Peripheral blood perfusion in patients with systemic lupus erythematosus and in primary Raynaud’s phenomenon

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Abstract

Objective: This study aims to evaluate blood perfusion (BP) in various cutaneous regions of the hands and face in patients with systemic lupus erythematosus (SLE) and primary Raynaud’s phenomenon (PRP) and healthy subjects (HS).

Methods: A total of 20 patients with SLE, 20 patients with PRP, and 20 HS were enrolled. BP was detected by laser speckle contrast analysis in different regions of the hand and at the facial level. The absolute nailfold capillary number (CN) was assessed by nailfold videocapillaroscopy.

Results: Patients with SLE and PRP had significantly lower BP levels than those of HS in 3 hand areas (fingertip, palm, and periangual; p<0.01). However, the SLE, PRP, and HS groups had comparable BP values at the hand dorsum and face. The BP and CN values revealed a positive correlation in the periangual, fingertip, and palm of hands (p<0.01), only in patients with SLE.

Conclusion: Our data demonstrated a correlation between functional and morphological microvascular impairment in patients with SLE.

Keywords: Connective tissue, systemic lupus erythematosus, autoantibodies, blood vessels, musculoskeletal system, microscopic angioscopy

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease, defined by numerous clinical and serological manifestations (1-4). Although these frequently include vascular involvement, few studies have evaluated morphological anomalies in microcirculation; literature on the functional alterations of the microcirculation is scant, without any investigation about the correlation between the morphological and functional damage (5-9).

Periungual nailfold videocapillaroscopy (NVC) is a validated and non-invasive diagnostic investigation to study microvascular alterations in routine clinical practice to help distinguish different connective-tissue diseases (CTD) and to distinguish primary from secondary Raynaud’s phenomenon (10, 11). Nailfold capillaries in primary Raynaud’s phenomenon (PRP) usually have a normal shape without any specific alterations or abnormal capillaroscopic findings, that is, giant capillaries and microhemorrhages (1, 11-14).

NVC is also used to monitor CTD, particularly systemic sclerosis (SSc), as many studies have reported that alterations in the microcirculation correlate with organ involvement, evolution, and prognosis of these diseases (14-16).

Few SLE studies have reported a variable prevalence of periungual capillary anomalies, with typical NVC changes in 40%-90% of cases, whereas others have only reported non-specific abnormalities (2-9). However, as far as we are aware, no clear evidence has been provided as to whether NVC can be useful in the diagnosis and long-term monitoring of patients with SLE.

In CTD, particularly in SSc, many studies have reported a link between the functional and morphological damage. Moreover, a decrease in perfusion that coincided with worsening of microangiopathic damage was observed (14, 16). Although numerous methods can evaluate peripheral blood perfusion (PBP), currently most groups use non-invasive techniques, such as laser Doppler flowmetry and speckle, which provide precise quantification of perfusion (11, 16). Laser speckle contrast analysis (LASCA) is a relatively recent
method, which is now widely used in research to evaluate perfusion in CTD, particularly in SSc, as it provides a safe and quick evaluation of a skin area (11, 16).

Although various reports have demonstrated a reduction of PBP in PRP (11, 16), few have investigated skin blood perfusion (BP) in patients with SLE (7-9).

This investigation was aimed at identifying any differences in the BP of patients with PRP and SLE and healthy subjects (HS) in determined hand and facial cutaneous regions.

Methods

Study population
A total of 20 patients with SLE (2012 criteria) (17) (average age, 51±14 [standard deviation, SD] years; average disease duration, 7±4 years), 20 patients with PRP (LeRoy criteria) (12, 13) (average age, 53±21 [SD] years; average Raynaud’s duration, 6±5 years), and 20 HS (average age, 53±17 years) were enrolled after obtaining written informed consent. The patients were chosen during routine clinical evaluations in our department in the winter period, and we selected patients with SLE with quiescent disease (to reduce the use of treatment that could have created a bias). The subjects to be enrolled in the 3 groups were of the same sex and similar age. Patients’ information is shown in Tables 1 and 2. All vasodilator drugs (such as calcium channel blockers) had been discontinued at least 4 weeks before the study entry. LASCA and NVC were carried out on all subjects involved.

