Editorial

Different techniques to assess microvascular damage in systemic sclerosis

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Blood perfusion

Systemic Sclerosis (SSc) is a connective tissue disease with multifactorial aetiology and autoimmune pathogenesis. SSc is characterized by structural and functional alterations of microcirculation, with important clinical implications, such as Raynaud Phenomenon (RP) and digital ulcers[1,2]. For these reason, morphological and functional assessment of the peripheral microvasculature is a must for diagnosis, prognosis and therapy in SSc patients [2].

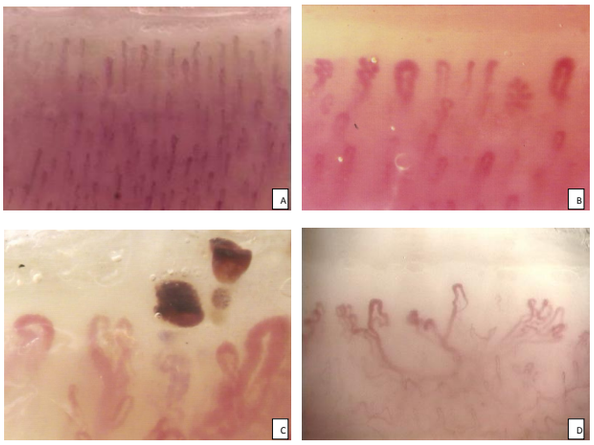
Nailfold videocapillaroscopy

Nailfold videocapillaroscopy (NVC) is the best safe and non-invasive method to detect morphological microvascular abnormalities. NVC allows to distinguish secondary RP from both primary RP and healthy subjects, identify morphological patterns that are specific to various SSc stages ('Early', 'Active' and 'Late' patterns of microvascular damage) and calculate the Microangiopathy Evolution Score (MES) to follow disease evolution[3,4].

The video-capillaroscope makes use of a magnification system (from 50x up to 500x magnification), and it has an optical/digital probe which can be moved over the surface of the finger nails from the 2nd to the 5th finger of both hands[2]. The normal NVC image is characterized by normal skin transparency, morphology of the capillary to "U" or "hairpin shape", morphological/structural homogeneity, 10-12 capillaries / linear millimetre, one capillary inside dermal papilla, diameters of capillary branches <20 μm, and lack of morphological atypia[2]. Nailfold capillaries are frequently normal in primary RP, but it is possible to observe capillaries with efferent branch enlargement or tortuous capillaries. Therefore in normal conditions, or in the presence of primary RP, the NVC examination is characterized by a regular array of capillary loops along the nailfold bed, without abnormal enlargements nor capillary loss[2]. Conversely, secondary RP is characterized by the morphological signs that represent the microvascular damage: these include giant capillaries, microhaemorrhages, capillary loss, 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 are described by the term "SSc pattern"[2,3].

NVC technique identifies morphological patterns specific to various stages of SSc ('Early', 'Active' and 'Late' patterns)[3,4]. The 'Early' SSc pattern is characterized by few enlarged/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 (more than 66%), frequent capillary microhaemorrhages, moderate (up to 33%) capillary loss, absent or mild ramified capillaries and a mild disorganization of the capillary architecture. In the 'Late' SSc pattern there is irregular enlargement of the capillaries, severe (>66%) capillary loss with evident avascular areas, ramified or bushy capillaries and a severe disorganization of the normal capillary array, although giant capillaries and microhaemorrhages are almost absent[3,4] (Figure 1). 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 (diagnostic parameters, such as irregularly enlarged capillaries, giant capillaries, microhaemorrhages; and progression parameters, such as reduced capillary number, capillary ramifications and capillary architectural disorganization) are evaluated by a semi-quantitative scale. Score 0-3 has been adopted for all these parameters[3,4].

Figure 1: Nailfold videocapillaroscopy images (x200) in healthy subject (A), early (B), active (C) and late patterns of scleroderma microangiopathy (D)



A ''Microangiopathy Evolution Score'' (MES) (the sum of progression parameters; score 0-9) was also selected to assess the vascular damage progression[3]. Data provided by NVC are also markers of SSc severity and progression, such as reduced capillary density, which has been associated with a high risk of developing digital skin ulcers and pulmonary arterial hypertension[5]. The inclusion of the NVC patterns in diagnosis could increase the sensitivity of classification criteria for SSc[6].

The analysis of the peripheral blood flow

Assessment of the peripheral circulation may also be useful for the evaluation of some drug effects, better if performed along with blood perfusion[7]. However, 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 as regular, granulous, or stasis[8]. The assessment and quantify of cutaneous blood perfusion in SSc may be performed by different laser techniques and by thermografy[9]. Other emerging technologies (e.g. optical Doppler tomography and spectroscopy) are possibly used to evaluate skin flow[10].

Different methods to analyse the peripheral blood flow Laser techniques

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 (1 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 [10,11].

Laser Doppler techniques

LDF is a non-invasive and user-friendly method to assess microvascular flow at a single skin point. It provides an index of skin perfusion by measuring the Doppler shift induced by coherent light scattering caused by moving red blood cells[11,12]. The blood perfusion evaluated by LDF is recorded as Perfusion Units (PU) and it is represented in a graph. Moreover, LDF technique may evaluate blood perfusion at 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[11,12].

