Peripheral Blood Perfusion Correlates with Microvascular Abnormalities in Systemic Sclerosis: A Laser-Doppler and Nailfold Videocapillaroscopy Study

MAURIZIO CUTOLO, CARMELA FERRONE, CARMEN PIZZORNI, STEFANO SOLDANO, BRUNO SERIOLO, and ALBERTO SULLI

ABSTRACT. ***Objective.*** To investigate possible correlations between fingertip blood perfusion (FBP) status, assessed by laser Doppler flowmetry (LDF), and morphological microvascular abnormalities, detected by nailfold videocapillaroscopy (NVC), in patients with systemic sclerosis (SSc). The effects on FBP of intravenous (IV) treatment with the prostacyclin analog iloprost were also investigated.

Methods. Thirty-four consecutive patients with SSc and 16 healthy subjects were evaluated. LDF was performed by analyzing blood perfusion at the fingertips in both hands. Patients with SSc were distributed into the appropriate NVC pattern of microangiopathy (early, active, and late). Iloprost was administered to inpatients with SSc by 24-hour IV infusion for 7 consecutive days (4 μg/h).

Results. FBP was significantly lower in patients with SSc ($p < 0.05$) compared to controls. Heating of the LDF probe at 36°C induced a significant increase of FBP in all subjects ($p < 0.001$), but the slope of variation was significantly lower in patients with SSc compared to controls ($p < 0.05$). Patients with SSc showing the late NVC pattern of microangiopathy had significantly lower FBP than patients with the active and early NVC patterns ($p < 0.05$). A negative correlation was observed between FBP and NVC rating of the microvascular damage ($p < 0.05$). After iloprost treatment, a significant increase of FBP was observed in patients with SSc ($p < 0.05$).

Conclusion. Patients with SSc show a decreased FBP partially reversible by local skin heating. The FBP correlated negatively with the extent of nailfold microvascular damage, and IV iloprost treatment increased the FBP. (First Release May 1 2010; J Rheumatol 2010:37; 1174–80; doi:10.3899/jrheum.091356)

Key Indexing Terms:
CAPILLAROSCOPY SYSTEMIC SCLEROSIS PERIPHERAL BLOOD FLOW
LASER DOPPLER FLOWMETRY ILOPROST MICROANGIOPATHY

Systemic sclerosis (SSc) is characterized by early and persistent microvascular impairment leading to functional [Raynaud's phenomenon (RP)] and organic manifestations (i.e., digital ulcers). Progressive deficiency in vasodilatory capacity of the vessels is proposed as a mechanism of the persistent vascular spasm; however, the mechanism of endothelial injury is still unclear[1]. Digital ulcers are considered to be related to tissue ischemia following several processes, including vasospasm, intimal fibroproliferation, and thrombosis of digital arteries[2].

The assessment of vascular involvement is still a matter of study, and several noninvasive techniques have been proposed. Peripheral microvascular impairment in SSc may be easily detected by nailfold videocapillaroscopy (NVC), and the morphological capillary abnormalities are classified into different patterns of microangiopathy and scored[3,4]. NVC may partially observe the column of red blood cells moving inside the capillary, but the technique does not allow measurement of the blood flow. Laser Doppler flowmetry (LDF) is the best technique to assess and to measure the blood perfusion at peripheral sites[5,6]. Blood flow has been found to be reduced in patients with SSc, compared with healthy subjects and patients with primary RP[6,7].

Recently, abnormal microvascular regulatory responses to hyperemia, neurovascular reflex, and iontophoresis were demonstrated in patients with SSc with a higher Medsger vascular score, suggesting that the degree of endothelial dysfunction might be related to the degree of peripheral microvascular involvement[8,9].

Our aim was to assess possible correlations between the altered blood perfusion at fingertips analyzed by LDF and the morphological microvascular abnormalities detected by NVC in patients with SSc. In addition, the effects of intravenous (IV) iloprost treatment (stable analog of prostacyclin

From the Research Laboratory and Academic Unit of Clinical Rheumatology, Department of Internal Medicine, University of Genoa, Genoa, Italy.

