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# Evidence of a selective nociceptive impairment in systemic sclerosis

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G. Bajocchi<sup>1</sup>, R. Terlizzi<sup>2</sup>, S. Zanigni<sup>2</sup>, G. Barletta<sup>2</sup>, D. Grimaldi<sup>2</sup>,  
G. Pierangeli<sup>2</sup>, P. Cortelli<sup>2</sup>

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<sup>1</sup>Rheumatology Unit, Arcispedale Santa Maria Nuova, Reggio Emilia, Italy;  
<sup>2</sup>Neurological Clinic, Department of Neurological Sciences, University of Bologna, Italy.

Gianluigi Bajocchi, MD  
Rossana Terlizzi, MD  
Stefano Zanigni, MD  
Giorgio Barletta, MD  
Daniela Grimaldi, MD  
Giulia Pierangeli, Professor  
Pietro Cortelli, Professor

Please address correspondence to:

Bajocchi Gianluigi, MD,  
Rheumatology Unit,  
Arcispedale Santa Maria Nuova  
Reggio Emilia, Italy.

E-mail: baiocchi.gianluigi@asmn.re.it

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**Key words:** Systemic sclerosis, Raynaud's phenomenon, cardiovascular autonomic dysfunction, heart rate variability, small fibres neuropathy.

## Abbreviations:

ANS: autonomic nervous system;  
SSc: systemic sclerosis;  
dcSSc: diffuse cutaneous systemic sclerosis;  
lcSSc: limited cutaneous systemic sclerosis;  
RP: Raynaud's phenomenon;  
BP: blood pressure;  
SBP: systolic blood pressure;  
DBP: diastolic blood pressure;  
HR: heart rate;  
HRV: heart rate variability;  
PSA: power spectral analysis.

Competing interests: none declared.

## ABSTRACT

**Objective.** To test for the autonomic neuropathy in systemic sclerosis (SSc) using cardiovascular reflex evaluation including the "cold face test", which elicits forehead cold receptors (C-fibres). These tests examine the induced bradycardia-hypertensive response and the integrity of nociceptive afferent and parasympathetic-sympathetic efferent pathways.

**Methods.** Twelve SSc patients were studied; including 5 with the limited cutaneous (lcSSc) involvement, and 7 with diffuse cutaneous involvement (dcSSc). All patients were matched with healthy controls. We performed cardiovascular autonomic tests (tilt-test, Valsalva manoeuvre, deep breathing, sustained handgrip and cold face) with continuous monitoring of beat-to-beat blood pressure (BP) and heart rate (HR). Baroreceptor sensitivity index (BRSI) and power spectral analysis (PSA) of heart rate variability (HRV) were also evaluated.

**Results.** SSc patients showed a statistically significant higher HR at rest ( $p < 0.01$ ), a lower increase of diastolic BP during tilt test ( $p < 0.01$ ). They had suboptimal hypertensive and bradycardic response to the cold face test (Systolic BP:  $p < 0.05$ ; Diastolic BP:  $p < 0.01$ ; HR:  $p = 0.08$ ). The Valsalva manoeuvre, deep breathing, isometric handgrip, BRSI and PSA of HRV results were within normal limits in the majority of SSc patients.

**Conclusion.** In this group of SSc patients cardiovascular reflexes were normal, whereas the cold face test which acts through cutaneous nociceptive sensory fibres was abnormal in almost all patients. These results suggest that insufficiency of epidermal small fibres (C-fibres) is involved in SSc.

## Introduction

The precise sequence of events preceding and triggering the deposition

of collagen in systemic sclerosis (SSc) remains unknown. Fibrogenesis of the skin and visceral organs is the endpoint of a complex network in which cytokines, vascular growth factors and neuropeptides have all been shown to have a role but in an uncertain hierarchy of cause/effect. (1).

To detect early evidence of immune-vascular-neurogenic imbalance, several pathological studies have focused on the pre-sclerotic skin of SSc patients and on Raynaud's phenomenon (RP). It is recognized that RP is present in more than 95% of SSc patients and may precede others signs of disease by months or years.

