Quantification of basal digital blood flow and after cold stimulus by laser doppler imaging in patients with systemic sclerosis

Marcelo José Uchoa Corrêa[1], Sandro F Perazzio[1], Luís Eduardo Coelho Andrade[2], Cristiane Kayser[3]

ABSTRACT

Objectives: The objective of this study was to investigate the dynamic behavior of the blood flow of the microvascular circulation of the fingertips before and after two cold stimuli (CS), using Laser Doppler Imaging with different intensities in patients with systemic sclerosis (SSc) and in healthy individuals. **Patients and Methods:** Fourteen SSc patients (51.2 ± 5.5 years) with Raynaud's phenomenon and 12 healthy controls (44.8 ± 9.0 years) were included in this study. Two CS protocols (submersion of the hands in water at 10 °C or 15 °C for 1 minute) were performed on the same day. Mean fingertip blood flow (FBF) of four digits of the left hand was measured using LDI (Moor LDI-VR, Moor Instruments) at baseline and at 1, 4, 10, 25, and 40 minutes after CS. **Results:** Baseline blood flow was significantly lower in both CS protocols in SSc patients when compared to controls (312.9 ± 102.7 *vs* 465.4 ± 135.4 PU, P = 0.006 at 15 °C; 305.2 ± 121.0 *vs* 437.9 ± 119.8 PU; P = 0.01 at 10 °C). In the control group, a significant decrease in FBF after CS, when compared to baseline, was observed 1 minute (P = 0.001) after CS at 15 °C and at 1 (P = 0.005) and 25 minutes (P = 0.001) after CS at 10 °C. In SSc patients, a significant decrease in FBF was observed in both CS protocols at 1, 4, and 10 minutes (P < 0.000; P = 0.002; P = 0.014, after CS at 15 °C; P < 0.000; P = 0.004; P = 0.001, after CS at 10 °C). **Conclusions:** Laser Doppler Imaging showed lower baseline fingertip perfusion and further reduction after CS in SSc patients compared to controls. Quantification of fingertip blood flow by LDI may be useful in the longitudinal monitoring of the disease status and therapeutic interventions in SSc.

Keywords: systemic sclerosis, Raynaud's phenomenon, microcirculation, Laser Doppler Imaging.

INTRODUCTION

Systemic sclerosis (SSc) is an autoimmune disease of unknown etiology characterized, clinically, by vascular involvement and fibrotic changes of the skin and internal organs.[1] The early detection of peripheral vascular changes, characterized by structural and functional abnormalities in small blood vessels and microcirculation, is possible in the majority of the cases.

Raynaud's phenomenon (RyP), seen as the first manifestation in 70% of the patients and in up to 95% of the cases during the evolution of the disease, is the most evident and earlier clinical manifestation of vascular involvement.[2] Raynaud's phenomenon is defined as an abnormal vasoconstriction in response to cold, causing recurring episodes of spasm of digital arteries, arterioles, and cutaneous arteriovenous shunts. It is characterized by typical color changes of the extremities, especially hands and feet, that classically have three phases: pallor, cyanosis, and rubor. Structural changes of the microcirculation, endothelial damage and dysfunction, and platelet activation, which usually make vasospastic events more severe, are present in RyP secondary to SSc, or diseases

Received on 03/17/2009. Approved on 02/24/2010. This study received a grant from the Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP, from Portuguese), and partial funding from the Research and Teaching Support Fund of the Brazilian Society of Rheumatology. The authors deny conflicts of interest.
Rheumatology Department, Escola Paulista de Medicina, Universidade Federal de São Paulo (UNIFESP), Brazil.
1. Post-graduate Rheumatology student at UNIFESP
2. Professor of Rheumatology at UNIFESP
3. Assisting physician and physician in charge of the Systemic Sclerosis Outpatient Clinic of the Rheumatology Department of UNIFESP
Correspondence to: Cristiane Kayser, Rua Botucatu, 740, 3° andar – São Paulo – SP. CEP 04023-062. E-mail: criskayser@terra.com.br

of the same spectrum.[3] Marked reduction in blood flow, and even complete occlusion of the vessel, can be seen. All those changes can lead to chronic tissue hypoxia and irreversible tissue damage with recurring ulcers, scars, and, in more severe cases, gangrene and amputation of the extremities.[4]