M. Cutolo, MD, Full Professor, Director, Research Laboratory and Academic Unit of Clinical Rheumatology; C. Ferrone, MD, Resident; C. Pizzorni, MD, Resident; S. Soldano, BS, Research Laboratory Assistant; B. Seriolo, MD, Academic Clinical Researcher; A. Sulli, MD, Academic Clinical Researcher, University of Genoa.

Address correspondence to Dr. M. Cutolo, Research Laboratory and Academic Unit of Clinical Rheumatology, Department of Internal Medicine, University of Genova, Viale Benedetto XV, no. 6, 16132 Genova, Italy. E-mail: mcutolo@unige.it

Accepted for publication January 27, 2010.

PGI2) on fingertip blood perfusion (FBP) were investigated in patients with SSc according to their SSc pattern.

MATERIALS AND METHODS

Patients. Thirty-four consecutive women with SSc (mean age ± SD 64 ± 12 years, mean RP duration 14 ± 12 years, mean SSc duration 7 ± 7 years; Table 1) and 16 healthy subjects (mean age 61 ± 20 years) were enrolled into the study. The controls were recruited from the Rheumatologic Outpatient Clinic and had been referred for localized soft-tissue diseases (mainly soft-tissue painful conditions). The patients met the American College of Rheumatology criteria for SSc, or the criteria for the classification of early SSc[10,11]. Patients were recruited from the Rheumatologic Service for the Diagnosis and Management of Vascular and Connective Tissue Diseases at the University of Genoa. Informed consent was obtained from all patients.

A complete medical history and comprehensive laboratory examinations were obtained from all patients, and a full medical examination was performed. All patients complained of secondary RP. The duration of both RP and SSc was evaluated by clinical interview and from clinical file data; the duration of SSc was calculated from the time of onset of clinical signs or symptoms clearly related to SSc (other than RP, i.e., skin fibrosis, dysphagia, etc.), or from the time of positive instrumental and/or laboratory investigations confirming the diagnosis (i.e., NVC, esophageal and/or pulmonary evaluation, detection of disease-specific serum autoantibodies). Esophageal evaluation was performed by manometry, pulmonary function by lung volume tests, diffusion capacity for carbon monoxide, and computed tomography; cardiac performance was investigated by Doppler echocardiography, renal function by laboratory tests and arterial Doppler echography.

All patients with SSc and healthy subjects were investigated by both NVC and LDF, in order to detect the morphological and functional microvascular status. LDF was performed in inpatients with SSc at basal time, after 24 hours, and after 1 week of continuous IV infusion of iloprost, in order to explore the reactivity of the microvascular circulation. NVC was performed at basal time and after 7 days of continuous IV iloprost infusion.

Any vasodilator drug or pharmacological treatment possibly interfering with vascular function was stopped 1 month before the clinical and instrumental examinations; this did not affect patient selection or recruitment. None of the patients was taking immunosuppressive agents for 3 months before the study. Patients had no concomitant serious systemic diseases other than SSc.

NVC was performed in each inpatient using a videocapillaroscopy optical probe equipped with a 200× contact lens and connected to image analysis software (Videocap, DS MediGroup, Milan, Italy). The same operator performed the NVC examination during the followup. Each subject was inside the building for a minimum of 15 minutes before the nailfold was examined, and the room temperature was 22–23˚C. The nailfolds of the second, third, fourth, and fifth digit were examined in each patient, after a drop of immersion oil was placed on the nailfold bed to improve the image resolution.

The first line of capillaries at the nailfold was evaluated, and the following capillaroscopic measurements were considered, according to previous observations: presence of enlarged and giant capillaries, hemorrhages, loss of capillaries, disorganization of the microvascular array, and capillary ramifications[12,13]. The measurements were defined as (1) enlarged capillary: an increase in capillary diameter (homogeneous or irregular) > 20 μm; (2) giant capillary: homogeneously enlarged loops with a diameter > 50 μm; (3) microhemorrhage: dark mass due to hemosiderin deposit; (4) loss of capillaries: reduction of the capillary number below normal range (the normal range was adopted from the literature: 9 capillaries per linear mm, counted at the first line of the nailfold, was chosen as normal lower limit); (5) disorganization of the microvascular array: irregular capillary distribution and orientation, along with shape heterogeneity of the loops; and (6) capillary ramifications: branching, bushy, or coiled capillaries, often originating from a single normal-size capillary[13-15].

