Assessment of skin microcirculation by laser Doppler flowmetry in systemic sclerosis patients

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Abstract

Introduction: First lesions to occur in the course of systemic sclerosis (SSc) involve microcirculation.
Aim: The study involved assessment of the suitability of laser Doppler flowmetry (LDF) in examination of the performance of skin microcirculation in the distal portion of the upper extremity in SSc patients.
Material and methods: Overall the study involved 27 patients with systemic sclerosis. The control group comprised age – and gender-matched 27 healthy individuals. All the study subjects underwent microcirculation perfusion measurement at rest (rest flow – RF) as well as microcirculatory flow challenge tests – reactive hyperaemia test (RHT) and thermal stimulation test (TST).
Results: The study did not show any differences in the skin microcirculation perfusion at rest between the test group and the control, while reactive hyperaemia test results revealed significantly lower skin microcirculation perfusion values during the cuff inflation in SSc patients, as compared to the controls. In the test group, a lower perfusion value was observed during secondary hyperaemia following cuff release. Comparative analysis of skin microcirculation perfusion changes during the thermal stimulation test revealed a significantly lower change of the perfusion value and longer time of return to the baseline in the test group as compared to the control group.
Conclusions: The study performed has shown the suitability of LDF in the assessment of the microangiopathy degree in systemic sclerosis patients. The skin perfusion value in SSc patients should be assessed on the basis of parameters obtained in microcirculation challenge tests.

Key words: sclerosis, scleroderma, microangiopathy, laser Doppler flowmeter.

Introduction

Systemic sclerosis (SSc) is a severe, chronic connective tissue disease. One of the first symptoms in the course of systemic sclerosis includes lesions in blood vessels of skin and internal organs. Lesions occurring in skin mcrocirculation manifest clinically as Raynaud's phenomenon that consists of reversible contraction of microcirculatory vessels in response to cold or emotional stress [1]. Frequent relapses of Raynaud's phenomenon result in interrupted blood inflow to tissues and, consequently, their ischaemia involving formation of necrosis and painful trophic ulcers on finger and toe pulps [1, 2]. In more advanced cases it may result in the lysis of distal phalanxes. Systemic sclerosis can be divided into two basic categories: limited systemic sclerosis or acrosclerosis (lSSc), where skin hardening lesions do not exceed 1/3 of the forearm length and occur also on the face and diffuse systemic sclerosis (dSSc) with generalised hardening [3].

Aim

The study provided an attempt to assess the suitability of laser Doppler flowmetry (LDF) in examination of the performance of skin microcirculation in the distal portion of the upper extremity in SSc patients.

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Material and methods

The study involved overall 27 systemic sclerosis patients (mean age 54.5 ±11.1 years; median: 52 years), including 17 patients with lSSc and 10 patients with dSSc; diagnoses were made on the basis of ARA 1980 criteria. Duration of the disease of 3–22 years (mean 11 ±7.5 years; median: 87 years) was determined on the basis of the onset of skin hardenings.

The control group comprised age – and gender-matched 27 healthy individuals (mean age 55.7 ±9.7 years; median: 55 years). Both the patients and individuals comprising the control group were informed about the aim of the study, which was conducted following the approval granted by the Bioethics Committee at the Medical University of Lodz (No. RNN/332/06/KB dated 26 Sep. 2006). The study subjects consented in writing to their participation.

Patients with lSSc (16 women and 1 man, aged 38–77 years) received vasodilating agents (calcium channel antagonists, benzodiazepines or angiotensin receptor antagonists, sometimes together with pentoxifylline) and vitamin E. Patients with diffuse systemic sclerosis (8 women and 1 man, aged 35–70 years) received immunosuppressive therapy (low-dose corticosteroids – prednisone at 0.5 mg/kg bw/day) in monotherapy or in combination with a cytostatic (cyclophosphamide at 1.5 mg/kg bw/day), as well as, similarly to limited systemic sclerosis patients, vasodilating agents.