Accurate methods for the evaluation and quantification of the changes present in the microcirculation in patients with RyP and SSc, may it be to study the pathophysiology of the disease or to differentiate patients with secondary RyP from those with primary RyP, have to be developed.[5] Besides, with the advent of new treatment modalities for SSc and associated RyP, objective and reproducible methods to evaluate the response to therapy are increasingly more necessary. Those methods could be associated, or not, with acute ischemia provocation tests - immersion of the extremities in cold water or insufflation of pneumatic devices.[6,7] Currently, a method considered the gold-standard to evaluate the blood flow in the microcirculation in patients with primary RyP or SSc does not exist. Laser Doppler flowmettrics, a non-invasive method that provides quantitative evaluation of the blood flow in the microcirculation, is based on the Doppler effect of beams reflected by moving blood cells.[8] Conventional Laser Doppler evaluates only a small circumscribed point of the skin each moment, and great signal variability among different areas represents an important limitation, making it a poorly reproducible method, limiting its use in therapeutic assays.[9,10] Laser Doppler Imaging (LDI) is based on a relatively recent technique that allows direct measurements of the blood flow of the cutaneous microcirculation over a wider area and not only in a single point. It also has the advantage of not requiring direct contact with the skin surface, eliminating changes secondary to pressure, or artifacts caused by movements.[11,12] Due to the precision and reproducibility, LDI is considered a promising method for the dynamic evaluation of the blood flow in RyP and in patients with SSc.[13]

In the present study, the response of the microcirculation to two cold stimuli of different intensities in healthy controls and in patients with SSc was evaluated. Our intention was to evaluate the dynamic behavior of the digital blood flow in healthy individuals and SSc patients.

PATIENTS AND METHODS

A transversal study with patients with the diagnosis of SSc, according to the ACR classification,[14] who also had RyP and periungueal capillaroscopy with SD (scleroderma pattern) microangiopathy,[15] from the Systemic Sclerosis Outpatient Clinic of HSP-UNIFESP, was undertaken. Healthy controls, paired by gender and age, were selected among the employees of Hospital São Paulo. Exclusion criteria were: smoking, diabetes, severe hypertension (diastolic blood pressure > 105 mmHg), or patients with clinically significant central vasculopathy and/or peripheral venous vasculopathy. Any vasodilators used by patients were discontinued three days before the tests. All patients should be older than 18 years and they had to sign an informed consent approved by the Ethics Commission of UNIFESP under protocol #1,298/06.

After an adaptation period (rest for one hour in a room the with temperature of 24 ± 1° C, with their hands most of the time in the axillary region to keep them warm), patients and controls underwent two consecutive protocols of cold stimuli on the same day with a minimal inter-test interval of 60 minutes. The specific conditions of both protocols were as follows:

Part I: Assessment of vasoreactivity of the cutaneous microcirculation in the presence of a cold stimulus at 15° C

A – Assessment of the baseline blood flow of the cutaneous microcirculation by Laser Doppler Imaging. The left hand of all individuals was placed on a flat surface (at the level of the heart), and the blood flow on the dorsal region of the distal phalange of four fingers (except the thumb) of the left hand was evaluated in basal conditions by Laser Doppler Imaging (Moor LDI, Moor Instruments, Axminster, UK). This device uses a low intensity helium-neon red laser beam with 633 nm with penetration in the skin surface of approximately 1 mm. The light beam is directed to the selected area of the skin by a mirror located at 40 cm from the skin surface. All images were captured at a rate of 4 pixels/millisecond, with acquisition time of 3 minutes and 15 seconds for each image. The mean blood flow of the selected area was determined with the help of the MoorLDI V5.2 software and expressed in arbitrary perfusion units (PU) in relation to an internal standard calibration of the device. The mean blood flow of the four fingertips (FBF) was considered for analysis.

B – Cold stimulus (CS). Cold stimulus was applied by immersion of both hands in water at 15° C for 60 seconds (UNITEMP 116, Fanem, Brazil).

