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Ivone SILVA[1]
Tiago LOUREIRO[2]
Andreia TEIXEIRA[3]
Isabel ALMEIDA[4]
Armando MANSILHA[5]
Carlos VASCONCELOS[4]
Rui ALMEIDA[2]

[1] Angiology, Vascular Surgery Service, Clinical Immunology Unit,
[2] Angiology and Vascular Surgery Service,
[3] Health Information and Decision Sciences Department,
Universidade do Porto
[4] Clinical Immunology Unit,
Centro Hospitalar do Porto,
Praça da Revista o Tripeiro,
n° 42, hab 23, 4415-789 Porto,
Portugal
[5] Unit of Angiology and Vascular Surgery; Faculty of Medicine of University of Porto

Reprints: I. Silva
<heitor.ivone@gmail.com>

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Digital ulcers in systemic sclerosis: role of flow-mediated dilatation and capillaroscopy as risk assessment tools

Aim: The aim of this study was to evaluate macrovascular endothelial dysfunction and microvascular damage as clinical markers of peripheral microangiopathy in patients with Raynaud's phenomenon (RP). *Patients and methods*: Seventy-seven secondary RP with systemic sclerosis, 32 primary RP and 34 healthy controls were included in our study. Secondary RP patients were divided into two subgroups: 39 with digital ulcers (DU) and 38 without digital ulcers (non-DU). *Results*: Patients with DU had significantly lower flow-mediated dilatation values (5.34 ± 7.49%) compared to non-DU patients (16.21 ± 11.31%), primary RP (17.96 ± 12.78%) and controls (20.17 ± 8.86%), $p<0.001$, favouring macrovascular endothelium dysfunction. Regarding microvascular damage, the DU group had a predominately capillaroscopic late pattern (71.1%) whereas non-DU patients had an active pattern (56.4%). The microangiopathy evolution score was significantly higher in the DU group compared to the non-DU group (4.79 ± 1.82 *vs*. 1.79 ± 1.56, $p<0.001$). Flow-mediated dilation was significantly lower in late pattern (6.13 ± 7.09%) compared to active (12.58 ± 10.66%) and early patterns (17.72 ± 14.90%), $p = 0.016$ and $p = 0.044$ respectively. *Conclusions*: Low flow-mediated dilatation and microvascular damage in capillaroscopy are early clinical markers of DU risk in RP patients.

Key words: Raynaud's phenomenon, systemic sclerosis, digital ulcer, endothelial dysfunction, flow-mediated dilatation, capillaroscopy

Raynaud's phenomenon (RP) is the earliest clinical presentation of some connective tissue disease and, in systemic sclerosis (SSc), for most authors, it is considered the first clinical manifestation of disease. Although microvessel involvement has largely been described, it is important to highlight that macrovascular disease may also affect more than half of SSc patients [1].
Endothelium is a dynamic endocrine organ that plays a critical role in vascular homeostasis by secreting substances that regulate vascular tone, platelet activity and coagulation factors. It participates in vascular inflammation, cell migration and proliferation [2]. An increased apoptotic state is involved in endothelium denudation and dysfunction [2], leading to impaired vascular tone, pro-inflammation and pro-thrombotic states [2], increased expression of adhesion molecules and oxidative stress [3]. Structural integrity of the endothelium is fundamental for a normal function. An impaired release and bioavailability of endothelium derived relaxing factor nitric oxide (NO) is observed in endothelial dysfunction [2]. NO is probably the major mediator of vasodilation and reduced NO bioavailability has been broadly accepted as a marker of endothelium dysfunction [4].
Endothelial dependent flow-mediated dilatation (FMD) measurement is a functional bioassay of NO bioavailability [4], a combination of the endothelium NO production and destruction by reactive oxygen species [5]. FMD is a simple, safe, cheap, non-invasive, sensitive, reproducible method and has been largely used for endothelium dysfunction assessment.
Microvasculopathy with alterations in morphology and function can easily be detected at the nailfold bed with nailfold videocapillaroscopy (NVC). Capillaroscopy is a valuable, accessible, non-invasive, easy, safe and cheap tool with important diagnostic and prognostic value in patients with SSc. Qualitative, semi-quantitative and quantitative indexes/scores may be performed. Capillaroscopy has been described as the best predictor tool of transition of primary to secondary RP [6]. Structural changes are present in more than 90% of SSc patients [7]. Structural morphological and functional changes in capillaries allows differentiation between PRP and SRP identification of different scleroderma evolution patterns, with a close monitoring of disease evolution.
In the clinical setting, qualitative evaluation is the most accessible and easy to perform and enables diagnosis of different stages of disease activity and progression. Architectural disorganization as a result of microvascular injury includes giant capillaries, microhemorrhages, capillary loss and avascular areas and morphological changes with branched/ramified/bushy capillaries, suggesting angiogenesis. Cutolo *et al.* described a useful and reproducible