All the SSc patients were asked about the duration of Raynaud's phenomenon occurrence when taking history. As no statistically significant differences in the duration of Raynaud's phenomenon occurrence in both clinical forms of sclerosis were found (mean 11.0 ±7.5 years; median: 8, though that period in lSSc patients and dSSc patients was: mean 14.2 ±9.4 years; median: 12.5 years and mean: 8.7 ±5.1 years; median: 7.5 years, respectively), all the patients were included into perfusion analysis as one group, without division into clinical forms.

Skin microcirculation perfusion was measured by means of PeriFlux System 5000 apparatus (Perimed, Sweden) generating laser light of wavelength 780 nm, able to penetrate skin up to 0.5–1 mm depth. The microcirculation perfusion value was expressed in relative scale of perfusion units (PU) and registered with PERISOFT software that enabled its analysis. Blood flow in skin was measured with a standard probe located on the dorsal side of the distal phalanx of the left index finger. The probe tip was enclosed in a rigid housing that adhered tightly to the skin and prevented any movements of the probe relative to the skin. The test was performed in a room of constant ambient temperature 20–23°C; before the test the patient was placed in a sitting position for 30 min to enable adaptation to the conditions in the room.

Following 5-minute continuous measurement of rest flow (RF), flow challenge tests were performed. The reactive hyperaemia test (RHT) consisted of the measurement of minimum flows with a sphygmomanometer cuff tightened on the left arm and inflated to 200 mm Hg for 60 s. Following its release, maximum flows were recorded. The thermal stimulation test (TST) consisted of cooling the patient's hand to 15°C and then local warming of a finger using a probe heated to 44°C.

Measurements of microcirculation perfusion of upper extremity distal parts in both test and control groups were performed in similar external weather conditions, in the afternoon, approximately 1 h after a meal.

Statistical analysis

Statistical analysis of the study groups involved calculation of structure factors. The factors were not expressed as percentages, due to small groups, but as fractions. For quantitative variables, arithmetic mean ($\bar{x}$), median (Me) and standard deviation (SD), as a measure of dispersion, were calculated. Minimum and maximum values also were provided.

The Mann-Whitney test was applied for the comparisons of the study subjects' age, duration of the disease, duration of Raynaud's phenomenon occurrence, as well as skin microcirculation perfusion values at rest and during the challenge tests. The assumed significance level was $p \leq 0.05$.

Results

The analysis of the test group for age and gender did not reveal any statistically significant differences ($p > 0.05$).

The mean value of rest flow in the systemic sclerosis patients was higher (47.00 ±46.35 PU) than in the control group (36.00 ±22.23 PU). However, the difference was statistically insignificant (Table 1, Figure 1).

Analysis of the microcirculation perfusion value during the RHT revealed statistically significant differences between both study groups. In majority of patients during cuff inflation, the minimal mean flow value in skin microcirculation was observed, and following cuff release, lower perfusion values were observed during secondary reactive hyperaemia. The mean value of skin microcirculation perfusion with a sphygmomanometer cuff tightened in the test and control groups was 8.00 ±3.49 PU and 11.425 ±2.99 PU, respectively ($p < 0.01$) (Table 1, Figure 2). On the other hand, the mean value of skin microcirculation perfusion following cuff release in the test and control groups was 105.00 ±78.63 PU and 223.50 ±71.99 PU, respectively ($p < 0.001$) (Table 1, Figure 3).

Similarly, challenge tests of microcirculation flow during the TST revealed statistically significant differences between both study groups. Change of the skin microcirculation perfusion index during warming of a previously cooled finger in the test and control groups was 3.130 ± 6.87 PU and 14.551 ±6.09 PU, respectively ($p < 0.001$) (Table 1,

Table 1. Value of skin microcirculation flow at rest (RF), skin microcirculation perfusion in the reactive hyperaemia test (RHT) and thermal stimulation test (TST) in test and control groups (in PU)

Group	RF		RHT				TST			
			Minimum flows		Maximum flows		Index of microcirculation perfusion change		Time of return of skin microcirculation to baseline	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Test	47.00	46.35	8.00	3.49	105.00	78.63	3.13	6.87	26.88	29.25
Control	36.00	22.23	11.42	2.99	223.50	71.99	14.55	6.09	2.82	0.85
Value of *p*	> 0.05		< 0.01*		< 0.001*		< 0.001*		< 0.03*	