Laser speckle contrast analysis
Skin BP was graded using LASCA (Pericam PSI, Perimed; Milan, Italy) in the SLE, PRP, and HS groups, as previously described (11, 15, 16). The same operator (BR) evaluated LASCA of all the subjects. Technical parameters, for example, working distance (14.3-14.8 cm), point density (1,386×1,036), frame rate resolution (10 images/s), and the width and height of the measurement area (12.5 cmx14/15 cm) were standardized for all evaluations (11, 15, 16). All the subjects stayed in the waiting room for 30 min at 22°C-23°C before all the evaluations. BP was registered at the level of the whole face and dorsal and volar aspects (Figure 1) of the hands for 30 seconds (11, 15, 16). Regions of interest were created at the central area of the fingertips and periungual areas from fingers II to V bilaterally, palm and dorsum of both the hands, as well as at the level of the face (forehead, tip of nose, zygomas, and perioral region) (11, 16).

Nailfold videocapillaroscopy
All patients were checked by NVC to evaluate the morphological microvascular impairment using a videocapillaroscopy optical probe (200xcontact lens) connected to an image analysis software (Videocap, DS Medica; Milan, Italy). The same operator (CP) conducted the NVC examination in all the subjects on the same day as the LASCA evaluation (CP was blind to its results).

Table 1. Clinical findings in patients with SLE and PRP and HS (# number).

|                  | Median (IQR) | Median (IQR) | Median (IQR) |
|------------------|--------------|--------------|--------------|
| SLE#20           |              |              |              |
| Age (years)      | 50 (25)      | 50 (27)      | 50 (29)      |
| Sex (Female/Male)| 20/0         | 20/0         | 20/0         |
| Smokers (number) | 1/19         | 1/19         | 2/18         |
| SLE duration (years) | 6 (3)   | Not applicable | Not applicable |
| Autoantibodies (dsDNA/negative) | 17/3 | 0/20 | 0/20 |
| SLEDAI           | 2 (1)        | Not applicable | Not applicable |
| Treatments (PPI/PRED/MMF/HCQ/AZA) | 2/13/4/12/6 | 2/0/0/0/0 | 1/0/0/0/0 |

SLE: systemic lupus erythematosus; PRP: primary Raynaud’s phenomenon; HS: healthy subjects; RP: Raynaud’s phenomenon; PPI: proton pump inhibitors; PRED: prednisolone (average; 5 mg/day); HCQ: hydroxychloroquine (average; 150 mg/day); MMF: mycophenolate (average; 1,500 mg/day); AZA: azathioprine (average; 50 mg/day); SLEDAI: systemic lupus erythematosus disease activity index; dsDNA: double stranded DNA; IQR: interquartile range.

Table 2. Clinical manifestations at the onset of SLE (# number).

|                  | SLE#20 |
|------------------|--------|
| Clinical manifestations |     |
| Malar rash       | 15     |
| Discoid lesions  | 1      |
| Photosensitivity | 7      |
| Oral ulcers      | 8      |
| Arthritis        | 7      |
| Myositis         | 2      |
| Serositis        | 1      |
| Renal disorder   | 3      |
| Hematologic disorder | 6     |
| Fever            | 8      |
| Lymphadenopathy  | 2      |

Serology tests

|                  | SLE#20 |
|------------------|--------|
| Antinuclear antibodies | 19    |
| Anti-double stranded DNA | 17    |
| Extractable nuclear antigens (Anti-Sm #3; Anti-U1-snRNP#1) | 4     |
| Low complement C3 or C4 decreased below lower limit of normal for lab | 12    |

SLE: systemic lupus erythematosus; anti-Sm: Anti-Smith antibodies; snRNP: Anti-snRNP antibodies.
as previously reported (10, 11, 14, 16). NVC was performed to evaluate the absolute nailfold capillary number (CN) per linear millimeter at the first distal row of the usual 8 fingers (8, 11, 18-20).