Skin biopsies from normal appearing skin of primary RP and SSc patients showed a loss of calcitonin gene-related peptide (CGRP) immunoreactive fibres when compared to normal subjects and this deficit was more pronounced in SSc. (2, 3). Distributed in the epidermis and in intimate apposition to the sub-epidermal capillary plexus, the CGRP fibers (C-fibers) function to control the neurovascular dilatory response, cold nociception and itching. Therefore, the loss of these peripheral unmyelinated afferent nerve fibers could explain the altered vasoactive response to cold in RP and the sensory disturbance (pruritus and pain) in SSc. Recently, Provitera (4) showed a direct correlation between CGRP fiber density and microvasculature. In three SSc patients he also demonstrate reversal of the cutaneous neuropathy by treatment with the vasodilator iloprost, prostacyclin analogue (5).

In support of this concept in the bleomycin-induced scleroderma model, CGRP gene knockout transgenic mice demonstrated increase skin fibrosis (6).

In parallel with the immunohistological data, several neurophysiological studies have been published indicating that subclinical autonomic involve-

ment is quite common in SSc patients. Certain features of the disease, such as Raynaud's phenomenon, abnormal oesophageal motility and gastrointestinal motor dysfunction, impotence and cardiac abnormalities could be explained in part by autonomic dysfunction (7-10). Cardiovascular autonomic function studies have been performed in SSc patients but the results have been contradictory (11-17).

Some authors found in SSc patients both parasympathetic and sympathetic impairment (11, 15, 16), while others found only parasympathetic deficit (12) or only sympathetic overactivity (13). Finally, one group who selected only CREST variant patients did not find autonomic function impairment between SSc patients and controls (14).

The discrepant results could be explained in several ways: a) difficulty in quantifying two reciprocal functions (sympathetic and parasympathetic); b) the different cardiovascular reflex tests used; c) patient age and patient sample; d) different target organs studied (oesophagus, intestine, heart, skin); e) concomitant diseases (diabetes, hypertension) and therapies. Based on this background, we assessed cardiovascular autonomic function in SSc patients. We evaluated cardiovascular reflexes with stimuli that explore not only the components of the baroreflex arc (sympathetic and parasympathetic pathways) but also the nociceptive sensory nerves of the skin that are thought to be impaired in SSc.

## Patients and methods

### *Patients and controls*

Local ethics committee approval and patient informed consent was obtained for this study in which the Rheumatology Unit of Arcispedale Santa Maria Nuova (Reggio Emilia, Italy) enrolled 12 consecutive patients with SSc. All the patients were compared with 25 age- and sex-matched healthy controls.

### *Rheumatologic assessment.*

All the patients were classified according to the 1988 proposal by LeRoy *et al.* considering two subsets of SSc (18), and were assessed using the EUSTAR-MEDS (EULAR Scleroderma Trials

and Research Group minimal essential data set) (19) and following the core set of clinical features as recommended by the same group of researchers (20). Disease duration was calculated from the onset of the first symptom attributable to scleroderma (Table I).

Laboratory investigations were performed based on signs and symptoms and included: chest x-ray, high resolution computed tomography (HRCT) scan, lung function study, standard ECG, doppler echocardiogram, esophagography, small bowel barium studies, endoscopy, capillaroscopy, antinuclear antibodies (ANA) using on HEP-2 as substrate and extractable nuclear antigens (ENA) by double immunodiffusion.

### *Neurologic assessment and autonomic control of the cardiovascular system*

A complete neurological examination was performed in all subjects. Patients were studied in a temperature controlled room ( $23 \pm 1^\circ\text{C}$ ) with continuous monitoring of systemic blood pressure (BP) (Finapres, Ohmeda 2300), electrocardiogram (ECG), heart rate (HR) (cardiotachograph B-R AO - FI), oronasal breathing (nasal thermistor connected to a preamplifier), abdominal breathing (strain gauge) and finger plethysmogram. All patients and controls were tested between 8 and 12 in the morning. Before the tests, patients fasted overnight but were allowed to drink water. All abstained from smoking cigarettes and drinking alcohol or coffee for 24 hours before the study. For ethical reasons, patients were allowed to take their usual medications but all of them delayed their morning doses until the end of the test. Patients were asked not to sleep or talk during the study.