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qualitative NVC "Scleroderma patterns" with 3 different evolutive patterns: early, active and late [8]. Several studies have been done in an attempt to identify NVC changes and correlation to organ disease, in order to identify a possible predictive role of NVC.
The aim of this study was to evaluate and compare macrovascular endothelial dysfunction and microvascular damage as potential clinical markers of peripheral microangiopathy in patients with Raynaud's phenomenon. Additionally we determined the relationship of these clinical markers with severity of vasculopathy, namely the presence of digital ulcers.

Patients and methods

Patients

All hundred and nine enrolled patients, 97 women; mean age 50.9 ± 12.4 years; range 14-79, attended the Multidisciplinary Raynaud Clinics of the Clinical Immunology Unit at Centro Hospitalar do Porto in Portugal.
Thirty two patients had primary Raynaud phenomenon (PRP), 25 women; mean age 49.9 ± 12.5 years; range 22-73, with negative autoantibodies (ANA), no digital ulcers and a bi- or triphasic colour change of fingers when exposed to cold.
Seventy-seven patients had secondary Raynaud phenomenon (SRP), 72 women; mean age 52.95 ± 12.6 years; range 14-79, and based on the 2013 classification criteria for SSc of the American College of Rheumatology [9], all SRP patients had SSc. According to the Leroy [10] classification, 13 (16.9%) had diffuse SSc (dcSSc) and 64 (83.1%) had limited SSc (lcSSc). SSc patients were divided into two subgroups: DU group - 38 patients with an active ulcer at time of study (painful area of 2 mm or greater in diameter with visible depth and loss of dermis, amenable to healing and localized to the fingertip), with or without a past history of DU, (34 women; mean age 52.7 ± 14.8 years; range 14-75) and non-DU group - 39 patients with no DU in the course of disease (38 women; mean age 53.2 ± 10.3 years; range 30-79). Onset of disease was defined at time of the first episode of RP.
A group of 34 (sex, age matched) healthy, non-obese, without self-reported cardiovascular risk factors: smoking, hypertension, diabetes or hyperlipidaemia, were invited to participate as controls (29 women; mean age 47.1 ± 10.96 years; range 23-66). No control subject was on any vasoactive medication.
The study was done in the winter months from November to February. Pre-menstrual women were assessed in days 1 to 7 in the menstrual cycle. Three patients with factors that could potentially interfere with FMD were excluded at the beginning of the study (smoking, diabetes, hypertension, hyperlipidaemia and a history of myocardial infarction). All subjects studied had ECG and echocardiographic measurements within normal ranges. Allen test was performed in all subjects for macrovascular impairment screening.
This study was approved by the Local institutional Ethical Committee and all subjects signed informed consent before the study.

Allen test

The Allen test was performed as follows: 1. instructing the patient to clench his/her fist; 2. Applying occlusive pressure to both ulnar and radial arteries by finger pressure; 3. Confirming palm and finger blanching with the patient's hand relaxed; 4. Releasing the occlusive pressure on the ulnar artery; 5. Positive test: If the hand flushes within 5-15 seconds it indicates that the ulnar artery has good blood flow and palmar arch is complete; Negative test: If the hand does not flush within 5-15 seconds, it indicates that ulnar circulation is inadequate, with an incomplete palmar arch.