A semiquantitative rating scale to score each capillary abnormality was adopted, according to previous studies (score 0–3 for every measurement: 0, no changes; 1, < 33% capillary alterations/reduction; 2, 33%–66% capillary alterations/reduction; 3, > 66% capillary alterations/reduction, per linear mm)[3,4]. The microangiopathy evolution score was also calculated (composite score 0–9, obtained from the sum of the scores for these measurements: loss of capillaries, disorganization of the microvascular array, and capillary ramifications)[4].

Based on the NVC abnormalities, patients with SSc were distributed into the appropriate NVC pattern, as described[3,12]. The patterns were (1) early NVC pattern (9 patients): few (< 33%) giant capillaries, few capillary hemorrhages, relatively well preserved capillary distribution, no evident loss of capillaries; (2) active NVC pattern (11 patients): frequent (> 66%) giant capillaries, frequent capillary hemorrhages, moderate (< 33%) loss of capillaries, mild (< 33%) disorganization of the capillary architecture, absent or mild ramified capillaries; and (3) late NVC pattern (14 patients): irregular enlargement of the capillaries, few or absent giant capillaries and hemorrhages, severe (> 66%) loss of capillaries with avascular areas, disorganization of the normal capillary array, and ramified/bushy capillaries.

Laser Doppler flowmetry. LDF was performed the same day, following the NVC examination, by Periflux System 5000 equipped with a thermostatic probe (Perimed, Milan, Italy), and each subject was inside the building at least 45 minutes before the assessment, at a room temperature of 22–23˚C. The subjects waited until the stated examination time seated in a waiting room. LDF was carried out analyzing the blood perfusion at second, third, fourth, and fifth fingertip bilaterally (central area), both at basal finger temperature and after heating the probe to 36°C. At basal temperature, FBP measurement was started 30–60 s after probe positioning on the central area of each fingertip, waiting for the minimal variation of the perfusion wave, and the recording was continued for 1 min in each finger; during the recording time the patient was relaxed and no noise was allowed. Subsequently the probe was heated to 36°C and a new assessment started. In the thermostatic probe the optical fibers are integrated in the heated area and thus the whole tissue area under the probe may be heated during blood perfusion measurement, to assess capillary dilation capacity. Using the thermostatic probe, perfusion assessment was started 1 min after the positioning of the probe on the fingertip: over this time, no further variation of blood perfusion due to heating was observed in our previous experiments, and the perfusion wave maintained a sinus form. The recording was continued for 1 min in every finger. In each patient, mean blood perfusion from the 8 digits was calculated, as well as the mean integration of the blood perfusion (area under the perfusion-time curve), by recording the Doppler signal. In particular, the mean value of blood perfusion was calculated by adding together the average perfusion values from the 8 fingers, and dividing the final value for 8 fingers. Results are expressed as perfusion units (PU)[16], and reported as median and interquartile range (IQR).

The reproducibility of LDF assessment was 90%. Different operators blindly performed LDF and NVC examinations.

Iloprost treatment. Iloprost was administered to inpatients with SSc by 24-h IV infusion for 7 days, following current local practice (4 μg/h, in physiological solution). Inpatients with SSc had LDF before, 1 day after, and 7 days after iloprost treatment. NVC was also performed at time 0 and day 7.

Autoantibody detection. Antinuclear antibodies (ANA), antitopoisomerase I (anti-Scl-70) antibodies, and anticentromere antibodies (ACA) were determined in all patients. ANA were detected by indirect immunofluorescence using HEp-2 cells as substrate (Euroimmun, Luebeck, Germany). Anti-Scl-70 antibodies and ACA were evaluated using ELISA (Euroimmun).

