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Nailfold avascular score and coronary microvascular dysfunction in systemic sclerosis: A newsworthy association

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ABSTRACT

Background and aims: We aimed to assess the relationship between nailfold videocapillaroscopy (NVC) abnormalities and coronary flow reserve (CFR), a marker of coronary microvascular dysfunction (CMD) in patients with systemic sclerosis (SSc).

Methods: We studied 39 SSc patients (33 females, mean ± SD age 54 ± 12 years, median disease duration 11 years, range 6–22) and 22 controls (matched for age and sex) without any evidence of cardiovascular disease. Clinical assessment was performed by modified Rodnan skin score (mRss) and EUSTAR score. Coronary flow velocities in the left anterior descending coronary artery were measured by transthoracic echocardiography. Average peak flow velocities, CFR and microvascular resistance at baseline (BMR) and in hyperaemic (HMR) condition were assessed. CFR ≤2.5 was considered marker of CMD. Six NVC-abnormalities were evaluated by a semi quantitative scoring system: enlarged and giant capillaries (diameter > 20 μm and > 50 μm, respectively), hemorrhages, disarray, capillary ramifications and loss of capillaries (avascular score). Statistic was performed using SPSS.

Results: CFR was lower in SSc patients than in controls (2.6 ± 0.5 vs 3.3 ± 0.5). CMD was detected in 24 patients (61.5%) vs 0 controls ($p < .0001$). CFR was inversely correlated with NVC-avascular score (*rho* = -0.750, $p < .0001$). Avascular and capillary ramifications scores ($p = .001$ and $p = .03$, respectively), mRss ($p = .003$) and EUSTAR score ($p = .01$) were higher in patients with CMD than in those without. At multivariable analysis, avascular score was independently associated with CMD ($p = .01$). HMR was directly correlated with avascular score (*rho* = 0.416, $p = .008$).

Conclusions: In our SSc patients NVC-avascular score was associated with CMD which seems to be the result of a structural microvascular remodeling.

1. Introduction

Systemic sclerosis (SSc) is an autoimmune connective tissue disease characterized by endothelial damage of small vessels resulting in a diffuse microangiopathy [1].

Microvascular lesions are a predominant feature in SSc and play a central pathogenetic role, leading to an abnormal fibroblast function and increased extracellular matrix production with a diffuse fibrosis of

Abbreviations: ACR, American College of Rheumatology; aDAP, Diastolic arterial pressure during adenosine infusion; aHR, Heart rate during adenosine infusion; APVb, Average peak velocities at baseline; APVh, Average peak velocities under hyperaemia; ARI, Arteriolar resistance index; aSAP, Systolic arterial pressure during adenosine infusion; ASE, American Society of Echocardiography; bDAP, Baseline diastolic arterial pressure; bHR, Heart rate at baseline; bSAP, Baseline systolic arterial pressure; BMR, Baseline microvascular resistance; CAD, Coronary artery disease; CFR, Coronary flow reserve; CMD, Coronary microvascular dysfunction; DLCO, Diffusing capacity for carbon monoxide; dSSc, Diffuse cutaneous form of SSc; EUSTAR, European Scleroderma Trials and Research; FVC, forced vital capacity; HMR, hyperaemic microvascular resistance; ILD, interstitial lung disease; IMR, resting myocardial resistance index; LAD, left anterior descending artery; lSSc, limited cutaneous form of SSc; LV, left ventricular; mPAP, mean pulmonary arterial pressure; mRSS, modified Rodnan skin score; NVC, nailfold videocapillaroscopy; RP, Raynaud's phenomenon; SSc, systemic sclerosis

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skin and internal organs (lung, kidney and heart) [1]. Raynaud's phenomenon (RP) is considered the earliest clinical sign of functional vascular damage in SSc as it may precede symptoms and clinical visceral involvement by several years [2,3].

