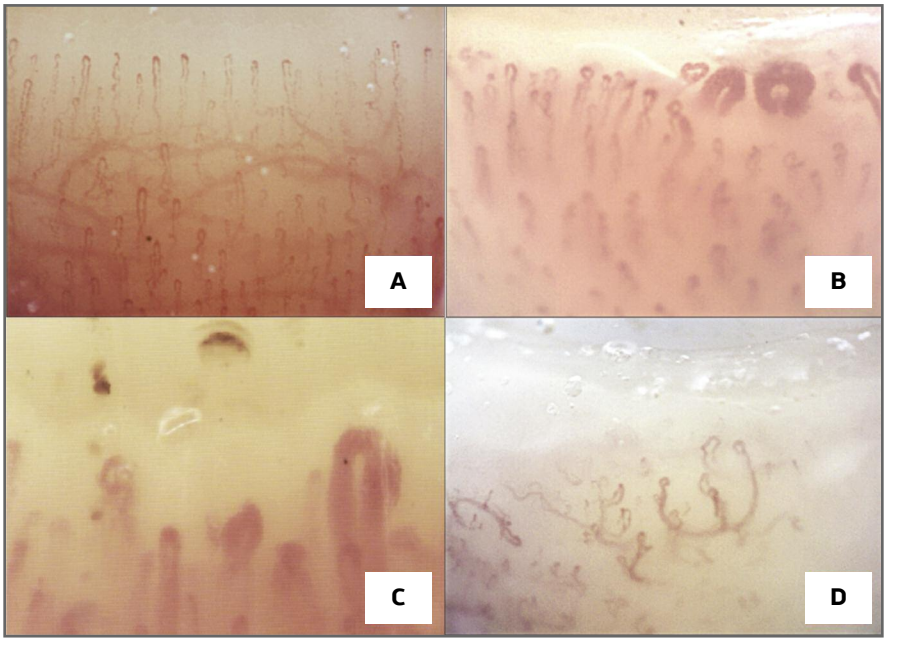

FIGURE 1. Nailfold videocapillaroscopy images (x200) in healthy subject (A), 'Early' (B), 'Active' (C)and 'Late' (D) patterns of scleroderma microangiopathy. Academic Division of Clinical Rheumatology – Genova, operator PC

considered significant if it is observed in at least two fingers of the subject, but in the case of giant capillaries their presence is highly predictive of a scleroderma-spectrum disease, even if detected in only one finger[3,4,11]. In this last case a short follow-up is recommended[2].

The "microangiopathy evolution score" (MES, sum of scores of progression parameters; score 0-9) is used to assess the vascular damage progression, as the scores of its parameters were demonstrated to significantly increase during the evolution of the SSc microangiopathy[3,4].

CLINICAL APPLICATIONS OF CAPILLAROSCOPY IN SYSTEMIC SCLEROSIS

NVC represents the best and validated method to assess the vascular damage in SSc.

For this reason, abnormal capillaroscopy is included among the parameters of the 2013 ACR/EULAR classification criteria, the 2001 LeRoy criteria for the classification of early systemic sclerosis and the 2013 VEDOSS criteria for very early diagnosis of SSc[5,16,17].

NVC analysis allows the detection of microvascular markers of severity and progression in SSc, such as reduced capillary density, which has been associated with a high risk of developing digital ulcers and pulmonary arterial hypertension[13,14].

The utility of NVC to predict digital ulcers (DU) is presented in the CAP study. CAP study is the first largest (500 patients), multicenter (70 centers), observational, clinical study with the aim of identifying NVC variables and clinical characteristics which predict the occurrence of new DU in SSc patients. The preliminary results of CAP study have been recently presented, and the study showed that the strongest predictors for new DU in SSc patients with DU history were NVC variables reflecting SSc microvasculopathy (mainly capillary number) and the number of DU at enrolment. The study showed that NVC imaging and assessment are feasible in multicentre trials[18].

NVC may be used also to monitor the effects of therapies in SSc treatment[19,20].