Statistical analysis. The statistical analysis was carried out by nonparametric tests. The Wilcoxon test was performed to compare paired groups and Mann-Whitney U test to compare unpaired groups of variables. Possible correlations between variables were assessed by Spearman rank correlation test. A p value < 0.05 was considered statistically significant.

RESULTS

FBP was significantly lower in patients with SSc at basal temperature ($p = 0.05$) and at 36°C [$p = 0.01$; median 29 (IQR 44) and 58 (IQR 74) PU, respectively], compared to healthy controls at basal temperature and at 36°C [median 123 (IQR 195) and 183 (IQR 192) PU, respectively; Figure 1]. The heating of the probe at 36°C induced a significant increase of FBP in all subjects ($p < 0.001$), but the magnitude of variation (difference between the means at the 2 temperatures) was significantly lower in patients with SSc compared to

Table 1. Main clinical and laboratory measures of the study patients with systemic sclerosis.

Measures	n = 34
Age, yrs ± SD	64 ± 12
Raynaud's duration, yrs ± SD	14 ± 12
SSc duration, yrs ± SD	7 ± 7
lcSSc	59%
dcSSc	41%
Ulcers	34%
Lung involvement	23%
Renal involvement	17%
Pulmonary hypertension	14%
Esophageal involvement	43%
ACA positivity	35%
Scl70 positivity	28%
ANA positivity	88%

SSc: systemic sclerosis; lcSSc: limited cutaneous SSc; dcSSc: diffuse cutaneous SSc; ACA: anticentromere antibodies; ANA: antinuclear antibodies.

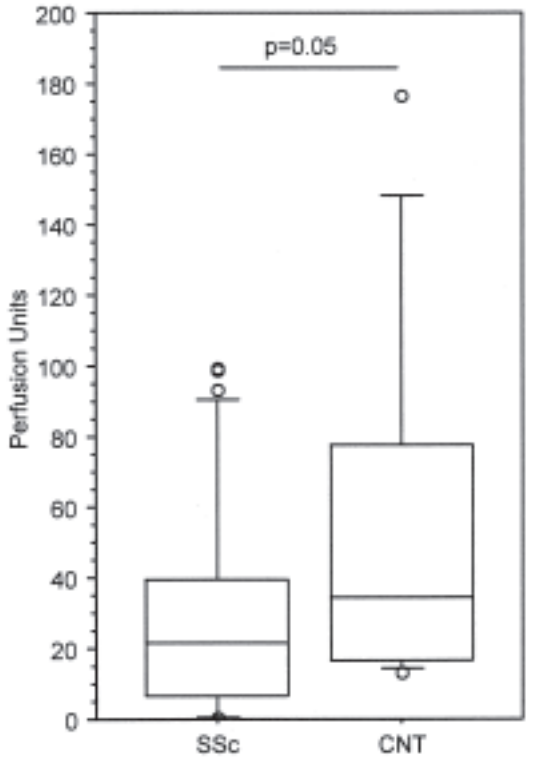

Figure 2. Magnitude of variation of fingertip blood perfusion from basal finger temperature to 36°C, in patients with SSc and controls (CNT). Results are reported as perfusion units. Data are given with the 5th, 10th, 50th (median), 90th, and 95th percentiles.

