



# Right ventricular overload and cardiovascular neuroendocrine derangement in systemic sclerosis<sup>☆</sup>

Michele Emdin<sup>a,\*</sup>, Carlo Marini<sup>b,c</sup>, Claudio Passino<sup>a</sup>, Dilia Giuggioli<sup>d</sup>, Bruno Formichi<sup>b</sup>, Clodoveo Ferri<sup>e</sup>, Jaleh Khabirinejad<sup>a</sup>, Roberta Poletti<sup>a</sup>, Concetta Prontera<sup>a</sup>, Annalisa Iervasi<sup>a</sup>, Antonio L'Abbate<sup>f</sup>

<sup>a</sup> Cardiovascular Medicine, CNR National Research Council, Institute of Clinical Physiology, Pisa, Italy

<sup>b</sup> Pulmonary Department, CNR National Research Council, Institute of Clinical Physiology, Pisa, Italy

<sup>c</sup> Cardiopulmonary Department, University of Pisa, Italy

<sup>d</sup> Department of Internal Medicine, Rheumatology Unit, University of Pisa, Italy

<sup>e</sup> Department of Internal Medicine, Rheumatology Unit, University of Modena, Italy

<sup>f</sup> Scuola Superiore di Studi Universitari S. Anna, Pisa, Italy

## KEYWORDS

Systemic sclerosis;  
Noradrenaline;  
Renin-angiotensin-  
aldosterone system;  
Cardiac natriuretic  
hormones;  
Right ventricular failure;  
Pulmonary hypertension

**Aim** Systemic sclerosis (SSc) may be associated with right ventricular overload, secondary to pulmonary hypertension. In heart failure patients, neuroendocrine derangements can influence clinical evolution and prognosis. The aim of this study was to investigate neurohormonal control affected in SSc patients with and without right ventricular impairment.

**Methods and results** A prospective series of 28 patients with SSc was studied. In addition to conventional evaluations, extensive neuroendocrine studies were done, including assays of both the vasoconstrictor system (plasma renin activity [PRA], aldosterone and catecholamines) and vasodilatory molecules (brain natriuretic peptide [BNP] and atrial natriuretic peptide [ANP]).

A significant relation was observed between echo-Doppler estimated pulmonary systolic pressure (PAP) and neurohormonal activation, in particular between PAP and BNP ( $R = 0.58$ ,  $p = 0.004$ ), ANP ( $R = 0.65$ ,  $p < 0.001$ ) and PRA ( $R = 0.45$ ,  $p = 0.032$ ). Patients with right ventricular overload (i.e., PAP > 40 mmHg confirmed at cardiac catheterization) had higher levels of ANP and BNP ( $147 \pm 26$  vs  $34 \pm 6$  pg/mL and  $344 \pm 86$  vs  $30 \pm 7$  pg/mL, respectively,  $p < 0.001$ ), PRA ( $6.4 \pm 1.9$  vs  $1.8 \pm 0.4$  ng/mL/h,  $p < 0.001$ ) and aldosterone ( $257 \pm 86$  vs  $114 \pm 22$  pg/mL,  $p = 0.02$ ). These patients had increased plasma noradrenaline, but not adrenaline ( $701 \pm 87$  vs  $452 \pm 66$  pg/mL,  $p < 0.001$ ).

**Conclusion** SSc patients with right heart failure have a neurohormonal derangement, showing overactivity of the vasoconstrictive system, counteracted by oversecretion of cardiac natriuretic hormones.

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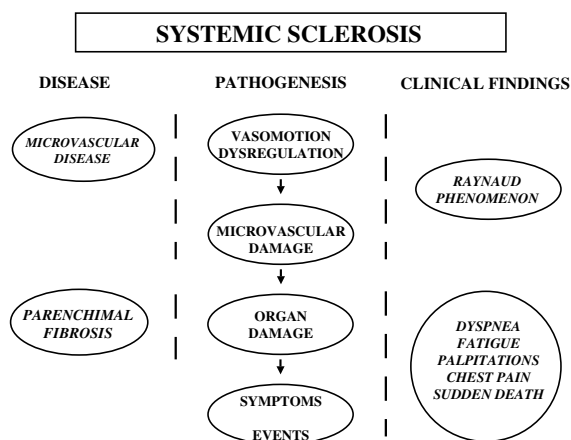
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\* Correspondence: Tel.: +39 50 3152189; fax: +39 50 3152109.