Treatments proven *in vitro* to interfere with endothelial cell functions and/or influencing mediators of the endothelial to mesenchymal cell transition (EndoMT), such as endothelin-receptor antagonists (ERA) that are involved in the fibrotic process, were found effective in preventing new DU in scleroderma, as well as to interfere with SSc microangiopathy progression[21,22]. The long term treatment with ERA in combination with a vasodilator (iloprost) of SSc patients affected by DU, was found to interfere with the evolution of the microvascular damage after at least one year, as assessed by both NVC and peripheral blood flow analysis[22,23].

Furthermore, immune-suppression with cyclophosphamide was demonstrated, by NVC, to improve the SSc nailfold microangiopathy[24,25].

CAPILLAROSCOPY AND ANALYSIS OF PERIPHERAL BLOOD FLOW

Capillary blood flow/perfusion cannot be quantitatively measured by NVC in standard conditions, as only a qualitative evaluation may be performed. Blood flow may be assessed by NVC only as regular, granulous, or stasis[6].

The real assessment and quantification of cutaneous blood perfusion in SSc may be performed by different laser techniques (see below)[7]. Also, thermography was employed to assess skin blood flow, and emerging technologies (e.g. optical Doppler tomography and spectroscopy) are under evaluation[7,26].

DIFFERENT LASER TECHNIQUES TO ANALYSE THE PERIPHERAL BLOOD FLOW

The different laser techniques most commonly used to assess vascular impairment in SSc are: laser Doppler flowmetry (LDF), that assesses and quantifies the blood perfusion at a single skin point (one mm^3); laser Doppler imaging (LDI), that measures blood flow of an area; laser speckle contrast imaging (LSCI), that quickly measures blood flow of an area (the contrast is calculated based on one pixel in a time sequence); laser speckle contrast analysis (LASCA), that quickly quantifies the blood flow of an area (the contrast is calculated based on multiple pixels in one image), allowing analysis of specific areas in a second time[7,8,26,27].

CAPILLAROSCOPY AND LASER DOPPLER

LDF is a non-invasive and user-friendly method, which provides an index of skin perfusion by measuring the Doppler shift induced by coherent light scattering

caused by moving red blood cells. LDF evaluates microvascular flow in perfusion units (PU) at a single skin point[7]. With LDF it is possible to assess the basal finger temperature (usually at the level of fingertips from 2nd to the 5th digit on both hands), and the capillary dilation capacity after having heated the probe to 36°C[23].

Some studies have demonstrated that SSc patients have a lower blood flow than both healthy subjects and primary RP patients and that patients with the 'Late' SSc pattern of microangiopathy on NVC had a lower blood flow at LDF than patients with 'Active' and 'Early' NVC patterns[23]. SSc patients have also an abnormal microvascular regulatory response to heat stimulation[23].

Cutolo *et al.,* in two studies have demonstrated that LDF is also efficacious for the evaluation of the variation of peripheral blood perfusion during treatment with vasoactive drugs within a few days or even over a long follow-up period of years[22,23]. In SSc patients, the correlation between blood perfusion, evaluated by LDF, and dermal thickness, measured by high frequency ultrasound, was also demonstrated at the finger level[9].

One disadvantage of this technique is the large site--to-site variation, which limits its efficacy in comparing blood flows between sites and in monitoring change over time[22,23].

Laser Doppler imaging (LDI), which evaluates blood flow over a skin area might overcome this problem[7,27]. In their article Murray *et al* demonstrated that NVC, LDI, and thermal imaging each independently provide good discrimination between patients with SSc and those with primary RP and healthy controls[7].