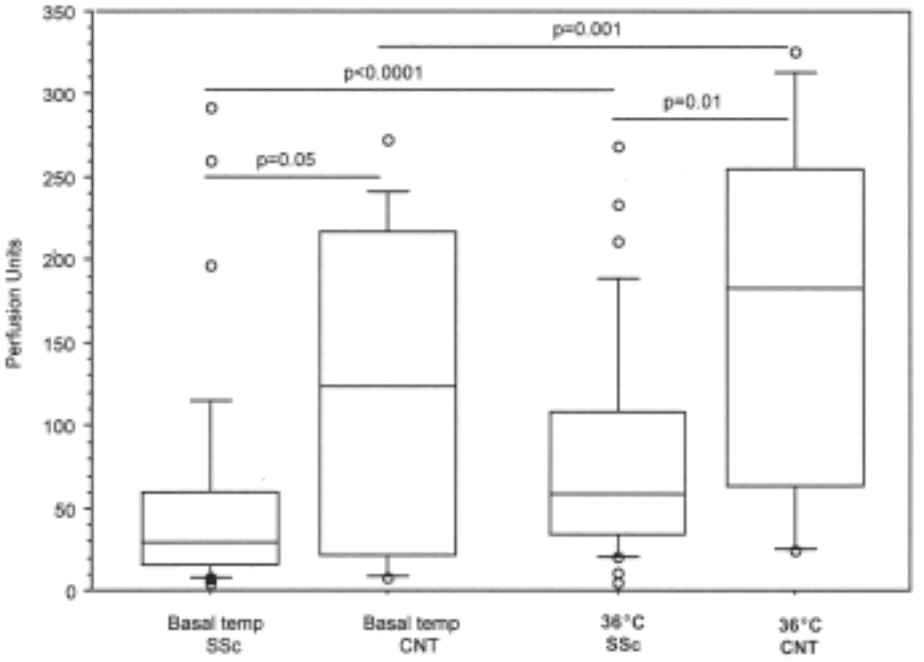

Figure 1. Fingertip blood perfusion in patients with SSc and controls (CNT) at basal temperature and after heating the probe to 36°C. Results are reported as perfusion units. Data are given with the 5th, 10th, 50th (median), 90th, and 95th percentiles.

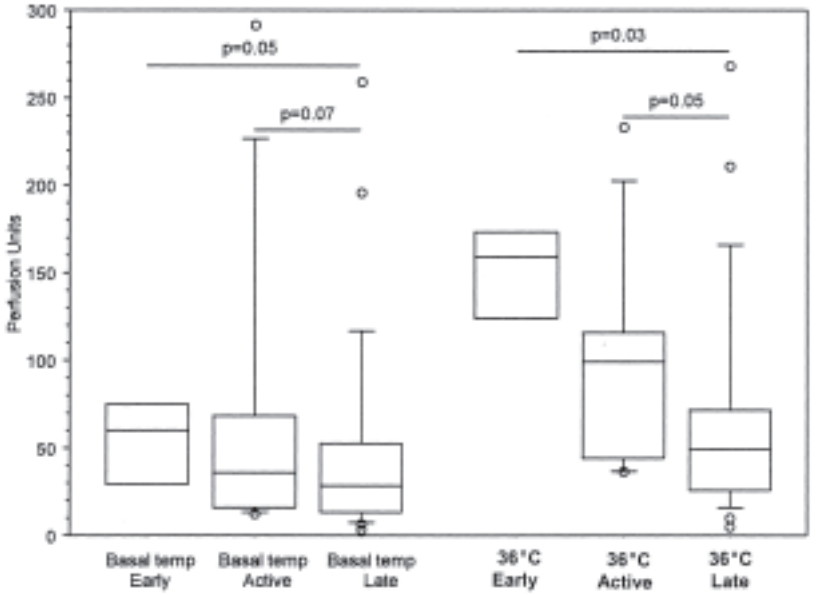

Figure 3. Fingertip blood perfusion in patients with SSc with different patterns of microvascular damage (early, active, and late), assessed by nailfold videocapillaroscopy, at basal temperature and after heating the probe to 36°C. Results are reported as perfusion units. Data are given with the 5th, 10th, 50th (median), 90th, and 95th percentiles.