Nailfold videocapillaroscopy (NVC) is a routine screening test to detect and evaluate the peripheral microangiopathy over the disease course. According to previous studies a qualitative analysis allows to detect three distinct “scleroderma patterns”: early, active, and late [4]. Several studies have shown a relationship between these different capillaroscopic patterns and severe organ involvement [5–8]. Sulli et al. created a semi quantitative rating scale for scleroderma microangiopathy by scoring each of the six altered microvascular features from 0 to 3. The capillary density score was identified as “avascular score” and was found to be a sensitive tool to quantify and monitor the SSc microvascular damage [9]. The same authors hypothesized that a regular NVC evaluation could be useful in the risk assessment of microvascular heart involvement in SSc, though no studies have proved it yet [10]. In the last years improved renal outcomes leave primary myocardial involvement as one of the main determinants in the prognosis of patients with SSc [11]. Primary myocardial involvement is common in SSc with a high prevalence at autoptical studies (30–80%), despite cardiac symptoms being detectable in only 20–35% of patients [12]. The microvascular origin (coronary microvascular dysfunction, CMD) of primary myocardial involvement was well documented in SSc patients with angiographically normal epicardial coronaries [13,14]. Coronary flow reserve (CFR) assessment by pulsed wave Doppler examination of blood flow velocity in the left anterior descending coronary artery (LAD) at rest and after maximum vasodilatation by adenosine infusion is a validated method to investigate CMD [15–17]. The presence of CMD is an independent risk factor of major adverse outcomes in several cardiovascular diseases [18].

In SSc patients with asymptomatic heart involvement, CFR is significantly reduced compared with controls [19,20]. The calculation of microvascular resistance, based on CFR data at baseline (BMR) and in hyperaemic (HMR) condition, allows to better understand the mechanism underlying CMD: a decreased BMR (and increased baseline coronary flow velocities) means that the mechanism responsible for CMD is functional; whereas the decrease in hyperaemic coronary flow secondary to high HMR may be due to a structural remodeling [21]. To the best of our knowledge, no studies investigating BMR and HMR in SSc by non-invasive CFR-measurement, in order to elucidate CMD mechanisms in these patients, have been published to date.

The aim of our study was to assess the relationship between NVC-derived scores and CFR-CMD in SSc patients asymptomatic for heart involvement, as expressions of the systemic microvascular damage. We also aimed to investigate the underlying mechanisms of CMD in SSc.

2. Methods

2.1. Study population

In this cross-sectional study, we enrolled 39 patients affected with SSc according to American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria [3].

Data collected at baseline included demographics, disease manifestations, antinuclear antibodies and treatments (Table 1). Modified Rodnan Skin score (mRSS) was assessed by the same physician, European scleroderma trials and research (EUSTAR) activity score was measured according to Valentini et al. [22] and the 10-year risk of cardiovascular disease was calculated by Framingham risk score [23].

The nonrandomized control group consisted of 22 healthy volunteers recruited from institutional personnel who were matched for age and sex. All subjects (patients and controls) were asymptomatic with no history of heart disease. Exclusion criteria for all subjects included any of the following conditions: cerebral vascular disease, carotid artery bruit, peripheral bruit or abnormal pulse, history of angina or myocardial infarction, alcohol intake > 10 oz. per week. All participants had normal ECG at rest and during adenosine-induced hyperaemia. Patients and control subjects came from the same geographic area. The absence of coronary artery disease (CAD) was evaluated by clinical history, physical examination, and ECG.

The study was approved by the institutional ethics committee and all patients gave written informed consent. All patients underwent to transthoracic Doppler echocardiography with CFR evaluation and NVC.

2.2. Nailfold videocapillaroscopy

All patients and controls underwent NVC, performed by the same

Table 1
Clinical, echocardiographic and capillaroscopic features in SSc patients with and without coronary microvascular dysfunction (CMD).