C – Monitoring the blood flow in the cutaneous microcirculation by Laser Doppler Imaging after the Cs. The FBF of the dorsum of the four fingers (except the thumb) of the left hand was measured continuously, as described, up to 25 minutes after the CS. The blood flow measured at 1, 4, 10, and 25 minutes after CS was considered for analysis.

Part II: Assessment of the vasoreactivity of the cutaneous microcirculation in the presence of a cold stimulus of 10° C

The protocol described in Part I was repeated, observing a minimal interval of 60 minutes between both CS. The main difference relied on the CS, which consisted on immersion in water at 10° C for 60 seconds. The blood flow in the cutaneous microcirculation was monitored up to 40 minutes after the CS and measured at 1, 4, 10, 25, and 40 minutes after the CS, represented another difference in relation to part I.

Statistical analysis

To better detect the dynamic changes in FBF secondary to the CS, the percentage variation in post-CS FBF in relation to pre-CS FBF, called ΔFBF, was used, in which:

$$\Delta FBF = \frac{\text{post-CS FBF} - \text{pre-CS FBF x 100}}{\text{Pre-CS FBF}}$$

Results were expressed as mean ± standard deviation. The Student *t* test was used for intergroup analyses, while ANOVA for repeated measurements was used to compare the groups along time. A level of significance of 0.05 was adopted in all the analyses.

RESULTS

Fourteen SSc patients, with mean age of 51.2 years (ranging from 41 to 60 years), and 12 healthy controls, mean age of 44.8 years (ranging from 33 to 54 years), were included in the study. Five patients (35.7%) had limited cutaneous disease and nine (64.3%) had diffuse cutaneous disease (Table 1). The duration of the disease in patients with the limited cutaneous type was 10.5 ± 4.2 years and the onset of RyP was 12.2 ± 5.1 years ago. In patients with the diffuse cutaneous type, the duration of the disease was 8.3 ± 5.6 years and the onset of RyP was 7.3 ± 6.4 years ago. Ten patients were being treated with vasodilators (calcium channel blockers, captopril, and losartan).

Basal FBF was significantly lower in SSc patients than in the controls in both CS protocols (312.9 ± 102.7 *versus* 465.4 ± 135.4 PU, P = 0.006, before CS at 15° C; 305.2 ± 121.0 *versus* 437.9 ± 119.8 PU, P = 0.01, before CS at 10° C) (Figure 1). Baseline FBF in healthy controls did not differ between the first and second CS (P = 0.341) or in SSc patients (P = 0.679), confirming the reproducibility of the method. The mean blood flow in the fingertips was significantly lower in SSc patients when compared to the control group at all times after CS at 15° C (P = 0.005, 1 minute after Cs; P = 0.009, 4 minutes after CS; P = 0.001, 10 minutes after CS; and P = 0.016, 25 minutes after CS), and at 1, 4, and 10 minutes after CS at 10° C (P < 0.001; P = 0.008; and P = 0.002, respectively) (Table 2).

An abrupt fall in FBF was observed 1 minute after both CS in SSc patients and in the control group, followed by a gradual recovery of the blood flow, which was delayed in SSc patients (Figure 2). In healthy controls, a significant reduction in FBF was observed after CS at 15° C, when compared to baseline levels, 1 minute after CS (*P* = 0.001). This group showed fast recovery of FBF and statistically significant differences among basal FBF and FBF 4, 10, and 25 minutes after CS at 15° C (P = 0.055; P = 0.55; and P = 1.00, respectively) were not observed. At 10° C, significant differences were observed in post-CS FBF at 1 and 25 min when compared to baseline levels (P = 0.005; P = 0.001, respectively) in the control group. In SSc patients, a significant reduction was observed in FBF 1, 4, and 10 minutes after CS at 15° C (P < 0.000; P = 0.002; and P = 0.014, respectively) and 10° C (P < 0.000; P = 0.004; P = 0.001, respectively). A recovery in FBF was observed only

Table 1
Demographic data of patients with systemic sclerosis (SSc) and healthy controls

	SSc patients (n = 14)	Healthy controls (n = 12)	P
Age (years)	51.2 ± 5.5	44.8 ± 9.1	NS
Gender	13F/1M	9F/3M	NS
Clinical type	5 Diffuse/9 Limited	–	–
Duration of RyP (years)	9.4 ± 6.1	–	–
Duration of the disease (years)	9.4 ± 5.8	–	–
Number of patients with active ulcers (%)	6 (42.8)	–	–
Positive ANF (n)	13	–	–
Positive Anti-Scl 70 (n)	2	–	–
Positive anti-centromere (n)	5	–	–

NS: non-significant; RyP: Raynaud's phenomenon; ANF: anti-nuclear antibody.