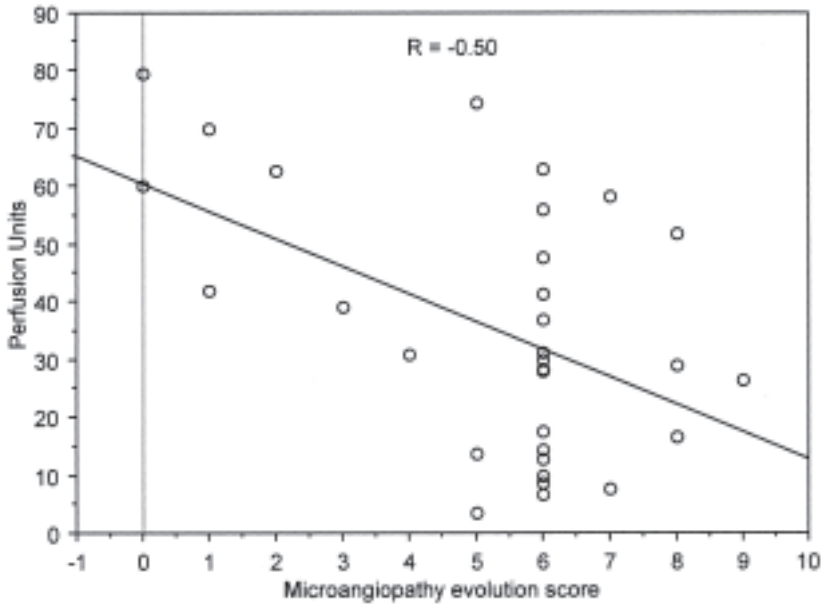

Figure 4. Correlation between fingertip blood perfusion and microangiopathy evolution score in patients with SSc, at basal temperature.

controls [median 22 (IQR 33) and 34 (IQR 61) PU, respectively; p = 0.05; Figure 2].

Patients with SSc who had the late NVC pattern of microangiopathy showed significantly lower (half as much) FBP than patients with the active and the early NVC patterns [median at basal temperature: 28 (IQR 40), 36 (IQR 53), 60 (IQR 45) PU, respectively; p = 0.05; Figure 3]. After heating the probe, the difference in FBP among the patterns became more evident: in patients with the late pattern SSc, it was half or even less, compared to the active (p = 0.05) and the early patterns [p = 0.03; median at 36°C: 49 (IQR 46), 99 (IQR 72), 159 (IQR 49) PU, respectively]. Further, a negative correlation was observed between the FBP and NVC microangiopathy evolution score (p = 0.04; Figures 4

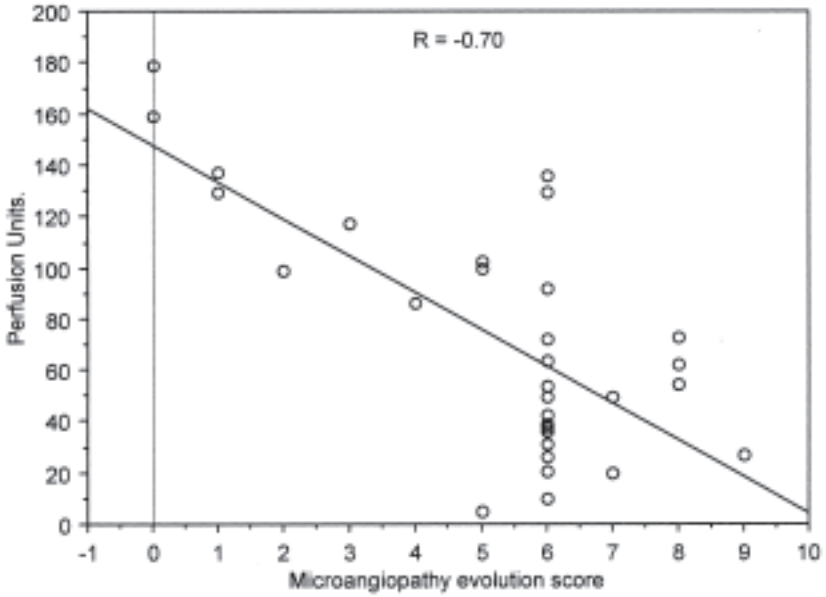

Figure 5. Correlation between fingertip blood perfusion and microangiopathy evolution score in patients with SSc, after heating the probe to 36°C.

and 5). Interestingly, a negative correlation was also observed between FBP and duration of RP ($p = 0.05$). Analyzing the integration of the FBP [area under the curve (AUC)], a similar statistical significance was observed.

