Blunted increase of digital skin vasomotion following acetylcholine and sodium nitroprusside iontophoresis in systemic sclerosis patients

M. Rossi, L. Bazzichi, C. Di Maria, F. Franzoni, K. Raimo, A. Della Rossa, G. Santoro and S. Bombardieri

Introduction

A number of studies [1–6] based on laser Doppler flowmetry (LDF) investigation of skin microcirculation have been recently performed in patients affected by SSc. The aim of these studies was to evaluate skin microcirculatory abnormalities in SSc, which have been suggested to be a primary vascular disease [7, 8] and to improve the understanding of its complex pathophysiology. In particular, LDF, together with iontophoresis of the endothelial-dependent vasodilator acetylcholine (ACh) and the endothelial-independent vasodilator sodium nitroprusside (SNP), has been used in SSc patients for evaluating the skin endothelial function [1–6]. The majority of these studies showed a reduced skin vasodilator response to both ACh and SNP iontophoresis in SSc patients [1, 2, 4, 5], a finding which cannot be formally ascribed to endothelial dysfunction, but only to a reduced skin microvascular wall compliance. A selective reduction in the skin vasodilator response to ACh, consistent with endothelial dysfunction, has been demonstrated only in two studies [3, 6]. Different LDF apparatus and iontophoresis protocols used in these studies, as well as different clinical characteristics of the studied patients, may explain these discrepancies.

More recently, spectral analysis of LDF tracings has been used for examining the cyclical variation in blood flow [9–12], the so-called flow motion, which has been demonstrated to be partly related to the periodic contraction and dilation of arterioles, the so-called vasomotion [13–15]. Vasomotion and the consequent skin blood flow motion have been suggested to have an important role in increasing the mean blood flow in the microvascular network by the functional recruitment of previously inactive microvascular units [16–18].

Spectral analysis of skin LDF tracing allows different spectral frequency ranges of flow motion to be identified in the total spectrum of 0.01–1.6 Hz. Three of them have been demonstrated to be selectively related to different mechanisms that control vasomotion: the LDF oscillations in the frequency range of 0.01–0.02 Hz, related endothelial activity [9–12], the LDF oscillations in the frequency range of 0.02–0.06 Hz, related to the local sympathetic activity [19, 20] and the LDF oscillations in the frequency range of 0.06–0.2 Hz, related to the spontaneous activity of the microvessel smooth muscle cells [9, 10]. The spectral amplitude of each of these LDF frequency range oscillations has been suggested to reflect the efficiency of the corresponding vasomotion mechanism [9–12]. Thus, this method has been recently proposed as a useful tool for investigating skin microvascular endothelial, sympathetic and myogenic function in clinical setting [11, 21]. A specific pattern of perturbed skin vasomotion was observed in hypertensive patients [21] and in the end-stage renal disease patients [22] during post-ischaemic reperfusion, as well as in diabetes patients under basal conditions [23, 24] in studies based on the spectral analysis of skin LDF tracing. On the contrary, a recent study based on the same method [25] showed no finger skin vasomotion (FSV) abnormalities in SSc patients under basal conditions. Nevertheless, according to dysregulated endothelial and smooth muscle control of skin microvascular tone found in SSc patients in some previous studies [1, 3, 6], we hypothesised that SSc patients could show a perturbed FSV when investigated under effect of vasomotor stimuli. To test this hypothesis, we performed spectral Fourier analysis of the finger skin LDF tracing registered either under basal conditions or following ACh or SNP iontophoresis in a cohort of SSc patients. A further aim of our study was to evaluate possible relationships between hypothetical skin vasomotion abnormalities and the severity and/or activity of SSc.

Objectives. To test the hypothesis that finger skin vasomotion (FSV), a known factor influencing microvascular blood flow motion, is impaired in SSc patients. Possible relationships between FSV abnormalities and the severity and/or activity of SSc were also investigated.