	All patients (n = 39)	CMD (n = 24)	No CMD (n = 15)	p-Value[a]
Age, years	54.0 ± 12.2	53.0 ± 13.1	54.4 ± 10.4	0.779
Female, n (%)	33 (85)	19 (79.2)	14 (93.3)	0.376
Clinical features				
Years after RP onset	16.0 ± 12.1	15.1 ± 9.2	17 ± 10	0.97
Disease duration, years	11 (6–22)	9 (6.2–21.7)	11 (4–22)	0.98
Diffuse cutaneous form, n (%)	21 (54)	16 (66.7)	5 (33.3)	0.042
Modified rodnan skin score	9 ± 7	11.67 ± 7.31	4.27 ± 4.27	0.003
PAH, n (%)	1 (3)	1 (4.2)	0 (0)	1.0
ILD on HRCT, n (%)	11 (28)	10 (41.7)	1 (6.7)	0.028
Digital ulcers, n (%)	16 (41)	15 (62.5)	1 (6.7)	0.001
EUSTAR score	1.7 ± 1	2.1 ± 1.12	1.17 ± 0.52	0.01
Framingham risk score	2.5 ± 3	3.17 ± 3.89	1.47 ± 1.18	0.05
Autoantibodies, n (%)				
ANA	39 (100)	24 (100)	15 (100)	1.0
Anti-topoisomerase I	16 (41)	11 (45.8)	5 (33.3)	0.517
Anti-centromere, n	15 (38)	7 (29.2)	8 (53)	0.446
Anti-RNA polymerase III	1 (2.6)	5 (20.8)	1 (13.3)	0.393
Treatment, n (%)				
Calcium channel blockers	39 (100)	23(95.8)	13(86.7)	0.547
Prostanoids	9 (23)	7(29.2)	2(13.3)	0.437
ET-1 inibithors	14 (36)	9(37.5)	5(33.3)	1.0
ACE inibithors	8 (20.5)	5 (20.8)	3 (20)	1.0
Cardioaspirin	13 (33)	8(33.3)	5(33.3)	1.0
Immunosuppressants	10 (26)	9(37.5)	1(6.7)	0.057
Echocardiography				
LVEF (%)	62.2 ± 4.8	63 ± 5	62 ± 4.8	0.620
E/A ratio	1.14 ± 0.39	1.1 ± 0.45	1.1 ± 0.39	0.776
Capillaroscopic features				
Enlarged capillaries (> 20 μm)	0.83 ± 0.42	0.84 ± 0.42	0.83 ± 0.45	0.9
Giant capillaries (> 50 μm)	0.37 ± 0.33	0.36 ± 0.32	0.38 ± 0.35	0.9
Hemorrhages	0.22 ± 0.21	0.22 ± 0.23	0.22 ± 0.19	1
Avascular score	0.52 ± 0.41	0.68 ± 0.55	0.17 ± 0.14	0.001
Disarray	0.54 ± 0.46	0.61 ± 0.48	0.42 ± 0.43	0.2
Capillary ramifications	0.14 ± 0.13	0.17 ± 0.15	0.08 ± 0.12	0.03

p-Value[a] between patients with and without CMD. ACE, angiotensin-converting-enzyme; ANA, antinuclear antibodies; CMD, coronary microvascular dysfunction; ET-1, endothelin 1; E/A, ratio of early transmitral diastolic flow velocity (E) and flow velocity during atrial contraction (A); EUSTAR, European scleroderma trials and research; HRCT, high resolution computed tomography; ILD, interstitial lung disease; LVEF, left ventricular ejection fraction; PAH, pulmonary arterial hypertension; RP, Raynaud phenomenon; SSc, systemic sclerosis.
Unless specified otherwise, values are mean ± SD or median (interquartile range).

operator blinded for clinical conditions, using an optical probe video-capillaroscopy equipped with magnification 200 x contact lens and connected to image analysis software (Videocap, DS MediGroup, Milan, Italy). Nailfold capillaries of distal row of all fingers (excluding the thumb) of both hands were examined in each patient. The same operator scored the following capillaroscopic features: 1) enlarged capillaries (increase in capillary diameter > 20 μm), 2) giant capillaries (homogeneously enlarged loops with a diameter > 50 μm), 3) hemorrhages, 4) loss of capillaries (the normal range adopted was 9 capillaries per linear millimeter), 5) microvascular disarray, and 6) capillary ramifications. For each field a semi quantitative rating scale was adopted to score each capillary abnormality from 0 to 3 and an average score value was calculated according to Sulli et al. [9]. The capillary density score was identified as "avascular score" [9].

2.3. Standard Doppler echocardiography and CFR assessment

Transthoracic Doppler echocardiography was performed with a commercially available ultrasound system (Vivid 7, GE Medical System, Inc., Hortem, Norway). From two-dimensional guided M-mode echocardiogram, left ventricular (LV) dimensions were measured according to American Society of Echocardiography (ASE); LV mass was calculated by the adjusted ASE method [24] and indexed for body surface area or height. In each subject, ejection fraction was measured and diastolic dysfunction was defined according to the ASE criteria [25]. These criteria integrate Doppler measurements of the mitral inflow and Doppler tissue imaging of the mitral annulus.

Coronary images were obtained in the distal part of the LAD with 7-MHz transducer. After baseline recordings of coronary flow velocity, adenosine was intravenously infused (140 $\mu g/kg^{-1}$/min) for 3 min, obtaining Doppler flow profiles. CFR was estimated as the ratio of hyperaemic to baseline peak diastolic coronary flow velocities. A CFR ≤ 2.5 was considered as a marker of CMD and the population was dichotomized accordingly [26]. All subjects were asked to avoid taking calcium-antagonists, endothelin-1 inhibitors and caffeine-containing drinks for at least 24 h before testing. In addition, prostanoids infusion was stopped at least 24 h before. CFR measurements were stored digitally for future offline analysis by 2 investigators blinded for all clinical variables. The following variables were evaluated: heart rate at baseline and during adenosine infusion (bHR and aHR), baseline systolic and diastolic arterial pressure (bSAP and bDAP, mmHg), systolic and diastolic arterial pressure during adenosine infusion (aSAP and aDAP, mmHg), average peak velocities at baseline and under hyperaemia (APVb and APVh, cm/s) and coronary flow reserve (CFR).