Ultrasound examination and FMD

Ultrasounds scans were performed using a two-dimensional ultrasonography General Electric Logic 7 with a 9 MHz Linear wideband multihertz imaging probe. All exams were performed by the same operator and recorded in the image analysis software.
For spectral waveform measurement, two cursors were placed on sonographic image of the braquial artery examined; sample gate cursor and alignment angle correct cursor. Three contiguous spectral waveforms were recorded for the determination of peak systolic velocity (PSV), end diastolic velocity (EDV) and Resistive Index (IR). The latter was calculated as (PSV-EDV)/PSV. All these parameters were measured for each waveform and obtained as the average of three measurements in each waveform.
Flow mediated dilatation of the braquial artery in the lower arm was evaluated following International Brachial Artery Reactivity Task Force guidelines [11] for the ultrasound assessment of brachial artery endothelial-dependent flow-mediated vasodilatation. Patients and healthy subjects were on overnight fasting for twelve hours before the study. The examinations were performed in the morning, after patients being kept in a quiet, temperature controlled room (22-24 °C) for a 20 minute rest. Vasoactive drugs were withheld for 10 half-lives. Patients did not exercise or ingest substances that could affect the response to ischemia, like caffeine, vitamin C, tobacco or high-fat foods for 24 hours.
The braquial artery was scanned in a longitudinal section below the antecubital fossa in 2 to 3 cm sections. Mean baseline braquial artery diameter was calculated as the result of the mean of 3 baseline measurements. A sphygmomanometric blood pressure cuff localized above the antecubital fossa was inflated 50 mmHg above the patient's systolic blood pressure (measured in the left upper arm) and kept inflated for five minutes. No examination had to be discontinued due to pain or discomfort. Before cuff deflation, images were recorded continuously from 15s before deflation to 180s after cuff deflation. Ultrasound images were analysed for 3 consecutive end diastolic frames (onset of R wave) at 45 to 60s after cuff deflation. The intraoperator variability was 3.6%. FMD measurements were performed blind with respect to the NVC evaluation.
FMD was calculated as the percentage of change of the peak diameter in response to reactive hyperaemia in [11]:

$$\text{FMD\%} = \frac{peak\ diameter - baseline\ diameter}{baseline\ diameter} \times 100$$

Nailfold videocapillaroscopy

Nailfold videocapillaroscopy (NVC) was performed with KK technology videocapillaroscopy with a 200x magnification lens. The same operator performed all capillaroscopies, with blind information regarding FMD. Images were recorded and kept anonymous in a KK image analysis software. The inter observer variability was 2.5%.

All subjects were in a quiet room with controlled temperature (21-24 °C). Nailfold distal row capillaries of 4 fingers, the 2nd, 3rd, 4th and 5th of both hands were examined.

Two classifications were used to describe capillaroscopic findings: a qualitative classification of scleroderma microangiopathy damaged as described by Cutolo [12] in 3 patterns, early, active and late. Early pattern was characterized by the presence of a small number of giant capillaries and microhemorrhages, no avascular areas and a relatively well preserved capillary distribution; active pattern was classified according to the presence of numerous giant capillaries and microhemorrhages, moderate capillary loss (20-30%) and a mild disorganized capillary architecture, with rare branches capillaries; Late pattern is characterized the by near-absence of giant capillaries and microhemorrhages, the presence of extensive avascular areas (50-70% of capillary loss) and the presence of many branched and ramified bushy capillaries (neoangiogenesis) and a complete disorganization of capillary array [12].

A semi-quantitative score, Microangiopathy Evolution Score (MES), was adapted from Sulli *et al.* [13]. The sum of three scores regarding loss of capillaries, disorganization of the microvascular array and capillary ramifications was assessed to study the progression of the vascular damage. A rating scale to score each capillary abnormality was used (0 = no changes; 1 $\leq$33% capillary reduction / changes; 2 = 33-66% capillary reduction / changes; 3 $\geq$66% capillary reduction / changes) per linear millimeter [13]. The average score values from the eight digits were added together and the final value divided by eight [13]. The resulting value represents the evolution score of microangiopathy (MES): 0-9 [14]. Following this, we categorized MES scores in 3 subcategories with the following cut-off values: subcategory 1 – from 0 to 3; subcategory 2 – from 4 to 6 and subcategory 3 – from 7 to 9, allowing a more detailed analysis between microangiopathy and endothelial dysfunction evaluation.

