Parasympathetic activity increases with digital microvascular damage and vascular endothelial growth factor in systemic sclerosis

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ABSTRACT

Objective. *The imbalance between angiogenic and angiostatic factors with derangement of the microvasculature are hallmarks of systemic sclerosis (SSc). Raynaud's phenomenon in SSc probably is due to the impaired neuroendothelial control mechanisms between vasoconstriction and vasodilatation. The aim of this study is to evaluate autonomic nervous system function using heart rate variability (HRV) analysis and to correlate with vascular endothelial growth factor (VEGF).*

Methods. *Twenty-seven SSc patients were enrolled. HRV was measured and markers of global sympathetic and parasympathetic system, respectively standard deviation of normal-to-normal RR intervals (SDNN) and square root of the mean of the sum of the squares of differences between adjacent NN intervals (RMSSD) were evaluated. Serum VEGF levels and nailfold videocapillaroscopy (NVC) were performed.*

Results. *A linear positive correlation was observed between RMSSD and VEGF ($p<0.01$, $r=0.55$), and RMSSD and disease duration ($p< 0.01$, $r=0.54$). The RMSSD median value was significantly increased ($p< 0.05$) with NVC damage progression. The RMSSD median value was significantly ($p<0.05$) higher in SSc patients with digital ulcers (DUs) than in SSc patients without DUs [44 (39.4–60.2) vs. 24.6 (23–37.1)].*

Conclusion. *In our study parasympathetic modulation increases in relation to VEGF. When microcirculation is modified with capillaroscopic pattern progression and DUs, autonomic system seems to stimulate vasodilatation trough parasympathetic system. We can conclude that parasympathetic activity increases with digital microvascular damage and promotes VEGF release.*

Introduction

The imbalance between angiogenic and angiostatic factors and the resulting derangement of the microvasculature are hallmarks of systemic sclerosis (SSc) (1).

The earliest and main clinical finding of SSc is Raynaud's phenomenon (RP) that is characterised by recurrent episodes of digital vasospasm (2). Herrick *et al*. have hypothesised that the pathogenesis of RP secondary to SSc is probably due to the impaired neuroendothelial control mechanisms of vasoconstriction and vasodilatation (3). Thus, in early stage of disease, microvascular damage results in endothelium dysfunction and promotes enlarged, giant and ramified capillaries. In late stage of SSc, vascular repair process results in reduced blood flow, hypoxia and neoformation of vessels (4). Loss of vasculature and tissue hypoxia in SSc promotes angiogenesis through the production of pro-angiogenic factors such as VEGF (1, 5). Angiogenesis implicates proliferation and migration of mature endothelial cells with release of angiogenic factor in all stages of the disease. The majority of studies showed upregulation of VEGF (6).

Global autonomic activity, evaluated by heart rate variability (HRV), is significantly lower in SSc patients than healthy controls. A correlation between renal vascular damage and autonomic dysfunction was observed in SSc patients. The intrarenal arterial stiffness, evaluated by resistive index, increases with sympathetic activity and decreases with parasympathetic activity (7).

The aim of this study is to evaluate autonomic nervous system function by HRV analysis and to correlate with VEGF.

Methods

Study population

Twenty-seven [21 females and 6 males with median age of 45 years (range 42–53) and a median disease duration of 8 (7-12) years] Caucasian subjects fulfilling the American College of Rheumatology/European League criteria for classification and diagnosis of SSc were prospectively enrolled in this study (8). Eight patients had limited cutaneous SSc and nineteen had diffuse cutaneous SSc according to LeRoy *et al*. (9).

Patients with pulmonary hypertension, heart failure with a preserved ejection fraction, heart failure with reduced ejection fraction, cardiac arrhythmias and conduction disorders, interstitial lung disease with significant gas exchange abnormalities [diffusion lung capacity for CO (DL_{CO}) ≤60% of predicted value] were excluded. Subjects with relevant systemic comorbidities, such as a history of uncontrolled systemic hypertension, dyslipidaemia, diabetes mellitus, valvular heart diseases, cerebrovascular and peripheral vascular diseases, hepatic or thyroid dysfunction, anaemia, coagulopathy were not eligible as well. Patients were not taking β-blockers, antiarrhythmic drugs, ACE-inhibitors or angiotensin receptor antagonists.

The study protocol is in accordance with the Declaration of Helsinki and was approved by the Ethics Committee at our Institution.

Heart rate variability

All patients underwent clinical evaluation, 24-h Holter monitoring and electrocardiography (ECG) to evaluate autonomic nervous system function by heart rate variability (HRV) analysis.

Autonomic nervous activity was analysed following the recommendations of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (10). The standard deviation of normal-to-normal RR intervals (SDNN) (ms) and the square root of the mean of the sum of the squares of differences between adjacent NN intervals (RMSSD) represent markers of global sympathetic and parasympathetic system, respectively. Data analyses were performed with software Del Mar Avionics Accuplus 363 (Irvine California, USA).