2.4. Doppler-derived resistance indexes

The mean pressure in coronary arteries without epicardial stenosis should be equal to the mean aortic pressure; thus it can be obtained by measuring mean arterial pressure with a sphygmomanometer. Microvascular resistance (mm Hg·s/cm) was calculated as the ratio between mean blood pressure (mean pressure = [2 × diastolic + systolic]/3) and average peak velocities (APV, cm/s) at baseline and under hyperaemia (BMR and HMR, respectively). The difference between BMR and HMR was defined as arteriolar resistance index (ARI) [21]. The ARI indicates vasodilatory capacity of the arteriolar vessels to dilate under maximal hyperaemia.

2.5. Statistical analysis

Categorical variables were compared by the χ^2 test or the Fisher exact-test as appropriate. Continuous data were compared with the 2-tailed paired or unpaired *t*-test or the Mann-Whitney *U* or Wilcoxon signed-rank test, as appropriate. Receiver operating characteristics (ROC) curve analysis was generated to test the predictive discrimination of patients with and without CMD. Bivariate correlations were assessed by the Spearman coefficient (*rho*). Unadjusted and multiple logistic regression analyses were performed between CFR and risk factors or clinical conditions. Variables included in the multivariable analysis were those achieving a $p \leq .1$ in unadjusted analysis. A combination of forward and backward selection procedures was used to aid in determining the best model of factors independently associated with CMD. Summary statistics for the regression models included the C statistic (a measure of association of predicted probabilities and observed prevalence of a binary outcome) and *R*2 (rescaled for use in logistic regression by the Cox and Snell method). All tests were two-sided and statistical significance was accepted if the null hypothesis could be rejected at $p < .05$. Intra-observer and inter-observer reproducibility of CFR was evaluated by linear regression analysis and expressed as the correlation of coefficients (r) and standard error of estimates and by the intraclass correlation coefficient (ICC). Reproducibility was considered satisfactory if the ICC is between 0.81 and 1.0. Intra-observer and inter-observer reproducibility measurements were calculated in all subjects. Data were analyzed with SPSS software version 22.0 (Chicago, SPSS, Inc., Chicago, Illinois).

3. Results

3.1. Baseline clinical features

Among the 39 patients (33 females, aged 54 ± 12 years), 21 were affected with diffuse cutaneous form of SSc (dSSc) and 18 with the limited cutaneous form (lSSc) [27]. Antinuclear antibodies were positive in all patients, anti-topoisomerase I in 16 (41%) and anticentromere in 15 (38%); only one patients had positive anti-RNA polymerase III. The mean disease duration was 11 years (range 6–22). The characteristics of the study population are summarized in Table 1.

3.2. CFR evaluation

CFR evaluation procedures were always well tolerated; all patients had normal ECG at rest and there were no significant electrocardiographic or LV wall motion abnormalities during adenosine infusion. Data on heart rate, arterial pressure at baseline and during adenosine infusion are reported in Supplemental Table.

Twenty-four patients (62%) were affected with CMD. Microvascular perfusion indexes and capillaroscopic features in SSc patients and in controls are reported in Table 2.

SSc patients compared with controls had lower CFR ($p < .0001$), APVb and APVh ($p = .005$ and $p < .0001$, respectively) and ARI ($p = .01$). By contrast, BMR and HMR were higher in scleroderma patients than in controls ($p = .003$ and $p = .001$, respectively).

Microvascular perfusion indexes in SSc with and without CMD are reported in Table 3: APVh but not APVb was lower in patients with CMD in comparison to patients without CMD ($p = .02$ and $p = .2$, respectively). Likewise, HMR but not BMR were significantly increased in patients with CMD ($p = .009$ and $p = .8$, respectively). ARI was decreased in the CMD group ($p = .003$).

3.3. Characteristics of patients with CMD (CFR ≤ 2.5)

Clinical and demographic characteristics, capillaroscopic features and echocardiographic variables in the entire SSc population and in patients with and without CMD are reported in Table 1.