Methods. FSV was investigated by means of spectral Fourier analysis of finger skin laser Doppler flowmetry (LDF) tracing, recorded before and following acetylcholine (ACh) or sodium nitroprusside (SNP) iontophoresis in 26 SSc patients and in 20 age-matched healthy controls. The power spectral density (PSD) of the 0.01–0.02, 0.02–0.06 and 0.06–0.2 Hz LDF oscillations (related to endothelial-, sympathetic- and myogenic-dependent FSV, respectively) was measured in PU² (perfusion units)/Hz.

Results. Compared with controls, SSc patients exhibited a significantly lower post-ACh and/or post-SNP percentage increase in PSD of 0.01–0.02 Hz (492 ± 297% vs 283 ± 167%; P < 0.005), of 0.02–0.06 Hz (336 ± 205% vs 239 ± 170%; P < 0.05) and of 0.06–0.2 Hz (223 ± 91% vs 194 ± 227%; P < 0.01) skin LDF oscillations. The post-SNP normalized PSD value of the 0.01–0.02 Hz and of the 0.02–0.06 Hz LDF oscillations was negatively related to SSc severity index (r = −0.407, P < 0.05 and r = −0.459, P < 0.05, respectively).

Conclusions. This study showed a selective abnormality of the endothelial, sympathetic and myogenic-dependent FSV in SSc patients, consistent with a parallel endothelial, sympathetic and myogenic macrovascular dysfunction. This study also suggests a possible role of endothelial and sympathetic dysfunction in the progression of SSc.

Key words: Finger skin vasomotion, Flow motion, Systemic sclerosis, Laser Doppler flowmetry, Spectral Fourier analysis, Acetylcholine, Sodium nitroprusside, Iontophoresis.
Subjects and methods

Subjects

The present study included 26 patients (22 females, 2 males; aged 55 ± 13 yrs) affected by SSC, according to the American Rheumatism Association Subcommittee for Scleroderma Criteria [26]. In all patients, RP was present at the time of the diagnosis of SSC. The extent of skin sclerosis was limited in 16 patients and diffuse in 10 patients. All patients were positive for ANA and seven of them were positive for anti-topoisomerase I (Scl-70) antibodies. Intersitial lung fibrosis was demonstrated in 22 of the studied patients. In all patients, nail-fold capillary microscopy examination showed a typical scleroderma pattern—that is, reduced capillary number and severely enlarged loops. The studied patients had an SSc severity score index [27] of 5.6 ± 2.9 (range: 1–12) and an SSc activity score index [28] of 2.8 ± 1.7 (range: 0–6). Seven patients had digital ulcers at the time of the study and two of them had history of it. No patients had diabetes mellitus, heart failure, renal failure, arterial hypertension or any other disease possibly responsible for microangiopathy. None of the patients was a smoker. Pharmacological treatment consisted of nifedipine in 10 patients, MTX in 3 patients, methylprednisolone in 3 patients and iloprost in 6 patients. Patients treated with nifedipine, methylprednisolone or MTX were studied after 1 day of therapy discontinuation. Patients treated with iloprost were studied after 1 week of this drug discontinuation. Twenty healthy non-smoker subjects (15 females, 5 males; aged 56 ± 13 yrs) were also recruited to form the control group. The clinical features of SSC patients and control subjects are reported in Table 1. The study protocol was approved by the local ethics committee and conformed to the principles outlined in the Declaration of Helsinki. All enrolled subjects gave their written informed consent to participate in the study.

Measurement of digital skin blood flux

Digital skin blood flux was measured by means of an LDF apparatus (Periflux PF4001, Perimed, Järfälla, Sweden) with the following characteristics: 780 nm wavelength, 10–19 kHz bandwidth, 0.1 s time constant, 32 Hz sampling frequency. Calibration was performed using colloidal latex particles whose Brownian motion provided the standard value. The LDF outputs were recorded continuously by an interfaced computer (Acer, Travelmate 202 T, Taipei, Taiwan) equipped with Perisoft dedicated software. This software allows measurement of LDF output in conventional perfusion units (PU): 1 PU = 10 mV. Investigations were performed in a quiet room with controlled temperature (22–24°C). All subjects fasted for at least 2 h before the study and refrained from caffeine and alcohol from midnight before the study day. Each subject had 20 min of acclimatization in supine position before the test.