E-mail address: emdin@ifc.cnr.it (M. Emdin).

## Introduction

Systemic sclerosis (SSc) is a connective tissue disorder of unknown aetiology characterized by fibrosis of the skin and visceral organs. Cardiac injury is frequent<sup>1,2</sup>



**Fig. 1** Microvascular dysfunction, characterized by vasomotor alterations and tissue ischaemia, and subsequent microvessel damage is the *primum movens* of the pathogenetic chain leading to clinically overt cardiovascular involvement in SSc patients.

and results from: (i) inflammatory and ischaemic lesions affecting the pericardium, myocardium, and heart conduction system, due to microvascular alterations ('cardiac Raynaud's phenomenon'); (ii) collagen overproduction by altered fibroblasts; and (iii) complex immune-system dysregulation<sup>1-3</sup> (Fig. 1). Myocardial fibrosis leads primarily to ventricular diastolic dysfunction,<sup>4</sup> whereas left ventricular systolic dysfunction is present in a minority of patients,<sup>5</sup> namely those with comorbid cardiovascular conditions (e.g., atherosclerotic coronary artery disease, which has the same prevalence in SSc patients as in the general population, and arterial hypertension, which may be worsened by renal scleroderma involvement). Rhythm and conduction disturbances are considered hallmarks of scleroderma heart disease<sup>6,7</sup> and are facilitated by autonomic dysfunction.<sup>8</sup> Right ventricular overload and failure may be responsible for a poor outcome in many cases of SSc,<sup>1-3,9,10</sup> and is frequently linked to lung fibrosis and pulmonary hypertension,<sup>9</sup> causing symptoms such as dyspnoea on effort, fatigue, and palpitations.

In patients with heart failure, irrespective of the initial 'noxa' affecting the myocardium, the 'neuroendocrine model'<sup>11,12</sup> is a widely accepted basis for interpreting clinical evolution and response to treatment. The possible neurohormonal derangements associated with chronic right ventricular overload and failure, as may occur in patients with SSc, have not been thoroughly investigated yet.

We, therefore, carried out this prospective study to evaluate the neuroendocrine characteristics of patients with SSc. In addition to conventional evaluations, we assayed vasoconstrictor (plasma renin activity [PRA], aldosterone and catecholamines) and vasodilatory (cardiac natriuretic hormones [CNH]) molecules with well-known diagnostic and prognostic value,<sup>13-16</sup> and thyroid hormones, whose concentrations may be altered in patients with heart failure, and which have prognostic implications.<sup>17-18</sup>

## Patients and methods

Twenty-eight SSc patients (25 females, aged  $52 \pm 2$  years, mean  $\pm$  S.E., range 21-76) and an age-matched control group of 28 healthy subjects without known systemic, immunological, and cardiovascular diseases (25 females; aged  $51 \pm 1$  years, range 25-70) were selected.

The diagnosis of SSc was made according to the American Rheumatism Association Subcommittee for Scleroderma Criteria.<sup>19</sup> Patients were classified into three subsets on the basis of the extent of skin sclerosis: limited, intermediate, and diffuse.<sup>20,21</sup>

At the time of the study, no patients were receiving diuretic, anti-adrenergic, ACE-inhibitor, angiotensin II-blocker, or spironolactone treatment.

All healthy subjects were non-obese and normotensive, and free from diseases. All of them had normal blood biochemistry (including creatinine, urea nitrogen, glucose, uric acid, albumin, enzymes, electrolytes), haematological indices (haemoglobin, red cell and white cell counts) and urine analysis. Asymptomatic heart disease was excluded in all control subjects by a complete cardiologic examination - including a standard electrocardiographic and echocardiographic examinations and a bicycle stress test.

All patients and control subjects gave their informed consent to participation in the study, which was carried out in compliance with the Declaration of Helsinki. The protocol was approved by our Institutional Ethical Committee.

## Clinical features and visceral involvement

An extensive evaluation of the signs and symptoms of SSc was carried out in all patients at the time of the study. In particular, the following parameters were recorded: extent of the cutaneous involvement, presence of telangiectasia, hypermelanosis, cutaneous ulcers, Raynaud's phenomenon, arthritis, oesophageal involvement (X-ray hypomotility with or without dysphagia), and nephropathy (proteinuria  $>300$  mg/24 h and/or serum creatinine  $>1.2$  mg/dL). Pulmonary involvement was carefully investigated: the physical examination was complemented by chest radiography, pulmonary function testing (total lung capacity, forced vital capacity, forced expiratory volume in one second), and measurement of single breath diffusing lung capacity for carbon monoxide (DLCO).