EUSTAR score and mRSS were higher in patients with CMD than in those without ($p = .01$ and $p = .003$ respectively). Digital ulcers ($p = .001$), interstitial lung disease (ILD) ($p = .028$) and dSSc ($p = .042$) were found to be associated with CMD. No significant differences in age, RP onset and disease duration of patients with and without CMD were found. Avascular and capillary ramifications scores were higher in patients with CMD than in those without ($p = .001$ and $p = .03$, respectively). Diastolic dysfunction and LV ejection fraction

Table 2
Microvascular perfusion indexes and capillaroscopic features in SSc patients and in controls.

	SSc patients (n = 39)	Controls (n = 22)	p-Value
Microvascular perfusion indexes			
CFR	2.6 ± 0.5	3.3 ± 0.5	0.0001
APVb (cm/s)	23 ± 7	32 ± 12	0.005
APVh (cm/s)	59 ± 18	104 ± 27	0.0001
BMR (mmHg.s/cm)	4.1 ± 1.3	3.2 ± 0.8	0.003
HMR (mmHg.s/cm)	1.6 ± 0.1	1.2 ± 0.04	0.001
ARI (mmHg.s/cm)	2.6 ± 1	3.2 ± 1	0.01
Capillaroscopic features			
Enlarged capillaries (> 20 μm)	0.83 ± 0.42	0.18 ± 0.13	0.0001
Giant capillaries (> 50 μm)	0.37 ± 0.33	0 ± 0	0.0001
Hemorrhages	0.22 ± 0.21	0.07 ± 0.1	0.002
Avascular score	0.52 ± 0.41	0.02 ± 0.06	0.0001
Disarray	0.54 ± 0.46	0.07 ± 0.01	0.0001
Capillary ramifications	0.14 ± 0.13	0.03 ± 0.06	0.002

APVb basal average peak velocity; APVh average hyperaemic peak velocity; ARI, arteriole resistance index; BMR, baseline microvascular resistance; CFR, coronary flow reserve; HMR, hyperaemic microvascular resistance; SSc systemic sclerosis. Values are mean ± SD.

Table 3
Microvascular perfusion indexes in SSc patients with and without coronary microvascular dysfunction (CMD).

	All patients (n = 39)	CMD (n = 24)	No CMD (n = 15)	p-Value*
CFR	2.6 ± 0.5	2.1 ± 0.3	3.1 ± 0.3	0.0001
APVb (cm/s)	23 ± 7	24 ± 7	21 ± 7	0.2
APVh (cm/s)	59 ± 18	53 ± 20	66 ± 13	0.02
BMR (mmHg·s/cm)	4.1 ± 1.3	4.1 ± 0.3	4 ± 0.2	0.8
HMR (mmHg·s/cm)	1.6 ± 0.1	1.7 ± 0.1	1.1 ± 0.1	0.009
ARI (mmHg·s/cm)	2.6 ± 1	2.2 ± 0.2	3.3 ± 1	0.003

p-Value* between patients with and without CMD. All values are reported as mean ± SD. APVb, basal average peak velocity; APVh, average hyperaemic peak velocity; ARI, arteriole resistance index; BMR, baseline microvascular resistance; CFR, coronary flow reserve; CMD, coronary microvascular dysfunction; HMR, hyperaemic microvascular resistance; SSc systemic sclerosis. Values are mean ± SD.

were similar in the two groups. The Framingham risk score tended to be higher in patients with CMD (p = .05).

3.4. Factors associated with CMD (CFR ≤ 2.5)

In unadjusted logistic regression analysis, the independent predictors of CMD were dSSc (p = .047), EUSTAR score (p = .031), ILD (p = .039), digital ulcers (p = .005) and avascular score (p = .038).

When modeled using a stepwise regression and adjusting for baseline differences, ILD and avascular score were the only factors independently associated with CMD (Table 4). To exclude the modulating effect of other variables, we also added Framingham risk score and diastolic dysfunction (marginally significant at univariate analysis, $p \leq .1$) (model 2). When also immunosuppressive agents and capillary ramifications were forced into the model (model 3), avascular score and ILD remained significantly associated with CFR ≤2.5 (p = .01 and p = .02, respectively).

In order to identify determinants of CMD, ROC curve analysis for avascular and capillary ramifications scores, EUSTAR score and mRSS was performed. The best curve was that of avascular score, where the area under the curve (AUC) was 0.840; a cut-off value of 0.21 showed 87% sensitivity, 73% specificity, 84% positive predicted value (PPV) and 78% negative predicted value (NPV), with a diagnostic accuracy of 82% (Fig. 1).