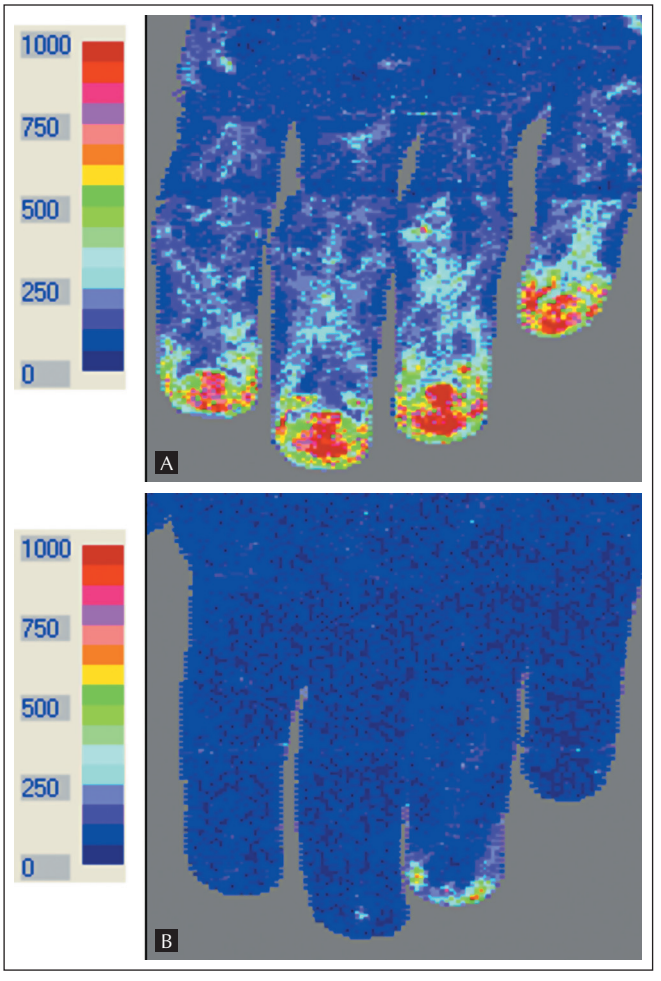

Figure 1
Baseline blood flow in a healthy control (A) and in one patient with systemic sclerosis (B) on Laser Doppler Imaging.

25 minutes after CS (P = 0.146 at 15° C; P = 0.25 at 10° C) (Figure 2). The magnitude of the reduction in FBF (ΔCS-FBF) was greater in SSc patients than in healthy controls 1 minute after CS at 10° C (-72.5 ± 63% *versus* -35.3 ± 15%) (Table 2).

DISCUSSION

In the present study, we undertook a novel evaluation of the dynamic behavior of the blood flow in the microcirculation of the digits, before and after two cold stimuli of different intensities, by comparing the LDI of patients with RyP secondary to SSc and healthy individuals. The FBF of patients with systemic sclerosis was considerably lower than in healthy controls. As expected, a significant reduction in FBF was observed in both groups after CS, both at 15° C and 10° C, and this reduction was more marked in SSc patients. Healthy controls showed fast recovery in FBF, especially after CS at 15° C (observed 4 minutes after CS); the same was not true for SSc patients, who had delayed recovery of FBF. The recovery curve after CS at 15° C was capable to better characterize both groups.