Capillaries were examined using a Leitz microscope and the findings interpreted according to the procedure described by Maricq et al.<sup>22</sup>

## Serological parameters

Standard techniques were used to screen all patients for the following serological markers: anti-nuclear antibody (ANA) and anti-nucleolar antibody (ANoA), by indirect immunofluorescence on rat liver at a dilution of serum of 1:20 and on a Hep 2 cell line at a dilution of 1:40, anti-centromere antibody (ACA), and anti-extractable nuclear antigen (ENA) antibodies, including Scl-70 antibodies.

## Cardiological investigations

Cardiac evaluation consisted of a standard 12-lead ECG examination, two-dimensional Doppler echocardiography, and 24-h Holter monitoring. The Holter monitoring revealed that six out of the 28 patients had >30 ventricular ectopic beats/hour: none had ventricular tachyarrhythmia.

Transthoracic 2D echocardiography and Doppler examination were performed in all patients with a Hewlett-Packard Sonos 5500 echocardiography system. A parasternal long axis view was used to measure left ventricular, right ventricular, left atrial and aortic root dimensions and end-diastolic septal and posterior wall thickness. The *E/A* ratio was computed from the apical four-chamber view, using pulsed-wave Doppler with the sample volume placed at the mitral valve leaflet tips. An inverted *E/A* ratio (<1) is considered indicative of left diastolic dysfunction. Left ventricular volumes and ejection fraction were calculated at baseline for each patient using a modification of Simpson's method. Systolic pulmonary arterial pressure (PAP) was estimated by continuous Doppler wave measurements of tricuspid regurgitation.

Right heart catheterisation was performed in all 11 patients with a Doppler-estimated PAP >40 mmHg. A 7 F triple lumen flow-directed thermodilution catheter (Baxter Edwards, Irvine, CA), was advanced into a lower branch of a main pulmonary artery under fluoroscopic guidance. Transducers were positioned at the mid-axillary line and zeroed at atmospheric pressure. Cardiac output was measured in triplicate (less than 10% variation) by the thermodilution technique using 5% dextrose in water as the indicator (Cardiac Output computer: Baxter Edwards); the cardiac index was calculated as the cardiac output divided by square metre of body surface area. Mean right atrial pressure, systolic and end-diastolic pulmonary artery pressures, and pulmonary wedge pressure were recorded.

## Neurohormone assays

Blood samples were withdrawn at 8 a.m. from an antecubital vein after a 30-min rest period in a supine position before the echocardiographic examination.

Plasma atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) were measured with two-site immunoradiometric assays, as described elsewhere.<sup>15</sup>

Blood samples for the catecholamine assays were drawn into pre-cooled plastic tubes containing EGTA (0.2 mL of solution at 9% w/v) as anticoagulant, and glutathione (0.012 g) as an antioxidant. Plasma was then separated from blood cells within 30 min by centrifugation (3000g) at 4 °C for 10 min and aliquoted and stored at -70 °C. The measurement of catecholamines in plasma was automatically carried out by HPLC 725 (Eurogenetics-Tosoh, Turin, Italy) by purification, derivatisation of catecholamines and fluorescence detection at 480nm with an excitation wavelength at 340 nm.

Plasma renin activity (PRA) and aldosterone were measured by radioimmunoassay (DiaSorin S.r.l., Saluggia, Italy), while serum cortisol was measured with a fluorescence polarisation immunoassay with the TDx system (Abbott Diagnostics). All assays were performed following the procedures indicated by the manufacturers.

Serum TSH, FT3 (free fraction of T3), free tetraiodothyronine (FT4, free fraction of T4), T3, and T4 were measured by an AIA 21 analyser (Eurogenetics-Tosoh, Turin, Italy). The concentration of TSH was determined with a sandwich enzyme immunoassay, while FT3, FT4, T3 and T4 were measured with a competitive enzyme immunoassay; all these methods use an enzymatically activated fluorescent label (4-methylumbelliperyl-phosphate).

## Statistical analysis

Statistical analyses were carried out on a Power Macintosh G3 personal computer using the Stat-View 5.0.1 programme (1992–1998, SAS Institute Inc., SAS Campus Drive, Cary, NC, USA). Because ANP and BNP values are not normally distributed, both the original and the logarithmic transformation of data were used for statistical analyses. Comparisons between two independent groups, and among more than two independent groups were analysed by ANOVA.