3.5. Correlation between CFR and clinical and capillaroscopic parameters

In whole group of SSc patients, the strongest correlation was between CFR and avascular score ($rho = -0.750$, $p < .0001$) (Fig. 2), followed by CFR and capillary ramifications ($rho = -0.539$, $p < .0001$), CFR and EUSTAR score ($rho = -0.447$, $p = .004$) and CFR and disarray ($rho = -0.381$, $p < .010$).

3.6. Correlation between avascular score and Doppler-derived resistance indexes

In the SSc group of patients, significant correlations between avascular score and HMR ($rho = 0.416$, $p = .008$) and between avascular score and ARI ($rho = -0.349$, $p = .03$) were found. No relations between CFR, BMR and HMR with disease duration were observed.

3.7. Intra- and inter-observer reproducibility of CFR by transthoracic echocardiography

Intra-observer and inter-observer reproducibility of CFR measurements were assessed by repeating CFR evaluation twice, 1 h apart, by the same operator (F.T.) in all subjects and by another operator (G.F.) in all subjects as well. The intra-observer reproducibility was high ($r = 0.92$, SEE = 0.12); ICC was 0.970. The inter-observer reproducibility was also high ($r = 0.91$, SEE = 0.11); ICC was 0.963.

4. Discussion

Our results show that the loss of nailfold capillaries (avascular score) is tightly associated with CMD in scleroderma patients, suggesting a common pathogenetic mechanism. In addition, other disease activity indexes and/or features (EUSTAR score, mRss, ILD and digital ulcers) were significantly associated with CMD.

NVC is an important screening tool included in the SSc classification criteria and it seems to be the best method to evaluate peripheral microvascular abnormalities [4]. Although the microvascular origin of the primary myocardial involvement has been well demonstrated in scleroderma [13,28,29], the assessment of CMD by CFR has not yet become a routine test in the cardiac evaluation of SSc patients. Nowadays, standard transthoracic Doppler echocardiography remains the routine diagnostic tool, even if it has shown a poor sensitivity in the early stages of primary myocardial involvement [28].

The results of our study confirm that CFR is lower in scleroderma patients than in controls, and that CMD has a higher prevalence in SSc patients.

In comparison with controls, our patients showed lower APVb with higher BMR, which could point out a loss of myocardial arterioles leading to a decrease in arteriolar bed and ultimately to an increase in resistance at rest. A similar pathogenetic mechanism has been suggested in previous studies in SSc patients [28–30]. By contrast, Pinter et al. found comparable resting myocardial resistance index (IMR) in SSc patients and in controls. However, both SSc patients and controls in that study showed similar CFR values, in contrast with any other reports [19,20].

To better understand the mechanism underlying microvascular dysfunction, we compared SSc patients with and without CMD. SSc patients with CMD showed lower APVh and higher HMR compared with CMD-free patients. These findings suggest an impairment of the microvascular vasodilatory function due to a structural microvascular remodeling [21]. In keeping with this hypothesis, the ARI was decreased in SSc patients with CMD, pinpointing a defective recruitment of CFR to maintain an adequate coronary blood flow commensurate with myocardial oxygen demand. Therefore, the main mechanism

Table 4
Multivariable logistic regression analysis for the association between CMD and clinical/capillaroscopic features of SSc patients.

	Model 1[a]			Model 2[b]			Model 3[c]		
	OR	95% CI	*p*	OR	95% CI	*p*	OR	95% CI	*p*
Avascular score	35	14–90	0.01	9.8	2.8–12.3	0.01	11	3.8–31	0.01
ILD	15.6	1.37–45	0.02	27	1.48–50	0.02	16.9	1.46–25	0.02
Diffuse SSc	1.28	0.17–9.2	0.8	4.2	0.47–8.2	0.1	1.51	0.94–24	0.7
Skin score	1.11	0.93–1.31	0.2	1.23	1.03–1.46	0.02	1.09	0.87–1.37	0.4
EUSTAR score	1.1	0.14–8.4	0.9	2.48	0.15–3.9	0.5	4.5	0.4–46	0.2

C-statistic for Model 1: 0.901, $p < .0001$; χ^2 0.74, $p < .0001$.
C-statistic for model 2: 0.900, $p < .0001$; χ^2 0.70, $p < .0001$.
C-statistic for model 3: 0.899, $p < .0001$; χ^2 0.68, $p < .0001$.
[a] Model 1: adjusted for significant ($p < .05$) variables at univariate logistic regression analysis.
[b] Model 2: Model 1 + adjustment for Framingham risk score and diastolic dysfunction (marginally significant at univariate analysis, $p \leq .1$).
[c] Model 3: Model 2 + immunosuppression and capillary ramifications (marginally significant at univariate analysis, $p \leq .1$). Other abbreviations as in Table 1.