The findings of lower baseline FBF levels in SSc patients confirm prior studies that reported conspicuous morphological changes, devascularization, and reduction in blood flow in the small vessels and microcirculation of those individuals.[16,17] The greater reduction in FBF observed in the majority of SSc patients might be due to changes in blood flow dynamics that are worsened by unfavorable conditions secondary to structural changes in the microcirculation observed in those individuals. The vasoreactivity of the cutaneous microcirculation is, usually, a protective response to heat loss. After a cold stimulus,

Table 2
Mean blood flow, in the fingertips of four fingers, in perfusion units (PU) and percentage variation, after cold stimulus at 15° C and 10 °C (ΔEF - FBF) in patients with systemic sclerosis (SSc) and healthy controls

		Cold stimulus a 15ºC				
	Baseline	**1 min**	**4 min**	**10 min**	**25 min**	
Controls	465.43 ± 135.38	273.27 ± 183.22	345.33 ± 196.84	424.57 ± 151.45	419.77 ± 147.08	
SSc	322.91 ± 102.72	115.89 ± 55.07	172.13 ± 108.88	223.56 ± 106.79	284.92 ± 117.83	
ΔEF-FPD controls		-41.2 ± 30.18%	-25.7 ± 20.2%	-8.7 ± 2.26%	-9.8 ± 6.45%	
ΔEF-FBF SSc		-64.1 ± 48.7%	-46.6 ± 7.2%	-30.7 ± 18.32%	-11.7 ± 5.0%	
P (FPD controls *versus* SSc)	0.006	0.005	0.009	0.001	0.016	
		Cold stimulus a 10ºC				
	Baseline	**1 min**	**4 min**	**10 min**	**25 min**	**40 min**
Controls	437.97 ± 119.79	283.43 ± 138.72	299.10 ± 169.63	381.20 ± 153.35	275.48 ± 107.55	312.75 ± 161.59
SSc	305.16 ± 121.00	83.76 ± 43.83	133.97 ± 118.39	199.3 ± 105.79	227.44 ± 126.82	255.50 ± 124.46
ΔEF-FBF controls		-35.2 ± 12.84%	-31.7 ± 12.4%	-12.9 ± 10.40%	-37.1 ± 25.10%	-28.5 ± 17.38%
ΔEF-FBF SSc		-72.5 ± 62.87%	-56.0 ± 21.2%	-34.6 ± 22.93%	-25.4 ± 6.39%	-16.2 ± 5.02%
P (FBF controls *versus* SSc)	0.01	0.000	0.008	0.002	0.312	0.247