Autoantibody detection

Antinuclear antibodies (ANA) were accessed by indirect immunofluorescence on Hep-2 cells (NOVAlite ANA, Inova Diagnostics, Inc., San Diego, CA, USA). Samples with a titer greater than or equal to 1:80 were considered positive. Autoantibodies anti-Scl-70, anti-centromere (ACA), anti-Ro52, anti-PM-Scl, anti-RNA-polymerase, anti-fibrillarin (AFA), and anti-NOR 90 were detected by immunoblotting using a Euroline Myositis Profile antibody test syst (Euroimmun, Lübeck, Germany). Quantification of anti-U1RNP, antibodies was carried out using a Fluoro Enzyme Immuno Assay (EliA™ U1RNP70; Phadia, Uppsala, Sweden)

Statistical analysis

For comparison of normally distributed scale variables, we used unpaired or paired two-sided student's t-test or analysis of variance (Anova). In these cases, data were described by mean ± standard deviation (SD) followed by the minimal and the maximal values (range). Normal distribution was tested by Q-Q plots. In cases of non-normally distributed variables, we used non-parametric tests: Mann-Whitney and Kruskal Wallis tests and data were described by median followed by the interquartile interval (Q_1-Q_2;), where Q_1 represents the first quartile (corresponding to 25% of data) and Q_3 represents the third quartile (corresponding to 75% of data). In Anova tests, when the homogeneity of variance was not satisfied, we used the Welch test. For comparison of categorical variables, we used Chi-square or Fisher's exact probability test. In cases of multiple testing, a Bonferroni correction or a Games-Howell test (for equal variances not assumed) was used. We considered p values <0.05 as significant. Data were analysed using the SPSS software (v.22.0, SPSS,Chicago,IL).

Results

Clinical assessment

The demographic and clinical characteristics of the 143 subjects are described in *table 1*. No major differences were observed between SSc patients, PRP patients and the control group regarding age, gender, mean arterial pressure and total cholesterol.

Disease duration was significantly different between groups ($p = 0.028$). Patients with PRP had a significantly longer history of RP (median value: 15 years) compared to SSc patients (median value: 10 years). The DU group had a median of 5 years of DU history.

The characteristics of the SSc patients with or without ischemic digital ulcer are summarized in *table 2*.

Allen test was positive in 71% of patients with DU and in 18% in SSc non-DU patients ($p<0.001$). Patients with SSc-DU had significantly more sclerodactily (55.3%, $p<0.001$), telangictasia (100%, $p<0.001$), digital pitting scars (84.2%, $p = 0.001$) and calcinosis (47.4%, $p<0.001$). Patients without DU had significantly more puffy hands (87.2%, $p = 0.008$).

Autoantibodies

There were no significant differences between SSc-DU and SSc non-DU patients regarding to ACA ($p = 0.301$), anti-Scl-70 ($p = 0.093$), AFA ($p = 1.000$), anti-PM-Scl ($p = 0.431$), anti-NOR 90 ($p = 0.240$), and anti-RNA-polymerase ($p = 1.000$). Although without statistical significance, anti-Ro52 showed a higher clinical prevalence in the SSc- DU group (52.6%) comparatively with the SSc non-DU group (30.8%) ($p = 0.052$).

Videocapillaroscopy

All SSc patients had a scleroderma pattern at NVC examination. Significant differences were found between the SSc DU and SSc non-DU groups regarding qualitative evaluation ($p<0.001$): late pattern was the most frequent pattern in DU patients (71.1%) whereas early and active patterns were the most representative patterns in non-DU patients (33.3% and 56.4%, respectively). Significant differences of

Table 1. Demographic and clinical data of subjects included.

Variables	PRP	SRPSS		Control	p-value
		DU	Non-DU		
Subjects, n	32	38	39	34	NA
Age (years), *mean ± SD (min-max)*	49.9 ± 12.5 (22-73)	52.7 ± 14.8 (14-75)	53.2 ± 10.3 (30-79)	47.1 ± 10.96 (23-66)	0.137[a]
Gender Women, n (%)	25 (78.1)	34 (89.5)	38 (97.4)	29 (85.3)	0.067[b]
Disease duration (years), *median (Q_1-Q_3)*	15 (11.25-30)	10 (5-23)	10 (7-20)	NA	0.028*[,c]
Mean arterial pressure (mmHg), *mean ± SD (min-max)*	87.6 ± 5.6 (76-102)	87.9 ± 6.04 (79-102)	88.3 ± 6.4 (77-103)	86.7 ± 6.8 (70-101)	0.75[a]
Total cholesterol (mg/dl), *mean ± SD (min-max)*	188.8 ± 8.8 (169-201)	191.3 ± 9.0 (169-216)	187.1 ± 12.1 (156-202)	190.6 ± 7.6 (170-201)	0.242[a]