**Statistically significant differences*

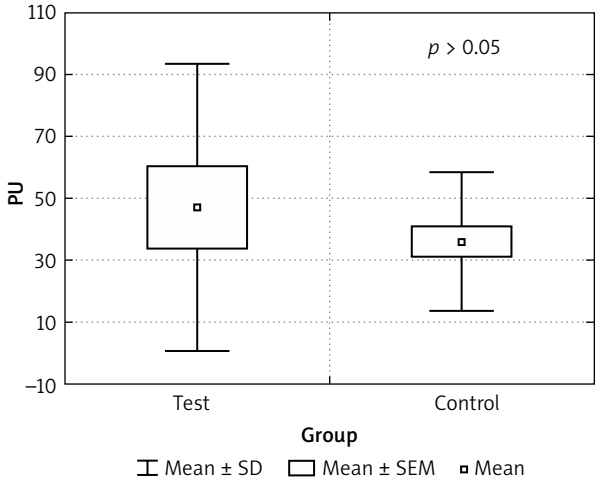

Figure 1. Skin microcirculation flow values at rest in the test and control groups

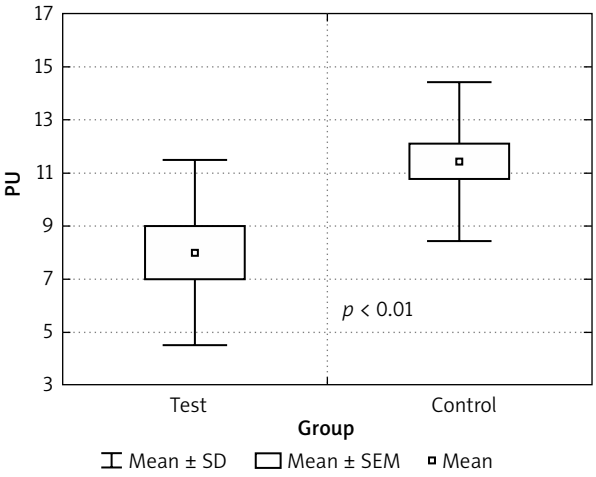

Figure 2. Mean value of skin microcirculation perfusion with a sphygmomanometer cuff tightened in the test and control groups

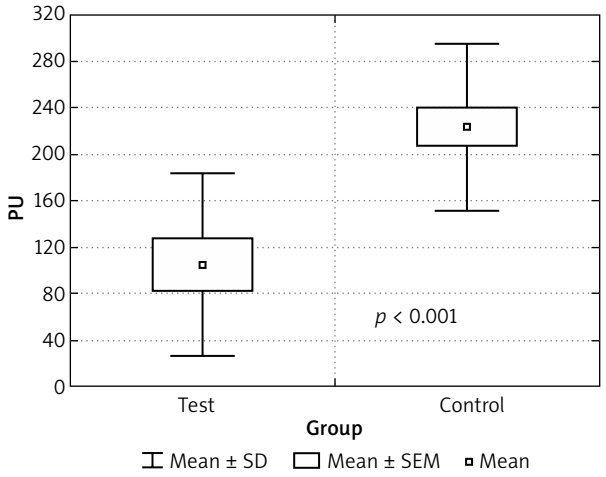

Figure 3. Microcirculation perfusion value following cuff release in the test and control groups

Figure 4). Time of return of microcirculation perfusion to baseline during warming of a previously cooled finger in the test and control groups was 26.883 ±29.25 min and 2.820 ±0.85 min, respectively ($p < 0.03$) (Table 1, Figure 5). In the challenge test involving finger cooling and warming, in the test group, a lower change of the microcirculation perfusion value and longer time of return to baseline during provoked temperature difference 15–44°C were observed.

In the flow challenge tests regular microcirculation response was observed only in the control group subjects, while impaired skin microcirculation response was observed in all the test group subjects. Differences between the study groups were statistically significant ($p < 0.05$) (Table 2).