**Ethical approval**

The study was approved by the Ethical Committee of San Martino Polyclinic Hospital on September 30, 2017, (30092017) and has been performed in accordance with the ethical standards laid down in the Declaration of Helsinki. All the patients gave their informed consent before their inclusion in the study.

**Statistical analysis**

Statistical analysis were performed using Prism version 5.02 (GraphPad Software; La Jolla, CA, USA). The Kruskal-Wallis and analysis of variance with Bonferroni/Dunn correction tests were used to compare continuous variables with nominal variables that had more than 2 levels. The Mann-Whitney U test was performed to compare unpaired groups of variables. Multiple and linear regression along with Spearman rank correlation tests were used to evaluate the relationship between variables. Any p values below 0.05 were considered sta-

|                | Median (IQR) | Median (IQR) | Median (IQR) | p       |
|----------------|--------------|--------------|--------------|---------|
|                | SLE#20       | PRP#20       | HS#20        | SLE vs. PRP | SLE vs. HS | PRP vs. HS |
| BP fingertips   | 116 (50)     | 83 (22)      | 188 (42)     | 0.03     | <0.001     | <0.0001     |
| BP periungual areas | 99 (42)   | 74 (20)      | 144 (45)     | 0.006    | 0.04       | 0.0002      |
| BP palm of hands | 94 (51)    | 75 (21)      | 120 (18)     | 0.02     | 0.001      | 0.0001      |
| BP dorsum of hands | 72 (39)    | 66 (20)      | 78 (30)      | >0.05    | >0.05      | >0.05       |
| BP tip of nose  | 139 (50)     | 142 (55)     | 146 (39)     | >0.05    | >0.05      | >0.05       |
| BP zygoma       | 146 (74)     | 150 (56)     | 153 (51)     | >0.05    | >0.05      | >0.05       |
| BP forehead     | 110 (36)     | 114 (50)     | 119 (51)     | >0.05    | >0.05      | >0.05       |
| BP perioral region | 100 (60)  | 103 (43)     | 106 (72)     | >0.05    | >0.05      | >0.05       |
| BP whole face   | 142 (42)     | 141 (43)     | 143 (45)     | >0.05    | >0.05      | >0.05       |
| CN              | 9 (2)        | 10 (2)       | 11 (2)       | <0.0005  | <0.0005    | >0.05       |

BP: blood perfusion; CN: capillaries number; SLE: systemic lupus erythematosus; PRP: primary Raynaud’s phenomenon; HS: healthy subject; IQR: interquartile range.

**Table 3.** BP and CN in SLE, PRP, and HS groups. BP evaluated by LASCA in different areas of hands (fingertips, periungual areas, and dorsum and palm of both hands) and whole face and different areas of face (tip of nose, zygoma, forehead, and perioral region). BP is reported as perfusion units (PU). (# number).

Figure 1. a-f. Nailfold videocapillaroscopy images (200x) (Videocap, DS MediGroup; Milan, Italy) in primary Raynaud's phenomenon (a), systemic lupus erythematosus (b), and healthy subjects (c). Laser speckle contrast analysis (Pericam PSI, Perimed; Milan, Italy) images in primary Raynaud's phenomenon (d), systemic lupus erythematosus (e), and healthy subjects (f), showing the regions of interest (white circles) created at the level of palm of hand and fingertips to evaluate blood perfusion.
tistically significant. The results are reported as median and interquartile range, average±SD, and correlation coefficients (r).