The results are expressed as mean ± SE; a *p* value < 0.05 was considered statistically significant.

## Results

The main clinical and serological findings of the SSc patients are summarised in Table 1.

### Cardiac function in patients with SSc

All but two of the SSc patients showed normal systolic left ventricular function (average ejection fraction 58.4 ± 2.1% on the whole patient population). Fourteen patients (50%) had abnormal left ventricular diastolic function (i.e., *E:A* ratio <1), whereas right ventricular overload with pulmonary hypertension (systolic PAP >40 mmHg at Doppler evaluation and confirmed by cardiac catheterisation) was detected in 11 patients. This subset showed a reduced cardiac output (3.7 ± 0.4 L/min), low cardiac index (2.27 ± 0.24 L/min/m<sup>2</sup>), increased pulmonary artery pressures (systolic 73 ± 7, diastolic 28 ± 2, mean 43 ± 4 mmHg) and elevated vascular pulmonary resistances (1480 ± 308 dyne s<sup>-1</sup> cm<sup>-5</sup>). The subgroups of SSc patients divided according to whether they did or did not have right ventricular dysfunction did not differ for the presence of ACA and Scl70 antibodies, or extent of skin sclerosis, but those with right ventricular involvement were characterised by a reduced lung diffu-

**Table 1** Clinical and serological data of 28 SSc patients

	Mean ( ± SEM )	Range
Age (years)	51 ± 2	21–76
Disease duration (years)	10 ± 1	3–20
	No.	(%)
Cutaneous involvement		
Limited	12	43
Intermediate	2	7
Diffuse	14	50
Raynaud's phenomenon	28	100
Telangiectasia	22	88
Oesophageal involvement	25	89
Lung involvement	24	85
Renal involvement	3	11
Autoantibodies		
ANA	14	50
ACA	7	25
Anti-Scl70	11	39

ANA: anti-nuclear antibody; ACA: anti-centromere antibody.

**Table 2** General characteristics and neurohormonal results of the SSc patients and the control group

	Patients	Healthy subjects	<i>p</i>
No.	28	28	
Age	52 ± 3	51 ± 1	NS
F/M ratio	25/3	25/3	NS
Body mass index (kg/m <sup>2</sup> )	24.3 ± 0.9	24.4 ± 0.4	NS
EF (%)	58.4 ± 2.1	60.1 ± 1.1	NS
ANP (pg/mL)	82 ± 16	22 ± 2	<0.001
BNP (pg/mL)	163 ± 47	8 ± 1	0.001
PRA (ng/mL/h)	3.76 ± 0.96	0.57 ± 0.08	0.002
Aldosterone (pg/mL)	183 ± 25	103 ± 10	0.05
Adrenaline (pg/mL)	45 ± 15	52 ± 5	NS
Noradrenaline (pg/mL)	550 ± 55	350 ± 31	0.002
fT3 (pg/mL)	2.5 ± 0.1	2.6 ± 0.1	NS
fT4 (pg/mL)	12.3 ± 1.2	11.6 ± 0.3	NS
TSH (pg/mL)	1.9 ± 0.3	1.4 ± 0.2	NS

F: females; M: males; EF: ejection fraction; ANP: atrial natriuretic peptide; BNP: brain natriuretic peptide; PRA: plasma renin activity; TSH: thyroid stimulating hormone.

sion capacity (DLCO 42 ± 4 vs 67 ± 5% of predicted value,  $p = 0.006$ ).

### Circulating levels of CNH and neurohormones in SSc patients and in healthy subjects

The circulating levels of CNH and neurohormones found in patients with systemic sclerosis and in a group of healthy subjects are reported in Table 2. CNH plasma levels were significantly higher in patients with SSc than in the healthy subjects. The SSc patients also had raised PRA and aldosterone plasma levels. Plasma adrenaline levels were not significantly different between the two groups, while noradrenaline values were increased in patients. Finally, no significant differences were found in TSH and thyroid hormone concentrations between the two groups. Plasma levels of neurohormones did not differ between groups of patients divided according to the presence or absence of Scl-70 or ANA antibodies or according to the extent of cutaneous involvement.