After 30 min of supine rest, the head up tilt test (10 min at  $65^\circ$ , HUTT), Valsalva manoeuvre (40 mmHg for 15 s), deep breathing (6 breaths/min), cold face test (60 s of application of cold compress ( $0 - 1^\circ\text{C}$ ) to the forehead and sustained handgrip (30% of maximal effort for 5 min) were performed using standard procedures (21, 22).

During the HUTT changes in systolic blood pressure (SBP), diastolic blood pressure (DBP) and HR were calculated

with respect to basal values. During the Valsalva manoeuvre, the following indices of autonomic activity were examined: a) the ratio between HR in phases II and IV (VR) and the overshoot during phase IV (difference between the highest SBP after expiration and the basal value); b) the baroreflex sensitivity index (BRSI) calculated during the strain ( $\alpha$  strain) and release ( $\alpha$  release) phase of the manoeuvre according to Goldstein *et al.* (23). On deep breathing: a) the sinus arrhythmia (calculated in beats per minute using the 10 longest R - R intervals during expiration and b) the 10 shortest R - R intervals during inspiration) and the I/E ratio (ratio between the mean of higher HR values during 10 deep inspiration and the mean of the lower HR values during expiration). With the cold face test, changes with respect to the basal values of SBP, DBP and HR were computed after 60 s of application of cold compresses to the forehead. On sustained handgrip, the changes with respect to the basal values of SBP, DBP and HR were calculated after 5 minutes of isometric effort.

These manoeuvres were carried out in the sequence described above, allowing a period of rest required to reach basal BP and HR values between sessions. The results of each test were automatically obtained by means of home developed software.

Autoregressive power spectral analysis (PSA) of HR variability (HRV) during supine rest and HUTT was performed using standard procedures (24). We evaluated a high frequency (HF), respiratory-linked component (centered to  $\sim 0.25$  HZ, reflecting vagal activity) and a low frequency (LF) component (centred at  $\sim 0.1$  HZ, reflecting sympathetic activity). The oscillatory components are presented in normalized form, dividing by the total power less the DC component, if present. The LF/HF ratio was used as an index of sympathovagal balance. Because of its inherent nonlinearity, the signal from the nasal thermistor was used only to assess the main respiratory frequency of the period considered for the evaluation of HRV. The power spectral component centered at  $\sim 0.25$  was considered HF only if coincident with the main respiratory frequency.

### Statistical analysis

Comparisons of group characteristics were performed using Student's *t*-test for unpaired data after evaluation for normal distribution. Values of  $p < 0.05$  were considered significant. All results are presented as mean  $\pm$  SD and data for PSA of HRV as normalized units. Regression analysis was used to test the correlation between age and disease duration and the cold face test results.

### Results

Table I shows the demographic and clinical characteristics of the patients. 11 were female. The mean age was 53 (range 30–69). Seven patients were classified as having diffuse SSc (dc-SSc), five had limited SSc (lcSSc). Ten of the 12 had disease duration less than 10 years, and 7 two years or less. Six had anti-scl70 antibody and one anticentromere. Other organ system involvement included esophagus (5), small intestine (2), pulmonary fibrosis (5), sicca symptoms (4) and renal crisis (1). The 25 control subjects included 23 females and 2 males (50 $\pm$ 15 years).

Neurological evaluation was normal in all subjects, namely pain, light touch and temperature sensation were normal in the face. Comorbidities were arterial hypertension in four patients, autoimmune thyroiditis in two, diabetes in two, and angina pectoris in one. Therapies at the time of testing were: low dose D-penicillamine in 6 (1, 3, 5, 10, 11, 12) Cyclophosphamide in one (7); low dose of prednisone 5–12.50 mg/day in 6 (1, 3, 6, 7, 10, 12); Calcium channel blockers in 6 (1, 4, 5, 6, 8, 12); Aspirin in five (4, 5, 6, 10, 11); insulin in 2 (5, 6); oral anti-diabetic drugs in one (5); ACE-inhibitors in 3 (5, 6, 12); Beta-blockers in one (6). Other supportive therapies included are: prokinetics, proton pump inhibitors, NSAIDs, paracetamol, vitamin D, calcium and various topical therapies.