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 microangiopathy pattern on NVC had a lower blood flow at LDF than patients with 'active' and 'early' SSc NVC patterns[11-13]. SSc patients have also an abnormal microvascular regulatory responses to heat stimulation[11]. LDF is also efficacious for the evaluation of the variation in peripheral blood perfusion during treatment with vasodilatation drugs within a few days or even over a long follow-up period of years[11,12]. Sulli *et al*[14] in a recent study have demonstrated, in SSc patients, the correlation between blood perfusion, evaluated by LDF, and dermal thickness, measured with high frequency ultrasound, at fingers level.

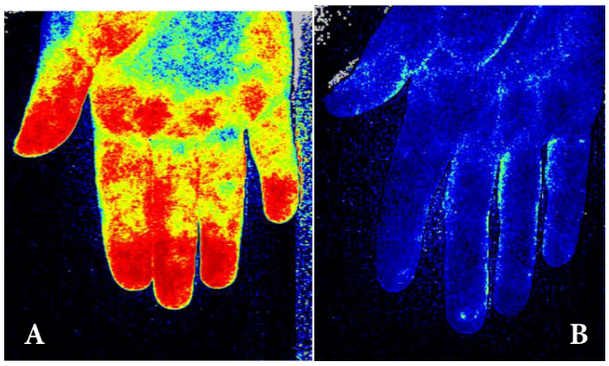
One problem with the LDF technique is the large site-to-site variation, which limits its efficacy in comparing blood flows between sites and in monitoring change over time[10,11]. Laser Doppler Imaging (LDI), which evaluates blood flow over a skin area might overcome this problem[9,10]. Murray and colleagues[9] showed that NVC, LDI, and thermal imaging (another technique to measure indirect blood flow) each independently provide good discrimination between patients with SSc and those with primary RP and healthy controls. In conclusion, they observed that the combination of all three techniques improves classification of SSc patients and that LDI and thermal imaging give equivalent information on dynamic changes in the cutaneous microcirculation. The study also confirms that NVC is the best method to classify patient groups.

Laser speckle contrast techniques

Laser Speckle Contrast Analysis (LASCA) is a relatively new technique to quantify blood perfusion. LASCA is based on the principle that when laser light illuminates a tissue, it forms a speckle pattern. Changes in the speckle pattern are recorded by a Charge-Coupled Device (CCD) camera and analyzed by a software. The static areas result in a stationary speckle pattern, in contrast the moving objects (e.g. red blood cells) cause the speckle pattern to fluctuate and appear blurred. Level of blurring (contrast) is analyzed and interpreted as blood perfusion[10,13,15]. LASCA has the advantages to quantify the blood flow

over an area and to be a non-contact technique. It is a fast imaging technique and with a high resolution. It combines perfusion image and real-time graphs (Figure 2). The blood perfusion is reported in perfusion units (PU)[13]. With LASCA it is also possible to create different regions of interest (ROI) and time regions of interest (TOI) to evaluate the perfusion. LASCA has been applied in research studies on RP and SSc[13,15,16]. One such study demonstrated that peripheral blood perfusion evaluated by both LDF and LASCA correlates to the extent of the microangiopathy[13]. 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[13].

Figure 2: Laser speckle contrast analysis images of hand palmar aspect 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 = higher blood perfusion



In another study we have showed that BP, as assessed by LASCA technique, is 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 exists between nailfold microangiopathy extent and BP values at the level of the same skin areas in SSc patients[16]. In our last study we have demonstrated that LASCA may safely monitor digital ulcers evolution in SSc patients, by evaluating their blood perfusion and area during standard treatment[17]. The instrumentation of Laser Speckle Contrast Imaging (LCSI) is similar to LASCA but the contrast is calculated in single pixel over a number of time frames. Spatial resolution increases five times over LASCA but poor temporal resolution. Processing takes quite longer time than LASCA due to calculation over number of frames.

Thermal imaging or infrared thermography

Thermal Imaging (TI) is a method that evaluates indirectly the perfusion, by using a thermal camera to image the temperature of the skin, and it was shown to be representative of underlying blood flow[9]. In several studies TI was used for the evaluation of RP, and the response to cold change was reported to be able to differentiate between primary RP and SSc. Distal dorsal difference > 1°C between one or more fingertips and the dorsum of the hand (fingers cooler) at 30°C is predictive of a SSc-spectrum disorder[18]. This method has several limitations (e.g. poor sensitivity to detect variations of blood perfusion and low spatial resolution).

Conclusions

The growing interest in the microcirculation caused a rapid development of new methods for its assessment, but all techniques require studies to validate their use in clinical practice. The NVC is currently the only validated method to study microvascular morphology for the evaluation of capillary abnormalities to distinguish secondary RP (associated to connective tissue disease) from primary RP. For this reason abnormal capillaroscopy is also one of the new classification criteria for SSc[6].

The evaluation of microvascular structure by NVC, in combination with function by laser techniques or thermal imaging, not only can help to distinguish between primary RP to secondary RP, but also to evaluate the response to therapy, and the disease progression[7, 11, 12]. It would therefore be desirable that these techniques should have a wide diffusion in the clinical rheumatological practice.

Key words: Systemic sclerosis, Cutaneous microcirculation, Nailfold videocapillaroscopy, Laser techniques

Competing Interest: None declared.

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