ACh and SNP iontophoresis

ACh and SNP were delivered to the skin by means of iontophoresis following a procedure previously described [12]. Iontophoresis was performed by means a battery-powered iontophoresis controller (Periiont 328, Perimed), equipped with a drug delivery electrode (PF 383, Perimed). An indifferent electrode (PF 384, Perimed) was also used to provide the current needed for ACh or SNP delivery.

ACh was delivered using a drug delivery electrode filled with 0.05 ml of 1% ACh solution, which was attached on the dorsal aspect of the third right finger by a double-sided adhesive disc. An indifferent electrode was attached on the dorsal aspect of the right hand. ACh was then delivered by means of nine iontophoretic pulses of 0.1 mA for 20 s with a 60-s interval between one and the other pulse. After ACh iontophoresis procedure, SNP was delivered using another drug delivery electrode filled with 0.05 ml of 1% SNP solution, which was attached to the dorsal aspect of the second right finger. An indifferent electrode was attached on the dorsal aspect of the right hand. SNP was then delivered by means of seven iontophoretic pulses of 0.2 mA for 20 s with a 180-s interval between one and the other pulse. Skin vasodilator response to each iontophoresis pulse was measured in PU as mean value during each interval from one pulse to the following pulse, as well as during 2 min following the last pulse.

Investigation of digital skin vasomotion

Digital skin vasomotion was investigated before and following ACh and SNP iontophoresis by means of the spectral Fourier analysis of the skin LDF signal, as previously reported [12, 21, 23–25]. Spectral Fourier analysis was performed on an LDF tracing segment registered during 10 min under basal conditions, immediately before ACh or SNP iontophoresis, as well as on a 10-min ‘steady state’ LDF tracing segment obtained during ACh or SNP iontophoresis.

Following previous studies [9–12], the LDF frequency range from 0.01 to 1.6 Hz was divided into five sub-ranges: the 0.01–0.02 Hz sub-range (associated with the endothelial-dependent vasomotion activity), the 0.02–0.06 Hz sub-range (associated with the sympathetic-dependent vasomotion activity), the 0.06–0.2 Hz sub-range (related to the myogenic-dependent vasomotion activity), the 0.2–0.6 Hz sub-range (related to the respiratory activity) and the 0.6–1.6 Hz sub-range (synchronous with heart activity).

For the spectral Fourier analysis of the LDF signal we used a Perisoft dedicated software [12, 21] that measures in PU²/Hz the power spectral density (PSD) of each LDF frequency range investigated. This software uses the basic fast Fourier transform algorithm, in which the beginning and end of each LDF sub-range investigated are attenuated by means of a windowing Parzen function to avoid the well-known leakage phenomenon (frequency components in the spectra leaking into other frequencies). In the windowing Parzen function, a short-time Fourier transform, with a different window length for each sub-range, was used. The highest PSD identified in each frequency range was considered as the PSD value of that frequency range. The PSD value of each frequency range was also expressed as normalized value, defined as the percentage ratio between the PSD value of that frequency range and the PSD value of the total spectrum from 0.01 to 1.6 Hz (obtained by the sum of the PSD value of each frequency range). The increase in PSD value following ACh or SNP iontophoresis for each frequency range was also calculated as percentage change from baseline.

Statistical analysis

Data are presented either as mean ± s.d. (or mean ± s.e.m. when specified) or as box plots.