Strongly disturbed microcirculation response was observed in 14 patients ($f = 0.52$), while moderately disturbed response in 13 ones ($f = 0.48$). The systemic sclerosis patients were divided into two subgroups, depending on the degree on microcirculation changes development: A (with strongly disturbed skin microcirculation response) and B (with moderately disturbed skin microcirculation response). Group A comprised 10 lSSc patients and 4 dSSc patients, while group B comprised 5 lSSc patients and 8 dSSc patients.

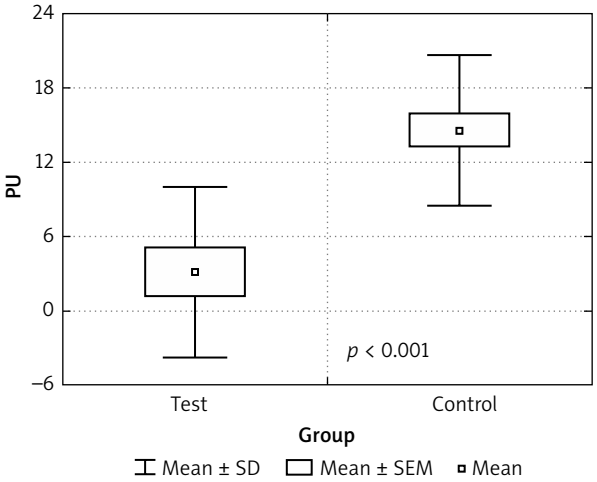

Figure 4. Analysis of changes of microcirculation perfusion value during warming of a previously cooled finger in the test and control groups

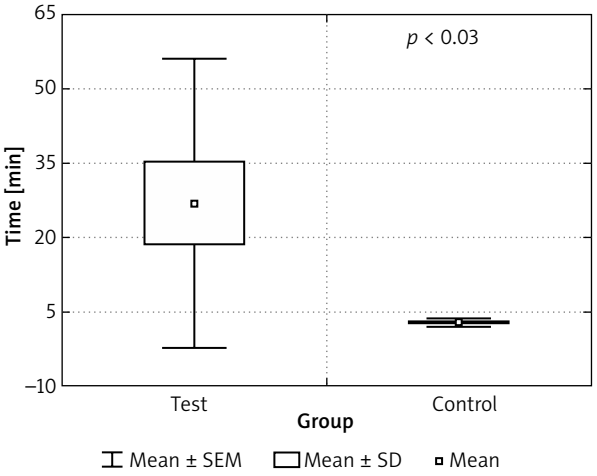

Figure 5. Time of return of skin microcirculation to baseline during warming of a previously cooled finger in the test and control groups

Table 2. Division of the test group in relation to the degree of microcirculation changes development

Test group (n = 27)	Clinical form of sclerosis	Microcirculation reactions	Fraction (f)	Significance level p (group A/group B)
A (n = 14)	lSSc (n = 10)	Strongly disturbed	0.52	< 0.05*
	dSSc (n = 4)			
B (n = 13)	lSSc (n = 5)	Moderately disturbed	0.48	
	dSSc (n = 8)			

**Statistically significant differences*

Group A comprised patients with low rest values of microcirculation perfusion (–3 SD relative to mean for the control group). Following cuff release a small increase in hyperaemia (–3 SD relative to mean for the control group) was observed, while slow and small change amplitude was observed in the thermal stimulation test. In the thermal stimulation test, microcirculation perfusion in these patients returned to baseline after 60 min.

Group B comprised patients with rest values of microcirculation perfusion similar to those observed in the control group. Following cuff release a significant increase in hyperaemia (–2 SD relative to mean for the control group) was noted, while slow and decreased change amplitude was observed in the thermal stimulation test. In the thermal stimulation test, microcirculation perfusion in these patients returned to baseline after 15 min.