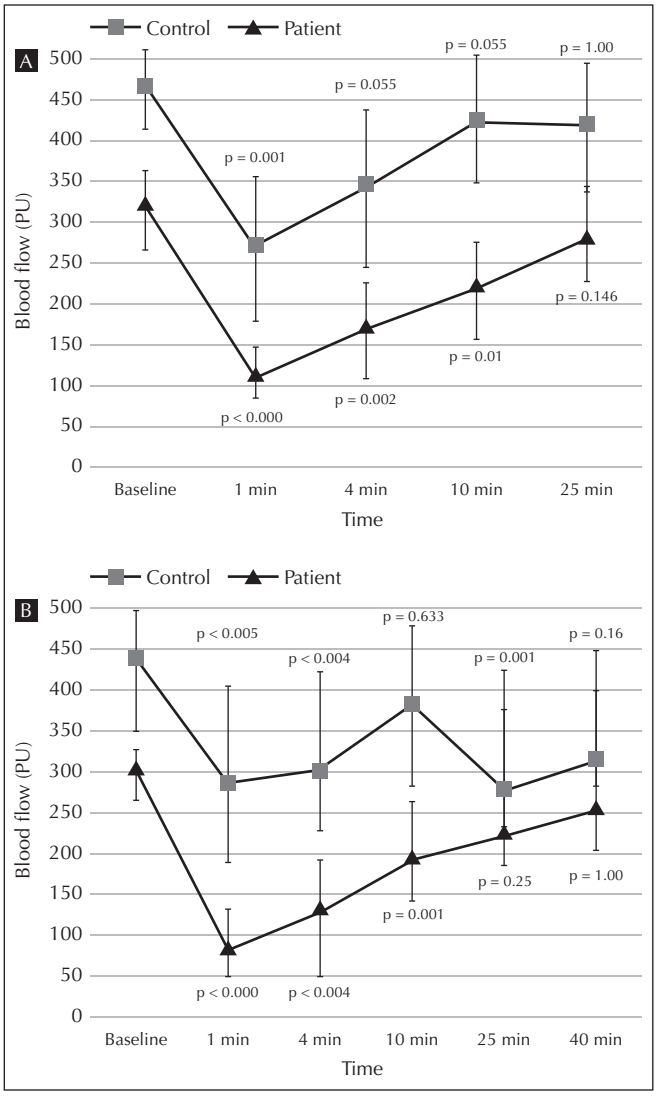

Figure 2
Mean blood flow, in perfusion units (PU), in the fingertips of patients with systemic sclerosis (SSc) and healthy controls before and after cold stimulus at15 °C (A) and 10 °C (B).

SSc patients show a knowingly exacerbated response to acute ischemia, with sudden increase in vascular tonus, marked reduction of the blood flow, and even possible closure of digital arterioles.[18,19]

The consistent and objective reproduction, in clinical practice, of the vasospastic events in patients with primary or secondary RyP is difficult. Several methods for the objective measurement of the severity of cold vasoreactivity in each patient with RyP, such as thermography, digital systolic blood pressure, fingertip lacticemy, plethysmography, conventional Laser Doppler, and LDI, are used.[5,12,19-23] Thermography evaluates, indirectly, the temperature of the skin of a specific area. Digital systolic blood pressure is useful to evaluate the blood flow of the digital artery; however, it does not evaluate the microcirculation. Plethysmography evaluates changes in blood volume in specific segments and it seems to be a poorly reproducible method.[5] On the other hand, LDI allows the evaluation of larger areas of the cutaneous microcirculation, and its results are more reproducible and representative when compared to conventional Laser Doppler. Clark *et al*.[10] used LDI to evaluate the blood flow on the back of the hands in patients with primary RyP and diffuse cutaneous SSc, limited to the room temperature of 23° C and 30° C. The authors observed significant differences in blood flow among the four study groups, especially when evaluating the maximal difference in blood flow among the different fingers of the same hand at a room temperature of 23° C. Note that the most expressive differences were observed in patients with limited cutaneous SSc and smaller differences in healthy controls.

Laser Doppler Imaging was also used to assess the therapeutic response to topical glyceryl trinitrate in patients with primary RyP and in SSc patients[24]. An increase in digital blood flow was observed in all patients after applying glyceryl trinitrate when compared to placebo. More recently, when evaluating the long-term treatment with intravenous N-acetylcysteine in RyP secondary to SSc, Salsano *et al*.[25] observed a significant increase in global perfusion of the hands on LDI after treatment. Those studies indicate that Laser Doppler Imaging is a powerful tool in the quantification of the blood flow in the microcirculation and objective monitoring of the therapeutic response in patients with RyP and SSc. However, until now, very few studies have evaluated the vasoreactivity of cutaneous microcirculation in response to a cold stimulus in patients with RyP and/or SSc using this method.[12,26] Picart *et al*.[26] evaluated digital perfusion before and after cold stimuli (cold plate at 5° C for 8 minutes) with LDI in eight patients with SSc, ten with primary RyP, and seven healthy controls. Similar to the results of the present study, SSc patients showed lower baseline blood flow and marked decrease after the cold stimulus when compared to healthy controls.

Different protocols of cold stimuli, such as immersion of both hands or one hand in water at different temperatures (ice until 20° C) and total body cooling are used for the objective evaluation of vasoreactivity in patients with RyP.[27-30] In our study, we performed two protocols aiming at standardizing CS test with LDI and quantify, objectively, changes in blood flow in the microcirculation related to the pathophysiology of RyP. To this end, we attempted to evaluate which CS would

be better to distinguish healthy individuals from patients with RyP secondary to SSc. Cold stimulus at 15° C for 1 minute was used by O'Reilly *et al*.,[30] and it was considered adequate to evaluate blood flow recovery curves in the presence of a CS. Our group has used the cold stimulus at 10° C, in association with lacticemy test of the fingertips, in several studies, showing that it is capable of differentiating patients with secondary RyP, primary RyP, and healthy controls.[21-23] However, CS at 10° C is more uncomfortable and potentially more damaging for patients with RyP. The present study demonstrated that very intense or prolonged CS is not necessary to produce vasospasm or distinguish patients with secondary RyP from healthy individuals by LDI. Besides, based on the results of the present study, CS at 10° C also seemed to cause severe vasoconstriction, even in healthy individuals, therefore hindering the discrimination between patients and healthy controls.