### Cardiovascular reflexes

Twelve patients completed all the tests correctly and were included in statistical analysis. The basal values of SBP, DBP during supine rest were normal and similar in SSc patients and controls. HR was significantly increased in SSc ( $p < 0.01$ ). (Table II). However, this effect

was mainly due to therapy with calcium channel blockers (CCB) (SSc CCB +: HR = 90 $\pm$ 15 bts/min; SSc CCB -: HR = 72 $\pm$ 9 bts/min;  $p < 0.02$ ).

During HUTT no patients showed clinical signs of orthostatic impairment but comparing cases to controls we found a lower DBP increase ( $p < 0.01$ ) in SSc whereas SBP and HR responses during HUTT were similar in both groups (Table II).

We did not find any statistically significant differences between SSc and controls on BRSI and the cardiovascular responses to Valsalva manoeuvre, deep breathing test or isometric exercise test (Table II).

The expected DBP and SBP increases during the cold face test were significantly lower in patients than in controls ( $p < 0.001$  and 0.05 respectively). Similarly, the expected bradycardia showed a blunted response in SSc compared with controls which did not reach sta-

tistical significance ( $p = 0.08$ ) (Table II). Regression analysis comparing disease duration and changes in HR, SBP and DBP during the cold face test showed a significant correlation with the reduction of HR ( $p < 0.05$ ) but not with  $\Delta$ -SBP and  $\Delta$ -DBP. No correlation was found between  $\Delta$ -HR,  $\Delta$ -SBP or  $\Delta$ -DBP during the cold face test or with patients age.

### Power spectral analysis

PSA of HRV was not obtained in three patients because of frequent ectopic beats. The PSA of HRV for the 9 SSc patients included in the statistical analysis was normal and similar to controls. (supine condition: LF=SSc 55.4 $\pm$ 15.7% vs. controls 61.5 $\pm$ 11.2%; HF=SSc 39.6 $\pm$ 15.3% vs. controls 33.5 $\pm$ 11.1%; LF/HF=SSc 1.88 $\pm$ 1.4 vs. controls 2.12 $\pm$ 1. tilt test: LF=SSc 65.1 $\pm$ 21.3% vs. controls 70 $\pm$ 17.1%, HF=SSc 28.4 $\pm$ 18.3% vs. controls 23.4 $\pm$ 13.6%; LF/HF=SSc 3.9 $\pm$ 3.9 vs. controls 4.8 $\pm$ 4).

**Table I.** Demographic and clinical features of 12 SSc patients.

	Sex	Age	SSc subsets	Disease duration (yr)*	First symptom	Total skin score	SSc-associated serum autoantibodies	Internal organ and other involvement
1	f	33	Diffuse	2	RP Puffy hands	21	Scl-70	Small Intestine Pulmonary fibrosis
2	f	51	Diffuse	26	RP Pulmonary fibrosis	0	Scl-70	Pulmonary fibrosis
3	f	52	Diffuse	1	RP Puffy hands	15	Scl-70	Esophagus
4	f	53	Diffuse	1	RP Puffy hands	4	Scl-70	Sicca symptoms Pulmonary fibrosis
5	f	68	Diffuse	7	RP	8	Scl-70	Pulmonary fibrosis Sicca symptoms
6	f	69	Diffuse	1.6	RP Puffy hands	13	Scl-70	Renal crisis Pulmonary fibrosis
7	m	41	Diffuse	1	RP Arthralgias	34	None	Sicca Symptoms
8	f	30	Limited	12	RP	11	None	Esophagus
9	f	43	Limited	1	RP Puffy hands	2	None	Small Intestine
10	f	51	Limited	2	RP	4	None	Esophagus Sicca symptoms
11	f	59	Limited	7	RP Puffy hands	5	None	Esophagus
12	f	66	Limited	7	RP	22	Anticentromere	Esophagus

\*Calculated from the first symptom onset. RP: Raynaud's phenomenon.