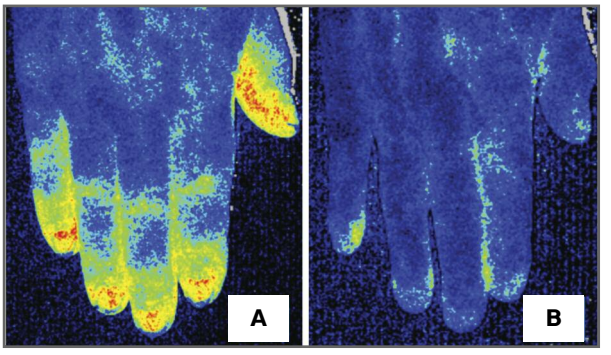

FIGURE 2. Laser speckle contrast analysis images of the hand dorsal aspect of the hand in healthy subject (A) and patient with a "Late" pattern of scleroderma microangiopathy (B). Blue colour = low blood perfusion, yellow colour = intermediate blood perfusion, red colour = high blood perfusion. Academic Division of Clinical Rheumatology – Genova, operator RB

CAPILLAROSCOPY AND LASER SPECKLE CONTRAST TECHNIQUES

Laser speckle contrast analysis (LASCA) is a non-contact technique that has also the advantages to quantify the blood perfusion (in perfusion units, PU) over an area (Figure2). With LASCA it is also possible to create different regions of interest (ROI) and time regions of interest (TOI) to evaluate the blood perfusion[10].

LASCA is based on the principle that when an object is illuminated by laser light, the backscattered light will form a random interference pattern made up of dark and bright areas. This pattern is the so-called speckle pattern and is stationary. When movement occurs, as in red blood cells in tissue, the speckle pattern will change over time and these changes will be recorded by a detector camera[28].

LASCA has been applied in research studies on RP and SSc[10,29,30]. One such study demonstrated that peripheral blood perfusion evaluated by LDF and LASCA correlates to the extent of the microangiopathy[10]. It also reported that when evaluated by both methods, patients with the 'late' SSc microangiopathy pattern had a lower blood flow than patients with the 'Active' or 'Early' SSc patterns on NVC[10].

In another study by LASCA technique, blood perfusion was found significantly lower in SSc patients in comparison with healthy subjects at the level of fingertips, periungual areas, and palm of hands, and a statistically significant negative correlation was observed between nailfold microangiopathy extent and blood perfusion values at the level of the same skin areas in SSc patients[29].

Furthermore, LASCA may safely monitor digital ulcers evolution in SSc patients, by evaluating their blood perfusion and area during the treatment[31].

THERMOGRAPHY AND OTHER EMERGING TECHNIQUES

Thermal imaging, or infrared thermography, is a method that indirectly evaluates the blood perfusion, by using a thermal camera to image the temperature of the skin. This was shown to be representative of underlying blood flow[7]. Murray *et al* used thermography, LDI and NVC to studies the vascular response of patients with SSc and primary RP compared with healthy controls. In conclusion they observed that the combination of all 3 techniques improves classification of

SSc patients and that LDI and thermal imaging give similar information on dynamic changes in the cutaneous microcirculation[7].

Other emerging technologies for example Doppler optical coherence tomography, photoacoustic tomography and hyperspectral imaging are potential techniques that could become established tools for clinical microvascular assessment[26].

CONCLUSIONS

NVC is currently the only validated method to detect peripheral microvascular morphology, and allows to classify and to score the capillary abnormalities in SSc patients[32]. Furthermore, it is the validated method to distinguish between primary and secondary RP, and to follow-up SSc microangiopathy.

Interestingly, the recent inclusion of capillaroscopy among the diagnostic tools for the ACR/EULAR classification criteria of SSc, has been found to increase their sensitivity and specificity[5].

Laser Doppler and laser speckle techniques represent the best tools to assess and quantitate peripheral blood flow/perfusion, showing good correlations with capillaroscopy analysis[10,30,33,34].

The growing interest in the microcirculation caused a rapid development of new methods for its assessment, but all new techniques require studies for its validation in clinical practice.

The use of NVC together with LDF and LASCA represents an essential set of safe and non-invasive tools for identification, quantification and monitoring of SSc microangiopathy. The use of these tools in rheumatology clinics allows to improve the detection of the SSc microvascular status and possible effects of the therapeutic intervention.

It would be therefore desirable that these techniques had a wide diffusion in the rheumatological clinical practice.

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