*PRP: primary Raynaud phenomenon; SRP: secondary Raynaud phenomenon ; SSc: systemic sclerosis; NA: no applicable. [a]:Anova test. [b]: Fisher's Exact test. [c]: Kruskal-Wallis test. *: statistical significance for a level of 5%.*

qualitative evaluation were found relative to disease subset (*p = 0.010*). Patients with diffuse disease had predominately late pattern (76.9%) while limited disease had a more active pattern (46.9%). Concerning the semi-quantitative MES score, significant differences were observed: the MES score was higher in the DU group compared to the non-DU group (4.79 ± 1.82 *vs*. 1.79 ± 1.56, *p<0.001*). Relative to MES subcategories, we found significant differences (*p<0.001*): subcategory 2 is the most frequent in SSc-DU patients (55.3%) while subcategory 1 is the most representative in the SSc non-DU group (89.7%).

Macrovascular ultrasound examination and FMD

Ultrasound patterns and FMD are described in *table 3*. Flow-mediated dilatation at 60 seconds after deflation was significantly lower in patients with DU (mean 5.34 ± 7.49%; range -9.22 - 21.67) when compared to SSc non-DU (mean 16.21 ± 11.31%; range -4.60 - 48.27; *p<0.001*), primary RP (mean 17.96 ± 12.78%; range 2.04 - 63.21; *p<0.001*) and control (mean 20.17 ± 8.86%; range 7.66 - 44.81; *p<0.001*). No statistical differences were found between SSc non-DU, PRP and control groups. When comparing the values of FMD in limited (n = 64) and diffuse (n = 13) SSc subjects, no statistical differences were found (mean 11.91 ± 11.35% *vs.* 5.63 ± 7.45%, *p = 0.06*). No linear regression was found between FMD and disease duration (*p = 0.542*).

Baseline artery diameter was similar between groups (*p = 0.620*). Absolute difference between mean baseline and post occlusive hyperemia dilatation at 60 seconds showed significant differences between SSc DU patients and all the other groups (*p<0.001*), see *table 3*.

Primary RP (*p<0.001*), SSc non-DU (*p = 0.001*) and SSc DU (*p = 0.002*) had significantly decreased basal state PSV compared to the control group (mean 94.99 ± 21.57cm/s; range 44.6-125.3). No differences were found between patients with and without DU (*p = 0.989*). After 5 minutes, braquial artery occlusion PSV and EDV were significantly reduced in SSc DU patients (PSV: mean

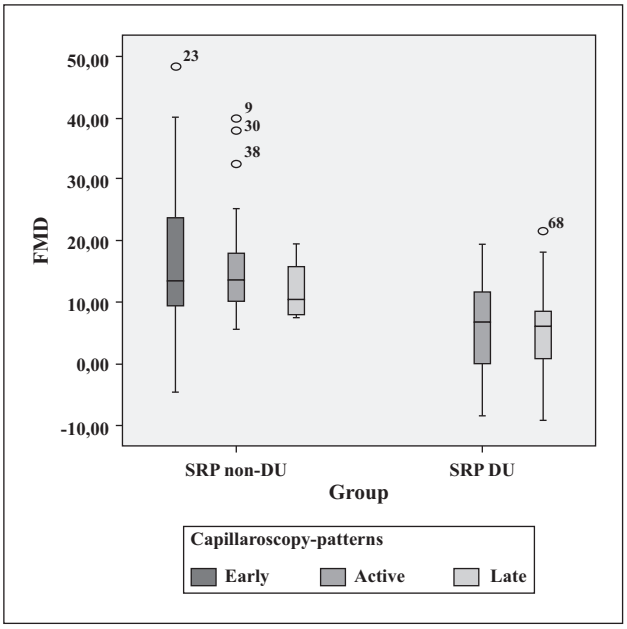

Figure 1. Comparison of FMD in different qualitative capillaroscopy patterns in patients with SRP non-DU and DU patients. *FMD: flow mediated dilation; SRP: Secondary Raynaud Phenomenon patients; DU: digital ulcers.*

148.78 ± 34.10cm/s; EDV: mean 58.03 ± 16.89cm/s) compared to PRP (PSV: *p = 0.003*; EDV: *p<0.001*), SSc non-DU (PSV and EDV: *p<0.001*) and controls (PSV and EDV: *p<0.001*). Resistance Index (RI) had significant differences between the groups (*p = 0.024*).