**Table II** Cardiovascular responses in SSc patients and controls.

	Basal values			Head-up tilt test		
	SBP (mmHg)	DBP (mmHg)	HR (beats/min.)	$\Delta$ SBP (mmHg)	$\Delta$ DBP (mmHg)	$\Delta$ HR (beats/min.)
SSc (n = 12)	113 $\pm$ 26	56 $\pm$ 12	81 $\pm$ 15	14.4 $\pm$ 11	8.1 $\pm$ 6.6	9.7 $\pm$ 4.9
Controls (n = 25)	122 $\pm$ 15	63 $\pm$ 9	64 $\pm$ 6	14.5 $\pm$ 9.8	14.2 $\pm$ 5.9	11.4 $\pm$ 5.7
p-value	NS	NS	0.0002**	NS	0.01**	NS
	Valsalva manoeuvre			Deep breathing		
	VR	overshoot (mmHg)	calculated during strain phase (mmHg/mms)	calculated during release phase (mmHg/mms)	I/E	$\Delta$ HR (beats/min.)
SSc (n = 12)	1.7 $\pm$ 0.4	48.9 $\pm$ 26.3	4.03 $\pm$ 2.5	3.11 $\pm$ 2.2	1.3 $\pm$ 0.2	17.4 $\pm$ 10.4
Controls (n = 25)	1.6 $\pm$ 0.3	49 $\pm$ 20.3	4.48 $\pm$ 1.8	2.41 $\pm$ 0.9	1.3 $\pm$ 0.1	19 $\pm$ 7.7
p-value	NS	NS	NS	NS	NS	NS
	Cold face			Isometric exercise		
	$\Delta$ SBP (mmHg)	$\Delta$ DBP (mmHg)	$\Delta$ HR (beats/min.)	$\Delta$ SBP (mmHg)	$\Delta$ DBP (mmHg)	$\Delta$ HR (beats/min.)
SSc (n = 12)	18.9 $\pm$ 16	3.08 $\pm$ 6.8	-4.1 $\pm$ 8.7	31.5 $\pm$ 13.9	17.5 $\pm$ 9.2	12 $\pm$ 6.7
Controls (n = 25)	33.3 $\pm$ 19.6	20 $\pm$ 14.3	-9.2 $\pm$ 7.1	39.5 $\pm$ 16.6	21.5 $\pm$ 8.8	14.2 $\pm$ 9.6
p-value	0.04*	0.0006**	0.08	NS	NS	NS

Values are expressed as mean  $\pm$  SD; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate;  $\Delta$ : change with respect to the basal value; VR: Valsalva ratio; overshoot: phase IV Valsalva; I/E: inspiratory/expiratory; \*  $p < 0.05$ ; \*\*  $p < 0.01$ .

## Discussion

The main result of this study is that autonomic control of cardiovascular reflexes was normal in our group of SSc, whereas the cardiovascular responses to cold face test were abnormal, suggesting integrity of the sympathetic and parasympathetic efferents but defective nociceptive skin afferents. Also in 2 scleroderma patients suffered from diabetes, we did not find evidence of the typical autonomic neuropathy, as proved by the normality of all autonomic test with the exception of the cold test. It seems highly unlikely that diabetic patients could have an isolated autonomic impairment limited to the afferent pathways without sensory or motor symptoms and signs.

In the supine condition at rest, we found normal and similar values of BP in SSc patients and controls whereas HR was significantly increased in the patients taking CCB. Cardiovascular responses to HUTT were qualitatively normal in the patients showing an increase in BP and HR but a lower DBP increase was noted in SSc patients compared to controls. This effect cannot be due to an efferent sympathetic dysfunction

because Valsalva manoeuvre and isometric exercise were normal in SSc. Again the influence of chronic drug intake on the cardiovascular system must be taken into account.

A normal parasympathetic and sympathetic efferent control of HR was also confirmed by PSA of HRV which showed a normal sympathovagal balance in both supine and orthostatic conditions in patients and controls. Valsalva manoeuvre and BRSI confirmed the normal function of the overall baroreceptor reflex arc in SSc, while deep breathing and isometric handgrip excluded a cardiovagal efferent and vascular sympathetic efferent dysfunction. Thus, SSc patients seem to have normal autonomic control of the cardiovascular system.