Within the limit of the small study population analyzed, no statistically significant correlation was found between FBP and presence of pulmonary, esophageal, or renal involvement in patients with SSc; however, patients with a history or presence of digital ulcers showed significantly lower FBP ($p = 0.05$) at 36°C than those without ulcers [median 27 (IQR 47) and 72 (IQR 75) PU, respectively]. ANA were present in 90% of patients with SSc, but no sta-

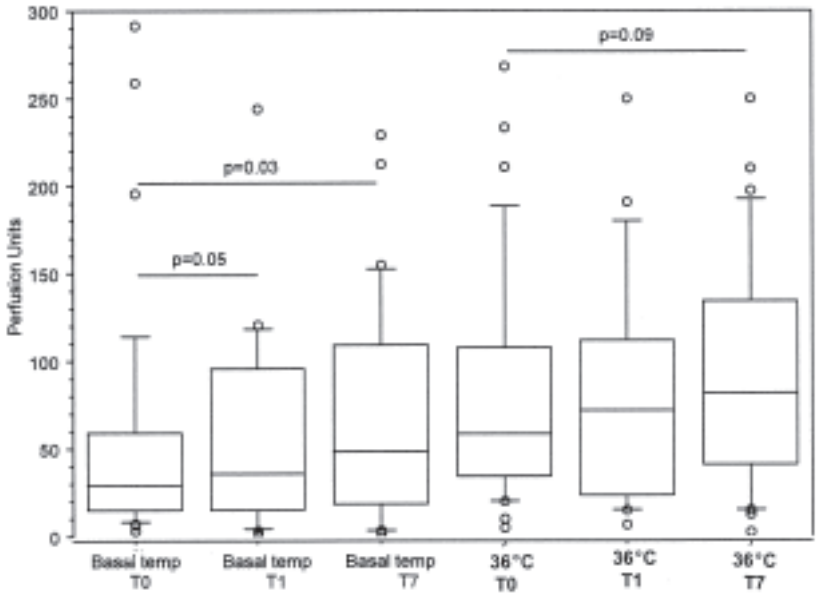

Figure 6. Fingertip blood perfusion in patients with SSc at basal time (T0) and after 24 hours (T1) and 7 days (T7) of intravenous iloprost infusion, at basal temperature and after heating the probe to 36°C. Results are reported as perfusion units. Data are given with the 5th, 10th, 50th (median), 90th, and 95th percentiles.

tistically significant correlation was observed between FBP and presence of either ACA or Scl-70 antibodies. No statistically significant difference in FBP was observed between patients with limited cutaneous SSc and those with diffuse cutaneous SSc.

Compared with the basal values [median 29 (IQR 44) PU], a significant increase of FBP was observed in patients with SSc after both 24 hours and 7 days of continuous iloprost treatment [median at basal temperature: 36 (IQR 80) and 48 (IQR 91) PU, respectively; $p = 0.05$ and $p = 0.03$; Figure 6]. The same trend was observed comparing the FBP values obtained after heating the probe at 36°C [median at 36°C: 58 (IQR 74), 71 (IQR 88), and 82 (IQR 93) PU, respectively], but the difference was not statistically significant ($p = 0.09$). The same statistical significance was observed analyzing the AUC.

No statistically significant changes were observed in the nailfold capillary abnormality scores, as assessed by NVC, after 7 days of continuous iloprost treatment. No serious adverse events were observed during iloprost infusions.

DISCUSSION

Our study clearly shows a correlation between the reduction of peripheral blood flow and the severity of nailfold microangiopathy in SSc. In particular, patients with SSc in the late NVC pattern of microangiopathy (characterized by advanced reduction of capillary number, capillary disorganization, and ramifications) showed a significantly lower FBP than patients with the active or the early NVC patterns.

Further, a statistically significant negative correlation was observed between FBP and microangiopathy evolution score. As recently demonstrated, tracking the scores of 3 capillaroscopic measurements (capillary loss, disorganization of microvascular array, and capillary ramifications) should be done together to survey the progression of SSc microangiopathy, since the relative scores usually increase during the progression of microvascular damage[4]. In addition, evaluation of the score for specific NVC measurements is useful to quantify the modifications of the SSc microangiopathy, when the qualitative variation between the different capillaroscopic scleroderma patterns (early, active, or late) is not evident. Qualitative and semiquantitative NVC scoring systems have been validated in a recent multicenter study[17]; the microangiopathy evolution score needs to be validated in larger studies. In summary, our investigation demonstrates a negative correlation between peripheral blood perfusion and the microvascular damage rating in SSc.