The three horizontal lines at the boxes are the 25th, 50th and 75th percentiles. The two lines out of the boxes represent the highest and the lowest values, respectively. Student’s t-test for non-paired data was used to compare basal LDF blood flux values obtained in SSC patients and in control subjects. Analysis of variance (ANOVA) for repeated measures (Scheffe’s test for

<table>
<thead>
<tr>
<th>Clinical feature</th>
<th>Control subjects (n=20)</th>
<th>SSC patients (n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>56 ± 13</td>
<td>55 ± 13</td>
</tr>
<tr>
<td>Sex (Male/Female)</td>
<td>5/15</td>
<td>2/24</td>
</tr>
<tr>
<td>BMI (± s.d.)</td>
<td>24.3 ± 3.3</td>
<td>24.6 ± 2.9</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg) (± s.d.)</td>
<td>124 ± 14</td>
<td>112 ± 7</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg) (± s.d.)</td>
<td>78 ± 6</td>
<td>71 ± 7</td>
</tr>
</tbody>
</table>
Results

Results of ACh and SNP iontophoresis

Figure 1 shows the digital skin blood flux response to ACh and SNP iontophoresis in SSc patients and in control subjects. SSc patients and control subjects did not differ in digital skin blood flux under basal conditions. SSc patients exhibited a significantly lower skin blood flux response to both ACh and SNP compared with control subjects ($P < 0.001$ for both procedures; ANOVA for repeated measures). No significant difference was observed between skin blood flux response to ACh and to SNP in SSc patients.

Results of skin vasomotion investigation

Table 2 and Fig. 2 show the results of vasomotion investigation performed under basal conditions and following ACh iontophoresis in SSc patients and in control subjects.

Table 3 and Fig. 3 show the results for vasomotion investigation performed under basal conditions and following SNP iontophoresis in SSc patients and in control subjects. As in the ACh iontophoresis test, no statistically significant difference was observed under basal conditions in the absolute PSD mean value of all the frequency ranges investigated, with the only exception of the 0.06–0.2 Hz range, which was significantly lower in SSc patients compared with control subjects ($P < 0.005$). Following SNP iontophoresis, there was a significant increase in the absolute PSD mean value for all the frequency ranges investigated, both in SSc patients and control subjects.

![Fig. 1. Digital skin laser Doppler blood flux values (mean±s.e.m.) obtained under basal conditions and following each pulse of ACh iontophoresis (A) or SNP iontophoresis (B), in the 26 SSc patients and in 20 control subjects enrolled in the study. The numbers under the abscissa represent the time of iontophoresis pulses.](image)

Table 2. Absolute and normalized (in parentheses) PSD (PU²/Hz) values (median ± S.D.) of each laser Doppler signal frequency range investigated under basal conditions and during ACh iontophoresis in the SSc patients and in the control subjects recruited in the study

<table>
<thead>
<tr>
<th>Frequency range and origin</th>
<th>Control subjects ($n = 20$)</th>
<th>SSc patients ($n = 26$)</th>
<th>Basal absolute PSD value (PU²/Hz) and normalized value (%)</th>
<th>Post-ACh absolute PSD value (PU²/Hz) and normalized value (%)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.009–0.02 Hz endothelial-dependent vasomotion</td>
<td>$1.40±0.89 (29±10)$</td>
<td>$5.20±2.33 (31±7)$</td>
<td>$&lt;0.0000005$</td>
<td>$1.22±1.40 (33±10)$</td>
<td>$2.54±2.49 (32±8)$</td>
</tr>
<tr>
<td>0.02–0.06 Hz sympathetic-dependent vasomotion</td>
<td>$1.24±0.69 (24±8)$</td>
<td>$3.48±1.64 (20±4)$</td>
<td>$&lt;0.000005$</td>
<td>$0.98±1.13 (28±24)$</td>
<td>$1.93±1.79 (24±6)$</td>
</tr>
<tr>
<td>0.06–0.2 Hz myogenic-dependent vasomotion</td>
<td>$1.39±1.30 (24±11)$</td>
<td>$1.87±0.90 (11±4)$</td>
<td>$&lt;0.000005$</td>
<td>$0.46±0.56 (14±5)$</td>
<td>$1.07±1.12 (13±8)$</td>
</tr>
<tr>
<td>0.2–0.6 Hz respiratory activity</td>
<td>$0.30±0.13 (6±2)$</td>
<td>$0.91±0.49 (5±2)$</td>
<td>$&lt;0.0000005$</td>
<td>$0.22±0.20 (8±4)$</td>
<td>$0.49±0.46 (7±4)$</td>
</tr>
<tr>
<td>0.6–1.6 Hz heart activity</td>
<td>$1.12±0.95 (20±11)$</td>
<td>$5.95±3.53 (33±10)$</td>
<td>$&lt;0.0000001$</td>
<td>$0.81±1.54 (17±10)$</td>
<td>$3.07±4.34 (24±15)$</td>
</tr>
</tbody>
</table>