### Neurohormonal activation in presence of right ventricular impairment

A significant relation was observed between neurohormonal activation and PAP, in particular between PAP and BNP ( $R = 0.58$ ,  $p = 0.004$ ), ANP ( $R = 0.65$ ,  $p < 0.001$ ) and PRA ( $R = 0.45$ ,  $p = 0.032$ ) (Fig. 2). Progressive neurohormonal activation was observed in patients with right ventricular overload (i.e., estimated PAP above 40 mmHg) (Fig. 3). The 11 patients with right ventricular overload showed higher ANP and BNP values (147 ± 26 vs 34 ± 6 pg/mL and 344 ± 86 vs 30 ± 7, pg/mL, respectively; both  $p < 0.001$ ) as well as higher PRA (6.4 ± 1.9 vs 1.8 ± 0.4 ng/

mL/h;  $p < 0.001$ ) and higher aldosterone (257 ± 86 vs 114 ± 22 pg/mL,  $p = 0.02$ ). Patients with right ventricular overload had raised plasma noradrenaline (701 ± 87 vs 452 ± 66,  $p < 0.001$ ). No differences were observed between the groups in plasma adrenaline, TSH, or thyroid hormone levels.

### Neurohormonal activation and lung diffusing capacity

Patients with systemic sclerosis had a marked reduction in lung diffusing capacity, their average DLCO being 57% of the predicted value (range 25–116). A significant relation was observed between DLCO and ANP ( $R = -0.60$ ,  $p = 0.002$ ), BNP ( $R = -0.55$ ,  $p = 0.007$ ), and PRA ( $R = -0.50$ ,  $p = 0.015$ ), whereas aldosterone, adrenaline and noradrenaline levels were not significantly related to DLCO. The impaired lung diffusing capacity in SSc was inversely proportional to the extent of right ventricular overload, as testified by the relation between PAP and DLCO ( $R = -0.47$ ,  $p = 0.025$ ).

### Discussion

There are few reports about the existence of neurohormonal activation in SSc and those that have been published provide contradictory data; Kazzam *et al.* reported an absence of adrenergic activation<sup>23</sup> and possible oversecretion of ANP.<sup>24</sup> To our knowledge, an extensive neurohormonal characterization in relation to disease evolution and myocardial dysfunction has never been performed in SSc patients.

Our data show that patients with SSc had significant activation of both the vasoconstrictor and vasodilator branches of the neurohormonal control of the cardiovascular system. In patients with right heart failure (at least in the early phase), the powerful stimulation of the adrenergic and renin-angiotensin-aldosterone systems should be considered compensatory, aimed at fluid and sodium retention, blood volume increase, and maintenance of cardiac preload.

Right ventricular involvement, typical of scleroderma heart disease<sup>9</sup> and determined by various degrees of pulmonary hypertension, is a powerful stimulus for eliciting secretion of cardiac natriuretic peptides, in line with previous reports of increased secretion in heart failure of any origin,<sup>25</sup> and in pulmonary hypertension.<sup>26,27</sup>

The pathophysiological linkage with cardiac disease is the ability of the myocardium to act as a gland in response to right ventricular pressure and volume overload, secreting these powerful natriuretic and vasodilatory hormones to counteract the activation of the vasoconstrictive, sodium-retaining neurohormonal systems (sympathetic nervous system, renin-angiotensin-aldosterone system, endothelins, and vasopressin, in the late phase of the disease).

There is now sound evidence of the value of BNP assays in diagnosis (of both systolic and diastolic ventricu-

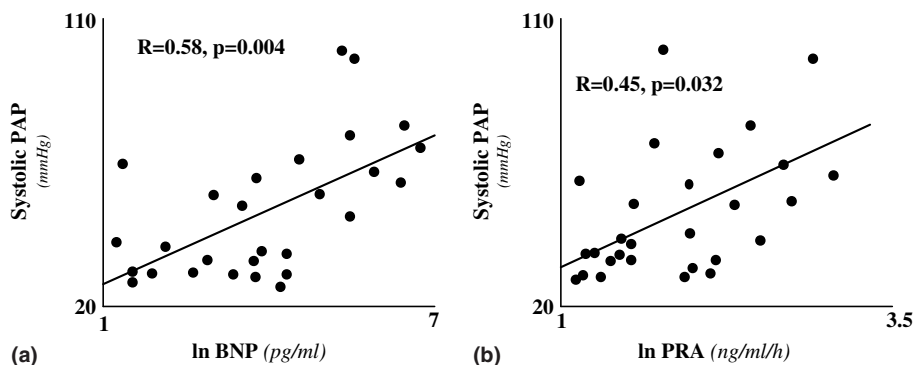


Fig. 2 Association between systolic pulmonary artery pressure in SSC patients, plasma level of brain natriuretic peptide (BNP) (a) and plasma renin activity (PRA) (b).