One should not forget that measurements of peripheral blood flow can be influenced be a series of factors, such as low environmental temperature, length of acclimatization, smoking, hormonal changes, use of vasodilators or vasoconstrictors, and stress.[31,32] In our study, we tried to minimize the influence of extrinsic factors by applying a homogenous protocol, consisting on individual adjustment in a calm environment with controlled temperature, exclusion of smokers, and discontinuation of vasodilators three days before the tests, to all participants.

In conclusion, the present study showed significant lower levels of basal FBF in SSc patients when compared to healthy controls. A significant reduction in FBF was observed in both groups after CS, especially 1 minute after CS in both protocols. Cold stimulus at 15° C differentiated better both groups. Quantification of FBF associated with a CS could, therefore, be useful in the evaluation for therapeutic assays and longitudinal follow-up of patients with RyP secondary to SSc. Prospective studies with a greater number of SSc patients and patients with primary RyP could better define the role of LDI in the diagnosis and monitoring of those patients.

REFERÊNCIAS

REFERENCES

1. Tan FK. Systemic sclerosis: the susceptible host (genetics and environment). Rheum Dis Clin North Am 2003;29:211-37.
2. Seilbold JR. Scleroderma. In: Kelley WN, Ruddy S, Harris ED & Sledge CB. Textbook of rheumatology. Philadelphia-USA: WB Saunders Company; 1997.
3. Campbell PM, LeRoy EC. Pathogenesis of systemic sclerosis: a vascular hypothesis. Semin Arthritis Rheum 1975;4:351-68.
4. Chung L, Fiorentino D. Digital ulcers in patients with systemic sclerosis. Autoimmunity Rev 2006;5:125-8.
5. Herrick AL, Clark S. Quantifying digital vascular disease in patients with primary Raynaud's phenomenon and systemic sclerosis. Ann Rheum Dis 1998;57:70-7.
6. Engelhart M. Clinical and physiological studies of Raynaud·s phenomenon. Dan Med Bull 1991;38:458-67.
7. Maricq HR, Weinrich MC, Valter I, Palesch YY, Maricq JG. Digital vascular responses to cooling in subjects with cold sensitivity, primary Raynaud's phenomenon, or scleroderma spectrum disorders. J Rheumatol 1996;23:2068-78.
8. Essex TJ, Byrne PO. A Laser Doppler Scanner for imaging blood flow in skin. J Biomed Eng 1991;13:189-94.
9. Anderson ME, Hollis S, Moore T, Jayson MI, Herrick AL. Non-invasive assessment of vascular reactivity in forearm skin of patients with primary Raynaud's phenomenon and systemic sclerosis. Br J Rheumatol 1996;35:1281-8.
10. Clark S, Campbell F, Moore T, Jayson MI, King TA, Herrick AL. Laser Doppler Imaging. A new technique for quantifying microcirculatory flow in patients with primary Raynaud's phenomenon and systemic sclerosis. Microvasc Res 1999;57:284-91.
11. Murray AK, Gorodkin RE, Moore TL, Gush RJ, Herrick AL, King TA. Comparison of red and green laser Doppler imaging of blood flow. Lasers Surg Med 2004;35:191-200.
12. Seifalian AM, Stansby G, Jackson A, Howell K, Hamilton G. Comparison of laser Doppler perfusion imaging, laser Doppler flowmetry, and thermographic imaging for assessment of blood flow in human skin. Eur J Vasc Surg 1994;8:65-9.
13. Herrick AL. Diagnosis and management of scleroderma peripheral vascular disease. Rheum Dis Clin N Am 2008;34:89-114.
14. Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1980;23:581-90.
15. Maricq HR, LeRoy EC, D'Angelo WA *et al*. Diagnostic potential of in vivo capillary microscopy in scleroderma and related disorders. Arthritis Rheum 1980;23:183-9.
16. Kahaleh MB. Vascular involvement in systemic sclerosis (SSc). Clin Exp Rheumatol 2004;22:S19-23.
17. Prescott RJ, Freemont AJ, Jones CJ, Hoyland J, Fielding P. Sequential dermal microvascular and perivascular changes in the development of scleroderma. J Pathol 1992;166:255-63.
18. Flavahan NA. Regulation of vascular reactivity in scleroderma: new insights into Raynaud's phenomenon. Rheum Dis Clin N Am 2008;34:81-7.
19. Kurki TS, Piirainen HI, Kurki PT. Non-invasive monitoring of finger arterial pressure in patients with Raynaud·s phenomenon: effects of exposure to cold. Br J Anaesth 1990;65: 558-63.
20. Clark S, Dunn G, Moore T, Jayson M IV, King TA, Herrick AL. Comparison of thermography and laser Doppler imaging in the assessment of Raynaud's phenomenon. Microvasc Res 2003;66:73-6.
21. Kayser C, Pucinelli MLC, Fontenelle SMA, Andrade LEC. Cold stimulus-fingertip lacticemy: standardization of the test in normal volunteers and diagnostic application for systemic sclerosis. Microvasc Res 2005;70:84-9.
22. Fontenelle SMA, Kayser C, Pucinelli MLC, Andrade LEC. Cold stimulus-fingertip lacticemy test – an effective method to monitor acute nifedipine effects on primary Raynaud's phenomenon and systemic sclerosis. Rheumatology 2008;47:80-3.