FMD and NVC

Regarding qualitative NVC patterns, statistically significant differences of FMD were found between the groups (*p = 0.005, figure 1*). FMD was significantly lower in the late pattern (mean 6.13 ± 7.09%; range -9.22 - 21.67) compared to the active pattern (mean 12.58 ± 1.07%; range

Table 2. Comparison between SSc-DU and SSc non-DU groups.

Variables	SRPSS-DU	SRPSS non-DU	p-value
Subjects, n	38	39	
dcSSc/lcSSc			0.001*,a
Limitada, n (%)	26 (68.4)	38 (97.4)	
Difusa, n (%)	12 (31.6)	1 (2.6)	
Onset of 1st ulcer (years)			
Median (Q_1-Q_2)	5 (3-13.25)	NA	NA
Puffy hands			
Positive, n (%)	23 (60.5)	34 (87.2)	0.008*,a
Sclerodactily			
Positive, n (%)	21 (55.3)	5 (12.8)	<0.001*,a
Telangiectasia			
Positive, n (%)	38 (100)	27 (69.2)	<0.001*,a
Digital pitting scars			
Positive, n (%)	32 (84.2)	19 (48.7)	0.001*,a
Periungeal haemorrhages *Positive, n (%)*	29 (76.3)	24 (61.5)	0.162a
Digital amputation			
Positive, n (%)	10 (26.3)	0 (0)	0.001*,b
Calcinosis			
Positive, n (%)	18 (47.4)	3 (7.7)	<0.001*,a
Hand/arm contractures			
Positive, n (%)	19 (50)	3 (7.7)	<0.001*,a
Allen Test			
Positive, n (%)	27 (71.1)	7 (17.9)	<0.001*,a
Autoantibodies			
ACA			
Positive, n (%)	22 (57.9)	27 (69.2)	0.301
Scl-70			
Positive, n (%)	12 (31.6)	6 (15,4)	0.093a
Anti-PM Scl			
Positive, n (%)	2 (5.3)	5 (12.8)	0.431b
Anti-RO 52			
Positive, n (%)	20 (52.6)	12 (30.8)	0.052a
Anti-NOR			
Positive, n (%)	0 (0)	3 (7.7)	0.240b
Anti-fibrilarin			
Positive, n (%)	0	1 (2.6)	1.000b
Anti U1 RNP			
Positive, n(%)	2 (5.3)	2 (5.1)	1.000b
NVC Pattern			
Early, n (%)	0 (0)	13 (33.3)	<0.001a
Active, n (%)	11 (28.9)	22 (56.4)	
Late, n (%)	27 (71.1)	4 (10.3)	
Normal, n (%)	0 (0)	0 (0)	
MES			
from 0 to 3	11 (28.9)	35(89.7)	<0.001*,b
from 4 to 6	21 (55.3)	4 (10.3)	
from 7 to 9	6 (15.8)	0 (0)	

SSc: systemic sclerosis; DU: digital ulcer; dcSSc: diffuse systemic sclerosis subset lcSSc: limited systemic sclerosis subset; SRP: secondary Raynaud phenomenon ; ACA: autoantibody anti-centromere; NVC: nailfold videocapillarosocopy; MES: microangiopathy evolution score;NA: not applicable.
*[a] : Chi-Square test. [b] : Fisher's Exact test. *: statistical significance for a level of 5%*

Table 3. Macrovascular ultrasound and flow mediated dilation in the study population.

Variables	PRP	SRPSS		Control	p-value
		DU	Non-DU		
Baseline artery (mm) *mean ± SD (min-max)*	3.51 ± 0.69 (1.48-4.51)	3.34 ± 0.51 (1.95-4.72)	3.44 ± 0.52 (2.31-4.17)	3.37 ± 0.55 (2.31-4.69)	0.620[a]
Absolute difference (mm) *mean ± SD (min-max)*	0.57 ± 0.29 (0.08-1.08)	0.17 ± 0.23 (-0.31-0.60)	0.52 ± 0.29 (-0.17-1.24)	0.65 ± 0.23 (0.28-1.09)	<0.001[*,a]
FMD (%) *mean ± SD (min-max)*	17.96 ± 12.78 (2.04-63.21)	5.34 ± 7.49 (-9.22-21.67)	16.21 ± 11.31 (-4.60-48.27)	20.17 ± 8.86 (7.66-44.81)	<0.001[*,a]
Baseline PSV (cm/s) *mean ± SD (min-max)*	65.77 ± 11.23 (49.10-94.80)	76.65 ± 19.72 (39.80-113.90)	75.26 ± 18.91 (37.10-113.50)	94.99 ± 21.57 (44.60-125.3)	<0.001[*,a]
PSV 60 sec after cuff deflation (cm/s) *mean ± SD (min-max)*	177.69 ± 26.69 (116.9-222,0)	148.78 ± 34.10 (51.7-207.8)	181.51 ± 38.10 (107.4-268.5)	199.77 ± 32.93 (122.7-262.8)	<0.001[*,a]
EDV sec after cuff deflation (cm/s) *mean ± SD (min-max)*	92.95 ± 35.05 (11.8-169.3)	58.03 ± 16.89 (24.6-90.3)	78.43 ± 24.37 (23.9-131.4)	93.70 ± 20.01 (50.5-131.9)	<0.001[*,a]
RI *mean ± SD (min-max)*	0.47 ± 0.23 (-0.38-0.94)	0.58 ± 0.23 (-0.60-0.85)	0.57 ± 0.09 (0.29-0.82)	0.53 ± 0.08 (0.33-0.70)	0.024[*,a]