Serum VEGF levels

Serum VEGF levels were determined in SSc patients by commercial ELISA kit (Human VEGF, Quantikine ELISA, R&D Systems, Minneapolis MN), with a sensitivity of 9 pg/ml and an assay range of 31.2- 2000 pg/ml.

Nailfold videocapillaroscopy

We performed nailfold videocapillaroscopy (NVC) in each patient by a videocapillaroscope (Pinnacle Studio Version 8) equipped with a 500 × optical probe. We examined the nailfold of the second, third, fourth and fifth finger, identifying the "SSc pattern" (early, active and late) according to Cutolo *et al*. (11)

Statistical analysis

The data were expressed as median and range. Commercially software (SPSS version 24.0) was used for statistical analysis. Group comparisons were madeby Kruskal-Wallis test. Spearman's coefficient (r) was used to evaluate an association between numerical variables. *P*-values <0.05 were considered significant.

Results

Table I shows SSc patients' epidemiological and clinical features, heart rate variability variables and vascular endothelial growth factors. Sixteen SSc patients had a history of digital ulcers (DUs). The NVC showed an early pattern on 8 SSc patients, active pattern on 10 patients and late pattern on 9. A linear positive correlation was observed between RMSSD and VEGF ($p<0.01$, r=0.55), conversely any correlation was found between SDNN and VEGF. A linear positive correlation was observed between RMSSD and disease duration ($p<0.01$, r=0.54), conversely any correlation was found between SDNN and disease duration. The RMSSD median value significantly increased ($p<0.05$) with NVC damage progression: early 24.4 (23–35.7), active 39.6 (36-43) and late 60.2 (56-69.9). The RMSSD median value is significantly ($p<0.05$) higher in SSc patients with DUs than in SSc patients without DUs [44 (39.4–60.2) *vs*. 24.6 (23–37.1)] (Fig. 1).

Table I. SSc patients' epidemiological and clinical features, heart rate variability variables and vascular endothelial growth factors. The data are expressed as median and range.

Sex (F/M)	21/6
Age, years	45 (42-53)
Disease duration	8 (7-12)
Modified Rodnan total skin score	12 (8-18)
Disease Activity index	1,5 (1-4,5)
Disease Severity Scale	4 (3-9)
dcSSc/lcSSc	19/8
SSc-specific autoanticorpi, n (%)	
Anti-topoisomerase I	17 (63%)
ACA antibodies	8 (29.6%)
None	2 (7.4%)
Digital ulcers (DUs), n (%)	16 (59.3%)
PAPs (mmHg)	27 (26-30)
DLCO (% of predicted)	75 (70-83)
NVC pattern, n (%)	
Early	8 (29.7%)
Active	10 (37%)
Late	9 (33.3%)
SDNN (ms)	120.2 (113.6-129.8)
RMSSD (ms)	39.4 (35.7-52.6)
VEGF (pg/ml)	222.8 (168.4-274.1)

PAPs: systolic pulmonary arterial pressure; DLCO: Diffusion Lung CO; NVC: nailfold videocapillaroscopy; SDNN: standard deviation of normal-to-normal RR intervals; RMSSD: square root of the mean of the sum of the squares of differences between adjacent NN intervals; VEGF: vascular endothelial growth factors.

Discussion

In our study parasympathetic activity showed a significant positive correlation with serum levels of VEGF. In previous studies, we have confirmed that global autonomic activity was significantly lower in SSc patients than healthy population [120.2 (86.8–161.6) ms] (7). In this study, the median value of SDNN is 120 ms. In subclinical renal vasculopathy a negative correlation was found between renal resistive index and global parasympathetic activity evaluated by RMSSD (7). In our study, RMSSD correlates with VEGF, disease duration, NVC damage and DUs history.

Several studies confirmed autonomic dysfunction by HRV evaluation. Autonomic dysfunction in SSc patients is characterised by parasympathetic dys-