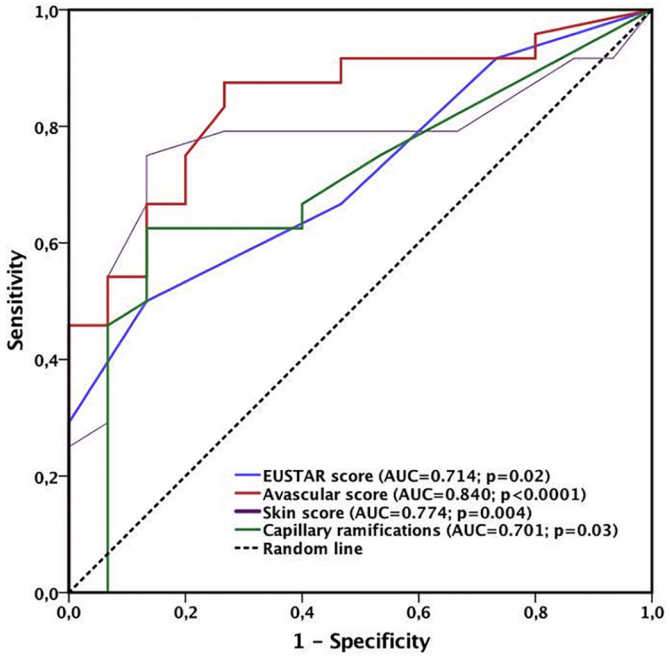

Fig. 1. Receiver Operating Characteristic (ROC) curve of the predictors of coronary microvascular dysfunction (CMD).

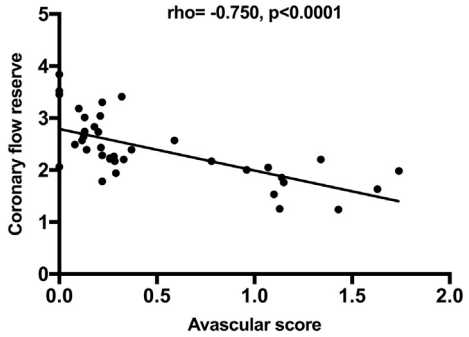

Fig. 2. Bivariate correlation analysis of CFR and avascular score.

underlying CMD in our patients appears to be structural microvascular damage, irrespective of the disease duration.

By contrast, in Pinter's study [31] hyperaemic IMR was similar in patients with CFR > and ≤2, denying the occurrence of a structural remodeling. However, it has to be pointed out that we used a different method and different cut-off values to define CMD (CFR ≤2.5 in our study vs ≤2 in Pinter's study) [26]. Moreover, the number of SSc patients affected by CMD was very small in Pinter's study (six).

The occurrence of a structural remodeling could also be due to the long disease duration in our patients (median 11 years) none of whom had a disease duration shorter than two years. Thus, we cannot rule out a potential functional impairment in the very early disease stages heralding the structural damage as in diabetes [21].

Likewise, avascular score identifies a structural damage in the nailfold capillaries. RP is considered the first clinical sign of vascular involvement in SSc and may precede visceral involvement by several years [2,3]. Considering the systemic impairment of the microvessels in SSc, a number of studies have investigated the correlation between capillaroscopic findings and organ involvements [6–8,32,33].

It has been suggested that the avascular score is the most reliable capillaroscopic feature in assessing peripheral microvascular damage. Two studies showed a significant correlation between avascular score and mean pulmonary arterial pressure (mPAP) in SSc. [32,33]. Furthermore, Sulli et al. [10] postulated that a regular NVC evaluation could be useful in the assessment of microvascular heart involvement in SSc, however this assumption has not been proved yet [10].

Among the clinical and capillaroscopic features considered in our study, avascular score was the only one independently associated with CFR at multivariable analysis. In addition, at multiple logistic regression analysis only avascular score and ILD remained significantly associated with CMD when modeled using a stepwise regression.