Our results are in contrast with other reports on cardiovascular reflexes (11-14) and PSA of HRV (8, 15, 16) in SSc patients which obtained contradictory results, suggesting both sympathetic and parasympathetic imbalance in SSc patients. Our different findings may be partly due to different methodologies. In particular we used in this study continuous BP monitoring instead of

intermittent arm-cuff inflators which is a more reliable and reproducible method and we calculated the results of cardiovascular reflex testing with an automatic operator independent method. Alternatively, different results may be due to true heterogeneity of autonomic nervous system function in SSc.

To our knowledge, the cold face test has not been used previously for the evaluation of cardiovascular reactivity in SSc patients. We decided to use this test because the painful stimulus (cold) eliciting the cardiovascular responses is applied to the forehead, a site where neurological examination of the skin was normal in our SSc patients. Thus, we could explore the function of afferent cutaneous C and A $\delta$  fibres without applying a cold stimulus to the hand (cold pressor test, perhaps not ethical and difficult to perform in SSc patients with severe sclerodactyly).

A cold stimulus applied to the face during spontaneous breathing evokes bradycardia and peripheral vasoconstriction as measured by increased systolic and diastolic blood pressure. It is a simple, non-invasive and reproducible test that activates a trigeminal auto-



onomic reflex through the nociceptive sensory nerve fibres of the skin innervated by the ophthalmic branch of the trigeminal nerve, evoking bradycardia (due to cardiovagal efferent activation) and hypertension (due to vascular sympathetic efferent activation) (25-27).

The cold face test in our SSc patients showed a blunted response of BP and HR. Bearing in mind that the other cardiovascular reflexes were normal in our patients, these results suggest a selective dysfunction of the afferent branch of the autonomic reflex that could be the result of C-fiber impairment localized to the skin.

Our patients did not show symptoms and signs of sensory peripheral neuropathy but clinical examination cannot detect a small fiber neuropathy that may be revealed by the cold face test. Since the cold face test depends on activation of a trigeminal autonomic reflex, and since the trigeminal nerve is a target in autoimmune disease, particularly in Sjögren's syndrome, and in SSc presenting mainly as a sensory neuropathy we looked closely at the results of the patients with sicca symptoms but no differences on cold test, resulted from all other patients.

Besides functional tests, in SSc skin biopsies (2-5) from the fingers, distal thigh and leg have shown a significant loss of unmyelinated and myelinated sensory autonomic nerve fibers (C fibers and A delta fibers) mainly associated with a reduction of the vascular bed. On the basis of these findings the authors hypothesized that ischemia may play a role in determining the neuropathic process. (11,12), but immuno-mediated or metabolic causes, as it was described in SLE, sicca complex, and Sjögren's syndrome (28-30), can not be excluded.

Why A delta and C fibre functions are selectively impaired in SSc subjects is unclear but their different quantitative and qualitative features may make them most susceptible to damage than the myelinated fibres. (31-33).

A protective role of nociceptive sensory nerve fibres (C-fibres) was proposed recently in the Bleomycin-induced scleroderma animal model (6), reinforcing our results suggesting a possible role of these fibres in the pathogenesis of SSc.

The correlation between disease duration and failure of HR decrease during the cold face test can be interpreted as consistent with the notion that, over the time, the parasympathetic part of the reflex is gradually more damaged. Noteworthy, the age of patients at the time of the study was not correlated with cardiovascular responses to the cold face test, indicating that the duration of the disease is more important than age in evaluating small fiber function in SSc.

A limitation of our study is the small number of SSc patients enrolled so the results has to be considered as preliminary but nonetheless in agreement with the observed loss of epidermal small fibers density (4). Further studies in a larger group of subjects and with other cardiovascular reflex tests useful to verify function small fibers in other cutaneous sites beyond face should be performed. Control disease patients with other autoimmune diseases will be necessary to determine the specificity of our results for SSc.

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