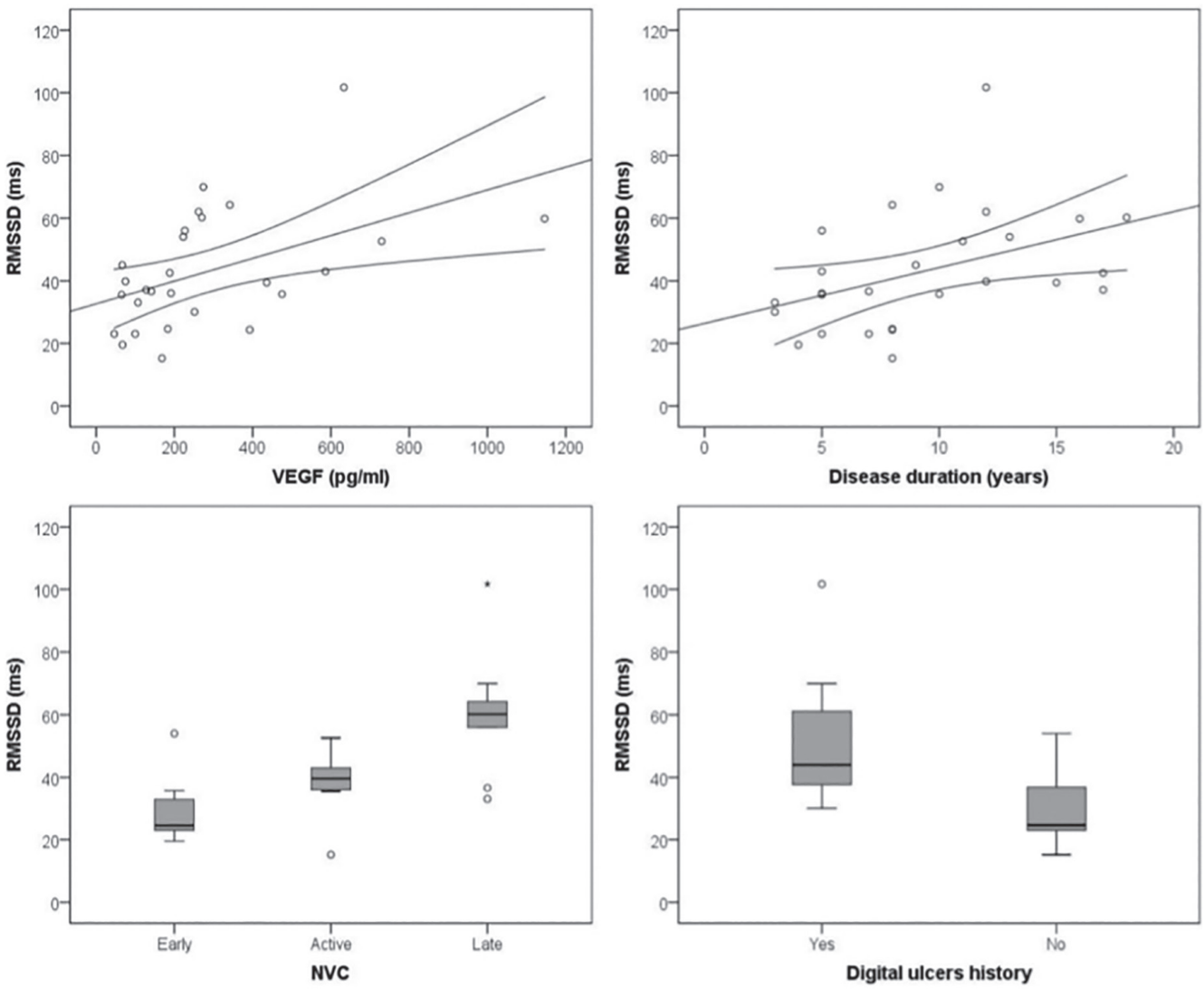

Fig. 1. Correlation between RMSSD and vascular endothelial growth factors (VEGF), disease duration, nailfoldvideocapillaroscopy (NVC) and digital ulcers history.

function, associated with sympathetic overactivity and depression of the circadian rhythm of heart rate. Autonomic dysfunction can explain many features of SSc as for impaired Raynaud's phenomenon (12). Othman *et al*. demonstrated a significant negative correlation between autonomic dysfunction and worsening of skin sclerosis, Raynaud's phenomenon and the presence of anti-SCL70 antibodies in the sera of SSc patients (13). In these patients autonomic dysfunction is associated with digital microvascular damage and intrarenal arterial stiffness (7).

The progressive derangement of the microcirculation leads to a state of chronic tissue hypoxia that stimulates the release of angiogenic and angiostatic factors such as VEGF and endostatin. Endostatin, an antiangiogenic mediator that represents the main antagonist of VEGF, is elevated in patients with SSc and to be relevant for the disease (14). Sildenafil improved digital blood flow and RP symptoms in SSc patients after 8 weeks of treatment without significant changes of serum VEGF levels (15). The effects of vasodilator drugs should be evaluated on global autonomic activity and serum level of VEGF.

Vasodilatation is mediated by parasympathetic system and we can speculate that VEGF expression is favoured by vasodilatation. Then, when architecture of microcirculation is altered, such as capillaroscopic pattern progression and DUs, autonomic system seems to stimulate vasodilatation through parasympathetic system. We can conclude that parasympathetic activity increases with digital microvascular damage and promotes VEGF release.

The main limitation of this study is the small sample size. In future studies it would be interesting to evaluate the correlation between endostatin and autonomic function. Largest studies are needed to evaluate the relationship between autonomic system and VEGF in SSc patients.

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