The $P$-values reported in the table are referred to the statistical difference in absolute PSD values between basal and following ACh iontophoresis in each group. All ± values are s.d.
As shown in Fig. 3, there was a significantly lower percentage increase in the absolute PSD mean value of the 0.01–0.02 Hz range, of the 0.06–0.02 Hz range and of the 0.6–1.6 Hz range following SNP iontophoresis, in SSc patients compared with control subjects \((P < 0.01, P < 0.01 \text{ and } P < 0.005, \text{respectively})\). The post-SNP percentage increase in PSD mean value of the 0.02–0.06 Hz range and of the 0.2–0.6 Hz range did not significantly differ between the two groups.

**Relation between skin microcirculatory parameters and SSc score index**

A negative relation was observed between the normalized PSD value of the 0.01–0.02 Hz frequency range following SNP iontophoresis and the severity SSc score index \((r = -0.407, P < 0.05)\), as well as between the normalized PSD value of the 0.02–0.06 Hz frequency range following SNP iontophoresis and the severity SSc score index \((r = -0.459, P < 0.05)\) (Fig. 4). No significant relation was found between the SSc activity or severity score index and the other skin vasomotion or the skin blood flux parameters investigated.

**Discussion**

To our knowledge, this is the first study which investigated FSV in SSc patients under the effect of the endothelium-dependent agonist ACh and the nitric oxide donor SNP. In a previous study [25], FSV was examined in SSc patients only under basal conditions, showing the absence of vasomotion abnormalities in the studied patients. On the contrary, in the present study, SSc patients exhibited a selective lower finger skin myogenic-dependent vasomotion under basal conditions, together with a selective lower increase in the endothelial-, sympathetic and myogenic-dependent vasomotion during ACh and/or SNP iontophoresis. Moreover, we observed a blunted digital skin vasodilator response to both ACh and SNP iontophoresis in SSc patients, as shown in some previous studies [1, 2, 4, 5].

The parallel blunted skin vasodilator response to ACh and SNP cannot formally be ascribed to vascular endothelium or smooth muscle dysfunction. On the contrary, the simultaneously blunted increase in finger skin endothelial-, sympathetic- and myogenic-dependent vasomotion, in response to the same stimuli observed in the studied SSc patients, is consistent with a parallel and selective dysfunction of the endothelial, sympathetic and myogenic mechanisms that control skin microvascular tone. The selective lower finger skin myogenic-dependent vasomotion observed under basal conditions in the same patients can be considered a further sign of vascular myogenic dysfunction.

In our study, SSc patients also exhibited a blunted post-ACh/SNP spectral increase in finger skin 0.2–0.6 and 0.6–1.6 Hz LDF oscillations, known to reflect central haemodynamic modifications synchronous with respiration and heart activity, respectively [9, 10]. This finding was probably a secondary phenomenon, due

![Fig. 2. Percentage increase from baseline in PSD (mean ± s.e.m.) following ACh iontophoresis for each of the different skin laser Doppler signal frequency ranges investigated in the 26 SSc patients and in the 20 control subjects enrolled in the study. **P < 0.01, between SSc patients and control subjects. *P < 0.001, between SSc patients and control subjects.](image1)

**Fig. 2.** Percentage increase from baseline in PSD (mean ± s.e.m.) following ACh iontophoresis for each of the different skin laser Doppler signal frequency ranges investigated in the 26 SSc patients and in the 20 control subjects enrolled in the study. **P < 0.01, between SSc patients and control subjects. *P < 0.001, between SSc patients and control subjects.**