Our study confirms that patients with SSc are characterized by lower peripheral blood perfusion than healthy subjects. However, the low resting blood flow in patients with SSc seems reversible because the vascular reactivity to local heating seems to be partially preserved. Indeed, the FBP under thermal heating (probe at 36°C) increased in patients with SSc, even if the extent of the variation was lower than in healthy controls.

Recurrent handwarming in hot water has been proposed as a treatment for secondary RP in SSc, and is supported by the evidence of an increased blood flow associated with clinical improvement[18,19]. Vascular response to heating seems primarily localized to the fingers, since no increase in perfusion due to heating was found in patients with SSc or in controls when evaluated in the dorsum of the hand or in the forearm[20,21]. The localized reactivity seems related to a selective abnormality of the endothelial, sympathetic, and myogenic-dependent finger skin vasomotion, but it is still not clear whether the impairment reflects functional and/or structural microvascular damage[8,9,21,22].

Further studies need to be done to understand whether morphological nailfold capillary abnormalities are the cause or the effect of digital blood hypoperfusion in patients with SSc. Recently, plasma levels of the vasoactive endothelin (ET-1) molecule were found to be higher in the more advanced stage of SSc microangiopathy, i.e., the late NVC pattern, and this might support the involvement of ET-1 in the pathogenesis and progression of microvascular damage in SSc[23].

Our study, limited by the small sample size of patients with SSc, was not able to demonstrate any statistically significant correlations between FBP and pulmonary, esophageal, or renal involvement, or correlations with the presence of specific autoantibodies. Patients with SSc with a history or presence of digital ulcers showed significantly lower FBP.

Both the rate of blood flow and the velocity of blood flow recovery after cold provocation have been found to be lower in patients with SSc, so that cold exposure can be considered a further risk factor for ischemia, and it should be prevented in patients with SSc[24].

Iloprost has been shown to be particularly effective in the treatment of digital ulcers in patients with SSc[25]. In a study of patients with SSc that compared low-dose and high-dose iloprost therapy over 21 days, 12% of the patients did not respond to iloprost therapy, but 78% experienced a long-lasting effect with all dosages[25]. Our study demonstrated a significant increase of FBP after both 24 hours and 7 days of continuous IV iloprost treatment in 90% of patients with SSc, compared with their basal FBP values. A further possible role of iloprost in promoting neoangiogenesis in SSc has been shown recently[26-28]. Because our investigation did not consider a control group of patients not treated with iloprost and evaluated sequentially at the same timepoints (ideally there should have been a placebo-treated control group), the positive effects shown by iloprost remain to be confirmed by further controlled studies.

Further, our study did not evaluate the clinical symptom changes to iloprost treatment in patients with secondary RP, but evaluated only the FBP, in order to search for correla-

tions between functional and morphological instrumental detection of SSc microangiopathy.

A possible limit of our study is that it was designed to globally assess the peripheral vasculopathy at fingertips in single patients with SSc by evaluating the average perfusion/microangiopathy over 8 fingers. In fact, the microvascular impairment is not homogeneous in patients with SSc: mild capillary alterations in some digits usually coexist with severe alterations in others, as observed by NVC, and some patients may show single fingers affected by vasospasm more than others. However, even the evaluation of a single finger over the long term might add other important information, and further studies are needed to confirm these data over a larger number of patients.

Our study demonstrated that patients with SSc showed a reduced FBP that was partially reversible by local skin heating. Peripheral blood perfusion correlated negatively with the extent of the nailfold microvascular damage, being lower in patients with SSc with the late NVC pattern of microangiopathy. IV iloprost treatment increased peripheral blood perfusion, confirming its usefulness in treatment of SSc[29].

The predictive power of these evaluations (NVC and FBP) is of prognostic importance for SSc, and they are also useful for early diagnosis of the disease.

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