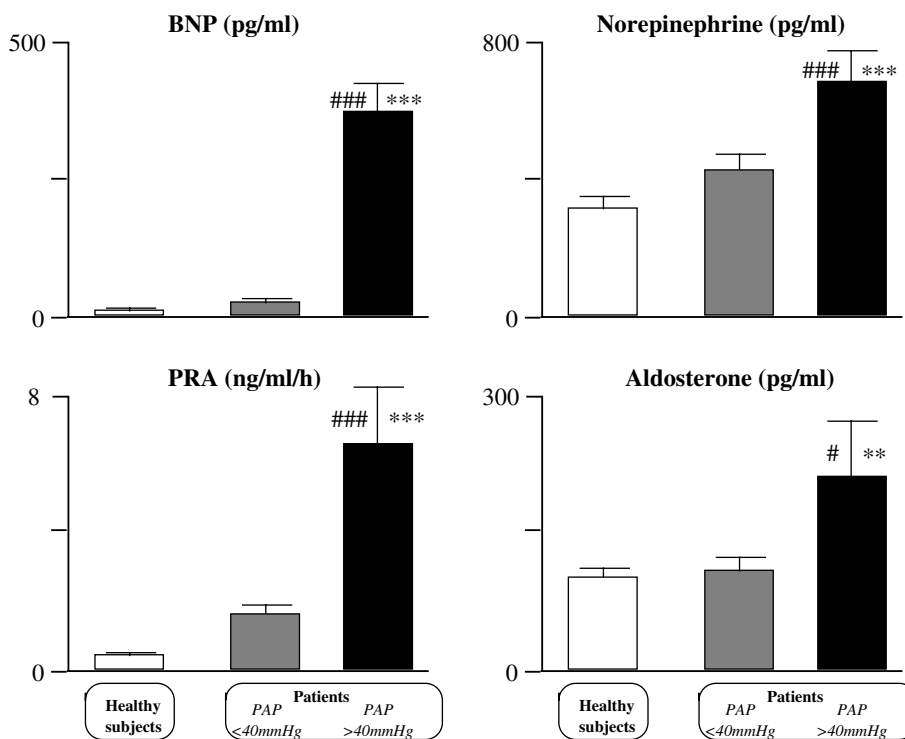


Fig. 3 The subset of 11 SSC patients with pulmonary systolic pressure >40 mmHg is characterized by overactivity of the main vasoconstrictor systems (adrenergic – top right, renin–angiotensin–aldosterone system – bottom) and by the counterregulatory secretion of cardiac natriuretic hormones (top left brain natriuretic peptide concentration), as compared to patients without pulmonary hypertension and healthy controls. \*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs healthy subjects; # $p < 0.05$ , ### $p < 0.001$  vs patients with PAP <40 mmHg.

lar dysfunction) and prognosis in symptomatic patients with no previous diagnosis, in unselected populations with a known increased cardiac risk,<sup>28</sup> and in patients with ascertained cardiac disease.<sup>29,30</sup> The present report suggests the possible diagnostic usefulness of cardiac natriuretic hormone (in particular BNP) assay, even in presence of isolated right ventricular dysfunction.

**Conclusions**

Our data confirm that SSC patients, particularly those with evidence of right heart failure have a progressive derangement of the neurohormonal systems, which worsens with disease severity. They have overactivity of the vasoconstrictive system, counteracted by oversecretion of CNH. Routine measurements of these neurohormones, which, except for the natriuretic peptides, is not currently a recommended strategy,<sup>16</sup> might represent a useful complement to the diagnostic work-up and a guidance to medical treatment even in SSC patients.

Several vasodilatory approaches, currently used and interacting with vascular paracrine vasoactive substances (prostacycline or NO/endothelin), may counteract peripheral and cardiac microvascular dysfunction, and fight the sequelae of pulmonary hypertension and right ventricular involvement. Furthermore, the recognition of a cardiovascular neuroendocrine derangement in SSc, associated with adrenergic and renin-angiotensin-aldosterone overexpression, and a relative imbalance between these vasoconstrictive systems and activation of the vasodilatory CNH system, opens up new promising therapeutic opportunities.

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