![Fig. 3. Percentage increase from baseline in PSD (mean ± s.e.m.) following SNP iontophoresis for each of the different skin laser Doppler signal frequency ranges investigated in the 26 SSc patients and in the 20 control subjects enrolled in the study. **P < 0.01, between SSc patients and control subjects. & P < 0.01, between SSc patients and control subjects. **P < 0.001, between SSc patients and control subjects.](image2)

**Fig. 3.** Percentage increase from baseline in PSD (mean ± s.e.m.) following SNP iontophoresis for each of the different skin laser Doppler signal frequency ranges investigated in the 26 SSc patients and in the 20 control subjects enrolled in the study. **P < 0.01, between SSc patients and control subjects. & P < 0.01, between SSc patients and control subjects. **P < 0.001, between SSc patients and control subjects.

**Table 3.** Absolute and normalized (in parentheses) PSD (PU²/Hz) values (median ± S.D.) of each laser Doppler signal frequency range investigated under basal conditions and during SNP iontophoresis in the SSc patients and in the control subjects recruited in the study.

<table>
<thead>
<tr>
<th>Frequency range and origin</th>
<th>Control subjects ((n = 20))</th>
<th>SSc patients ((n = 26))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal absolute PSD value ((\text{PU}^2/\text{Hz})) and normalized value (%)</td>
<td>Post-SNP absolute PSD value ((\text{PU}^2/\text{Hz})) and normalized value (%)</td>
</tr>
<tr>
<td>0.009–0.02 Hz</td>
<td>1.34 ± 0.83 (25 ± 8)</td>
<td>3.38 ± 2.06 (27 ± 7); (&lt; 0.00005)</td>
</tr>
<tr>
<td>0.02–0.06 Hz</td>
<td>1.33 ± 0.59 (26 ± 7)</td>
<td>2.34 ± 1.22 (19 ± 4); (&lt; 0.005)</td>
</tr>
<tr>
<td>0.06–0.2 Hz</td>
<td>1.30 ± 1.44 (22 ± 11)</td>
<td>2.51 ± 1.89 (20 ± 12); (&lt; 0.0005)</td>
</tr>
<tr>
<td>Myogenic-dependent vasomotion</td>
<td>0.42 ± 0.23 (9 ± 5)</td>
<td>0.83 ± 0.60 (7 ± 4); (&lt; 0.01)</td>
</tr>
<tr>
<td>0.2–0.6 Hz</td>
<td>0.94 ± 0.68 (18 ± 10)</td>
<td>3.78 ± 3.11 (27 ± 11); (&lt; 0.001)</td>
</tr>
</tbody>
</table>

The P-values reported in the table are referred to the statistical difference in absolute PSD values between basal and following SNP iontophoresis in each group. All \(\pm\) values are s.e.m.
is in keeping with the previous data showing a strong positive
and the sympathetic-dependent vasomotion. The first relationship
normalized post-SNP increase in both the endothelial-dependent
negative relationship between the SSc severity score index and the
or severity score index of the studied patients. We found a
between the different skin vasomotion data and the SSc activity
pathological significance of the skin vasomotion abnormalities
patients [29].

In order to gain new insights into the potential physio-
pathological significance of the skin vasomotion abnormalities
observed in SSc patients, we performed regression analysis
between the different skin vasomotion data and the SSc activity
or severity score index of the studied patients. We found a
negative relationship between the SSc severity score index and the
normalized post-SNP increase in both the endothelial-dependent
and the sympathetic-dependent vasomotion. The first relationship
is in keeping with the previous data showing a strong positive
relation between free radical damage (a well-known cause of
endothelial dysfunction) and the severity of both the lung
involvement and of the perturbed video-capillaroscopic pattern
in SSc patients [30]. The inverse relationship between the SSc
severity score index and the normalized post-SNP increase in the
sympathetic-dependent vasomotion is in agreement with the
previous data showing that autonomic dysfunction was associated
with a more severe microvascular damage in SSc patients [31]. If
skin microcirculation mirrors the state of the microcirculation in
other vascular beds, as suggested by recent studies [32, 33], then
the aforementioned relationships may confirm the role of the
endothelial and sympathetic dysfunction in the progression of the
overall microvascular involvement in SSc. The parallel absence of
any relationship between the severity SSc score index and the finger
skin vasodilator response to ACh or to SNP suggests a possible
greater sensitivity of skin vasomotion investigation in order to gain
new insights in the physiopathology of SSc. However, further
studies on FSV in SSc patients should include not only healthy
subjects but also patients with primary RP as control groups.