Summary of results: the study did not show any differences in the skin microcirculation perfusion at rest between the test group and the control group. Reactive hyperaemia test results revealed significantly lower skin microcirculation perfusion values during the cuff inflation in SSc patients, as compared to the controls. In the patient group, a lower perfusion value was observed during secondary hyperaemia following cuff release. Comparative analysis of skin microcirculation perfusion changes during the thermal stimulation test revealed significantly lower change of the perfusion value and longer time of return to the baseline in the test group as compared to the control group. The results of challenge tests of flow indicate the possibility to divide SSc patients according to the degree of vascular changes development, which may be of importance for prognosis, as well as for the selection of optimal therapy.

Discussion

The earliest lesions to occur in the course of system sclerosis involve microcirculation, that is small arterial vessels and capillaries, that become occluded and then atrophied. At an early stage of the disease, inflammatory infiltrations develop around vessels involved, and cytokines and reactive oxygen species released from them provide one of the factors contributing to angiogenesis disturbances. Vascular loops become distorted, and endothelial damage results in the increase in vessel permeability and abnormal tone of their walls [1, 4].

In our own studies, microcirculation perfusion in systemic sclerosis patients was determined within the skin of the dorsal side of the distal phalanx of the left index

finger. For all the subjects of the study and test groups, the right upper extremity was the dominant one.

Results obtained by other authors determining the mean value of rest flow in skin microcirculation in distal phalanxes in systemic sclerosis patients are equivocal. The authors have proven that the value may be higher [5] or lower than results obtained in healthy individuals [6–8]. In our own studies, the mean value of rest flow in the patient group was similar to the control.

The results of our studies and literature data confirm that the microcirculation perfusion value determined at rest does not allow reliable assessment of the degree of lesion development at the capillary level. Perfusion challenge tests, which force the skin vascular bed for maximum dilatation or contraction in response to the challenge stimulus applied, provide much more information about the functional status of microcirculation. Thus, flow challenge tests, namely the reactive hyperaemia test and thermal stimulation test, are used for objective assessment [9, 10].

It has been demonstrated that microcirculation response to reactive hyperaemia or local warming depends at an early stage on neurogenic regulation and on metabolic-mediated one at a later stage. Literature data indicate that nitric oxide is the primary causative factor [6, 11, 12]. Microcirculation response in the cooling test indicates myogenic auto-regulation of the vascular lumen diameter [13, 14]. Perhaps the degree of response to post-occlusive or thermal burden to microcirculation indicates the degree of capillary damage.

Our own results concerning impaired hyperaemic response following cuff application in systemic sclerosis patients are consistent with previous literature data [5, 6]. In our own studies, the skin microcirculation perfusion value was decreased after cooling in the majority of SSc patients; however, the value did not return to the baseline before 15 min in any patient of the test group. In 52% of the patients, a slow return of perfusion as late as after 60 min was observed. Available study results concerning microcirculation response in the cooling test may suggest that reduction in perfusion of capillary network occurs in healthy individuals, as well as in patterns with primary or secondary Raynaud's phenomenon. In healthy individuals, the perfusion value always returned to the baseline within 15 min, while in systemic sclerosis patients, the return to the baseline required much more time. On the other hand, in patients with primary Raynaud's phenomenon, the results were within the intermediate range or similar to those in the control group [13, 14].

Data analysis should also take into account the fact that, due to the severity and chronic nature of the disease, almost all the patients examined took vasodilating agents before the study, some of them in combination with immunosuppressive agents, which may affect the results obtained to some extent.

Conclusions

Results of our studies indicate that the microcirculation perfusion value determined at rest does not allow reliable assessment of the degree of lesion development on the capillary level. The challenge test results obtained in SSc patients indicate persistent capillary contraction, abnormal tone of their wall and lack of neurogenic, myogenic and metabolic auto-regulation. Obtained values of microcirculation perfusion change in response to post-occlusive or thermal burden to microcirculation indicates the degree of capillary damage. The study results confirm the suitability of LDF in the assessment of the degree of microangiopathy development in systemic sclerosis patients. Precise assessment of the skin perfusion value in SSc patients should be only performed on the basis of parameters obtained in microcirculation challenge tests.

Acknowledgments

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