**Results**

Patients with SLE had higher BP values than those of patients with PRP evaluated by LASCA and measured using perfusion units in the fingertip (p=0.03), palm (p=0.02), and periungual (p=0.006) areas but not on the back of the hand, whole face, or various single facial areas (Table 3). Patients with SLE and PRP had a significantly lower BP level than that of the HS at the fingertip level, periungual, and palm areas (Table 3, Figure 1). In contrast, the SLE, PRP, and HS groups had similar BP at the dorsum level, whole face, and individual facial areas (p=0.4) (Table 3).

A positive correlation between BP and CN was noted in patients with SLE at the level of the fingertips (p<0.0001, r=0.84), palm (p=0.01, r=0.74), and periungual (p=0.001, r=0.76) areas, but there was no statistically significant correlation between BP and CN at the dorsum, whole face (p=0.10), or individual facial areas (p>0.05). BP and CN did not correlate to any areas examined (p>0.05) in PRP or HS. Patients with SLE had a significantly lower CN than both PRP and HS (median 9.2 vs. 10.2 vs. 11.2, respectively, p<0.0005) (Table 3, Figure 1). In contrast, no statistically significant difference in CN was observed between PRP and HS.

There was no correlation between SLE disease activity index (SLEDAI) and both BP and CN in patients with SLE (p>0.05).

Our investigation involved a few tobacco and alcohol users; however, no statistically significant differences, regarding these most important risk factors for microvascular alterations, were observed in patients with SLE and PRP and HS.

**Discussion**

To our knowledge, this pilot study is the first to investigate BP by LASCA in various cutaneous regions in patients with PRP and SLE and compare it with that of HS. It also investigated the possibility of a correlation between functional and morphological alterations in microcirculation in determined cutaneous regions of the hands and face in patients with SLE and compared the said values in patients with PRP and HS.

The LASCA investigation demonstrated that patients with SLE have a reduced peripheral perfusion compared with that of HS.

A likely explanation is that patients with SLE may present a subclinical microangiopathy. In contrast, only patients with PRP displayed microvascular dysfunction with a lower peripheral cutaneous BP than both patients with SLE and HS.

This is different from the studies by other authors, which have reported that the vascular responses observed in patients with SLE did not differ from those in HS. The different results could be explained by the fact that they evaluated microvascular perfusion by laser Doppler flowmetry and reported the changes in BP in response to acetylcholine-iontophoresis (6, 7, 9). However, our study evaluated the perfusion by LASCA in various skin areas (11, 16, 20). Moreover, perfusion was evaluated at basal condition.

We are aware that the limited number of subjects caused by single-center recruitment poses a limitation. Another limitation is that we assessed microvascular function in patients with SLE with quiescent disease (to reduce possible bias), which could explain the lack of correlation among BP, CN, and SLEDAI in patients with SLE.

Finally, for the NVC evaluation, we decided to consider the changes in CN because it appears the best validated NVC parameter and is nowadays quantifiable with automated systems (8, 18). Furthermore, the research on microcirculation in SLE is expanding quickly as very recently reviewed and reported (2, 8).

Many articles have reported that NVC is the most suitable technique for the early identification and evaluation of morphological microvascular damage in several rheumatic diseases, including SLE (1, 5, 8, 20-24), and LASCA is a relatively new but promising technique to evaluate the functional alteration in microcirculation that could characterize many rheumatic diseases (11, 16). In this study, we used the 2 methods together to analyze the possibility of subclinical microvascular damage in SLE, which is a complex disease with unknown origins (25).

We would also like to emphasize that it is important that clinicians do not underestimate the risk of subclinical microvascular dysfunction in rheumatic diseases, such as SLE, characterized by endothelial and vascular alterations at different levels. Our study has demonstrated that patients with SLE have a subclinical microangiopathy and patients with PRP have a functional alteration as demonstrated in other studies (8, 10, 11, 16). Further studies are ongoing to ascertain the clinical application of these findings.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the Ethical Committee of San Martino Polyclinic Hospital (Approval Date: September 30, 2017; Approval Number: 30092017).

**Informed Consent:** Written informed consent was obtained from the patients who participated in this study.

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