One possible limitation of our study is that we did not formally
assess the reproducibility of the data obtained from the spectral
analysis of skin LDF signal. However, no significant difference
was observed among skin vasomotion data basally obtained from
different fingers in control subjects or in SSc patients. This may
minimize the aforementioned limitation.

Another potential limitation of the present study is that we
cannot exclude a reduction in the effective ACh or SNP delivery to
the finger skin during iontophoresis procedures in SSc patients,
due to the increased electrical or ‘mechanical’ resistance of
sclerodermatous skin to the passage of the vasoactive drug.
However, a previous study showed no difference in the finger
blood flow response to a transdermal nitro patch between SSc
patients and control subjects [34], suggesting that the fibrosis of
sclerodermatous skin did not influence the drug delivery to the
skin through microcirculation in our patients. On the other hand,
SSc patients exhibited a preserved ability of the brachial arteries to
dilate in response to sublingual nitroglycerine in another study
[35]. This last finding suggests that an increased electrical
resistance of the sclerodermatous skin could partly account for the
blunted finger skin vasodilator response to SNP observed in
SSc patients in the present study.

A possible source of misinterpretation of the findings obtained
in the present study could arise from the instability of LDF
tracing during ACh or SNP iontophoresis. However, the ACh and
SNP iontophoresis protocol we used elicited a rapid and
progressive increase in the laser Doppler signal beginning from
the first iontophoretic pulse, followed by a ‘steady-state’ laser
Doppler tracing. This makes the results of the skin vasomotion
investigation sufficiently reliable in our study.

Finally, we cannot formally exclude that the results of the
present study have been influenced by nifedipine, methylpredni-
isolone or MTX therapy, which was discontinued for only 1 day
before the tests. On the other hand, no study investigated the
effects of these drugs on skin vasomotion. However, it is very
unlikely that at least nifedipine, which is able to increase blood
flow in peripheral microcirculation, could have negatively
influenced skin vasomotion in our SSc patients.

Conclusions
In conclusion, our study showed a perturbed pattern of FSV in
SSc patients. This pattern was characterized by a lower myogenic-
dependent vasomotion under basal conditions, together with a
blunted increase in the endothelial-, sympathetic- and myogenic-
dependent vasomotion in response to ACh and/or SNP ionto-
phoresis. A negative relation between the severity of SSc and the
normalized spectral increase of the endothelial- and sympathetic-
dependent vasomotion was also observed in SSc patients. The first
of these findings suggests a parallel and selective dysfunction

Fig. 4. (A) Relation between the post-SNP normalized PSD value of the 0.01–
0.02 Hz frequency range (related to the endothelial-dependent vasomotion) and
SSc severity score index in the SSc patients enrolled in the study. (B) Relation
between the post-SNP normalized PSD value of the 0.02–0.06 Hz frequency range
related to the sympathetic-dependent vasomotion) and SSc severity score index
in the 26 SSc patients enrolled in the study.
of the endothelial, sympathetic and myogenic mechanisms that control finger skin microvascular tone in SSc patients. The second finding suggests a role of the endothelial and sympathetic dysfunction in the progression of the overall microvascular involvement in SSc patients.

**Rheumatology key messages**

- Spectral analysis of skin laser Doppler tracing is useful to investigate digital skin vasomotion FSV in patients with SSc.
- FSV response to vasoactive drugs is blunted in SSc patients, consistent with selective endothelial, sympathetic and myogenic microvascular dysfunction.

**Disclosure statement:** The authors have declared no conflicts of interest.

**References**