23. Pucinelli MLC, Fontenelle SMA, Andrade LEC. Determination of fingertip lacticemy before and after cold stimulus in patients with primary Raynaud's phenomenon and systemic sclerosis. J. Rheumatol 2002;29:1401-03.
24. Anderson ME, Moore TL, Hollis S, Jayson MI, King TA, Herrick AL. Digital vascular response to topical glyceryl trinitrate, as measured by laser Doppler imaging, in primary Raynaud's phenomenon and systemic sclerosis. Rheumatology 2002;41:324-8.
25. Salsano F, Letizia C, Proietti M *et al*. Significant changes of peripheral perfusion and plasma adrenomedullin levels in N-acetylcysteine long term treatment of patients with sclerodermic Raynaud´s phenomenon. Int J Immunopathol Pharmacol 2005;18:7761-70.
26. Picart C, Carpentier PH, Brasseur S, Galliard H, Piau JM. Systemic sclerosis:blood rheometry and laser Doppler imaging of digital cutaneous microcirculation during local cold exposure. Clin. Hemorheol 1998;18:47-58.
27. White CJ, Phillips WA, Abrahams LA, Watson TD, Singleton PT Jr. Objective benefit of nifedipine in the treatment of Raynaud's phenomenon. Double-blind controlled study. Am J Med 1986;80:623-5.
28. Engelhart M, Kristensen JK. Colour changes during Raynaud's phenomenon and finger blood supply during direct and indirect cooling procedures. Clin Exp Dermatol 1987;12:339-42.
29. Rademaker M, Cooke ED, Almond NE *et al*. Comparison of intravenous infusions of iloprost and oral nifedipine in treatment of Raynaud's phenomenon in patients with systemic sclerosis: a double blind randomised study. BMJ 1989;298:561-4.
30. O'Reilly D, Taylor L, El-Hadidy K, Jayson MIV. Measurement of cold challenge responses in primary Raynaud's phenomenon and Raynaud's phenomenon associated with systemic sclerosis. Ann Rheum Dis 1992;51:1193-6.
31. Cleophas TJ, Fennis JF, van't Laar A. Finger temperature after a finger-cooling test: influence of air temperature and smoking. J Appl Physiol 1982;52:1167-71.
32. Bartelink ML, Wollersheim H, Leesmans E, De Boo Th, Thien Th. A standardized finger cooling test for Raynaud›s phenomenon: diagnostic value and sex differences. Eur Heart J 1993;14:614-22.