Mueller et al. [34] found that a reduced capillary density was the capillaroscopic feature more strongly associated with all-cause mortality in patients with RP, irrespective of the presence of a connective tissue disease. Moreover, Kaiser et al. [35] found that among several disease and capillaroscopic features an avascular score higher than 1.5 was the only independent predictor of death. Our study showed an association between avascular score and CMD in SSc patients, which could explain why avascular score emerged as the only independent risk factor for death in Kayser's study. Notably, the Authors did not evaluate any clinical variables markers of primary myocardial involvement.

Avascular score seems to mirror the structural impairment of microcirculation (presence or absence of capillaries) in scleroderma, as CMD. In support of this hypothesis, we found a significant correlation between avascular score and both HMR and ARI but not with BMR, suggesting a common structural mechanism.

Regarding the other clinical features, some studies found a more severe decrease in CFR in patients with dSSc than in those with lSSc [19,20]. Likewise, we found that dSSc was a risk factor for CMD, although it was not independently associated with an impaired CFR in multivariable logistic regression analysis (Table 4). We found a correlation between CFR and disarray and capillary ramifications. The latter (also named arborescent capillaries), stands for an inefficient neoangiogenesis attempt in response to nailfold capillary loss. Thus, the

inverse correlation between CFR and arborescent capillaries suggests that inefficient neonagiogenesis could play a role also in the development of CMD, as in the nailfold bed.

We also found a significant inverse correlation and association between CMD and EUSTAR score which suggest that primary heart involvement might occur during the active stages of the disease, supporting the role of inflammation in triggering endothelial injury and dysfunction in CMD [36,37].

Framingham risk score, as expected, was higher in patients with CMD, confirming that traditional cardiovascular risk factor can affect CFR. However, the Framingham risk score was very low in our patients and we adjusted data at multivariate regression analysis, accordingly.

ILD and digital ulcers were other significant and independent risk factors for a decreased CFR in our study. Notably, persistent and repeated injuries to endothelial cells are considered primarily linked to the pathogenesis of lung involvement in SSc. Castellvi et al. [38] uncovered a correlation between diffusing capacity for carbon monoxide (DLCO) or forced vital capacity (FVC) and avascular score. Mihai et al. [39] demonstrated that digital ulcers are a risk factor for a more severe disease, heralding cardiovascular events and a poor survival in patients with SSc. These findings support the close link between ischaemic digital damage (represented by digital ulcers and avascular score) and mortality due to cardiac involvement in SSc.

Moreover, digital ulcers and functional pulmonary involvement are included in the EUSTAR score [22] and, therefore, can be considered as markers of disease activity. Our findings support the hypothesis that microvasculature of different anatomical areas may be affected by the same pathogenetic mechanism, mainly during more active stages of the disease.

We did not find any significant associations between cardiac resistance and velocities at rest and in hyperaemic condition, CMD, CFR, and disease duration probably due to the great heterogeneity of scleroderma onset and evolution, which can range from very slow and mild phenotype to severe and rapidly life-threatening condition. Ultimately, our data suggest that CMD is more closely linked to the concomitant presence of other clinical involvements driven by microvascular impairment, rather than disease duration itself.

4.1. Study limitations

A reduced CFR is detectable also in the presence of significant epicardial coronary stenosis [15]. One of the limitations of our study is the lack of systematic evaluation of epicardial coronaries in patients with a decreased CFR; thus, the presence of coronary significant stenosis cannot be ruled out. In spite of this, the likelihood of coronary plaques is low among our patients for several reasons. First of all, the risk of cardiovascular disease, as assessed by the Framingham risk score, was low. Second, exclusion criteria for participation in this study included recent acute cardiovascular events, history of angina or myocardial infarction as well as cerebral vascular disease, carotid artery bruit, peripheral bruit or abnormal pulse. Third, many previous studies demonstrated that in asymptomatic scleroderma patients the CFR impairment is due to a primary dysfunction of the coronary microvasculature and not to an epicardial coronary stenosis, which was actually ruled out by invasive test performed in these studies [13,14]. Notably, the extent of fibrosis does not correspond to any epicardial artery distribution in autoptical and magnetic resonance imaging studies, confirming previous hypotheses [40]. Finally, the relatively small number of patients, in particular patients with abnormal CFR, limits statistical power. Further studies will be required to confirm our results in a larger population of SSc patients.

4.2. Conclusions

This study showed that NVC-avascular score is associated with coronary microvascular disease in our SSc patients. NVC may identify a subset of patients with low CFR and higher cardiac risk. In these patients a tight cardiologic and rheumatologic follow-up is recommended. The main mechanism for SSc-CMD seems to be a structural microvascular remodeling.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.autrev.2018.09.002.

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