PRP: primary Raynaud phenomenon; SRP: secondary Raynaud phenomenon ; SSc: systemic sclerosis; DU: digital ulcer; FMD: flow mediated dilatation; PSV: peak systolic velocity; EDV: end diastolic velocity;RI: resistive index. [a]: Anova test. [*]: statistical significance for a level of 5%.

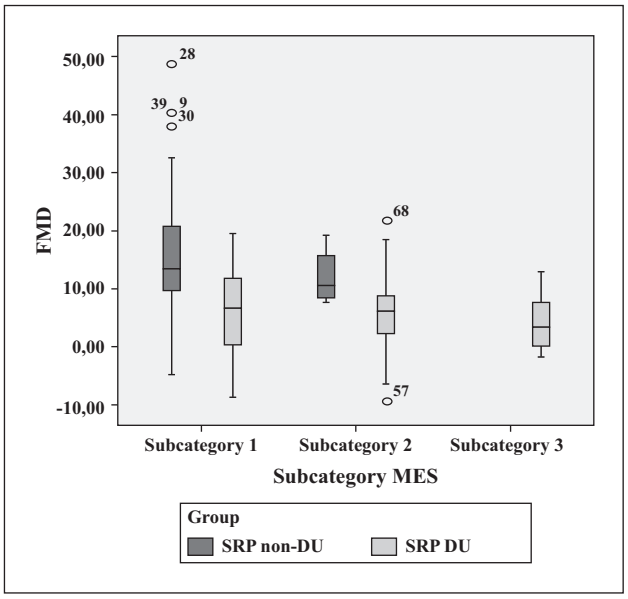

Figure 2. FMD and MES sub-categories in SSc patients with and without digital ulcers. MES Sub-category 1 (*n= 46)*; Sub-category 2 (*n= 25)* and Sub-category 3 (*n=6). FMD: flow mediated dilation; MES:Microangiopathy evolution score; SSc Systemic sclerosis.*

-8.49 - 40.04, *p = 0.016*) and the early pattern (mean 17.72 ± 1.49%; range -4.60 - 48.27, *p = 0.044*). No differences found between active and early patterns (*p = 0.506*).

FMD and MES

Flow-mediated dilatation was statistically different in MES subcategories (*p = 0.006, figure 2*): lower in subcategory 3 (mean 4.37 ± 5.55% range -1.49 - 12.98) compared to subcategory 1 (mean 14.03 ± 1.21% range -8.49 - 48.27) and subcategory 2 (mean 6.55 ± 7.44%; range -9.22; 21.67).

Discussion

Vascular dysfunction is a key element of SSc pathogenesis [14]. Functional and structural changes involve microvessels, digital arteries and small elastic conduit arteries, such as radial and braquial arteries. Vascular changes in SSc can be classified into two subgroups: destructive vasculopathy with progressive loss of capillaries early in disease course and proliferative obliterative vasculopathy characterized by proliferation of vascular cells, with luminal narrowing due to intimal hyperplasia and intimal fibrosis [15]. Although microvessel involvement has largely been described, macrovascular disease may affect more than half of SSc patients [1] but there are few studies regarding small elastic conduits, such as brachial, ulnar and radial arteries [16-18].

Comparing the results of FMD across studies is troublesome. The absolute mean FMD varies among studies from -1.9 to 19.2% [19]. These differences may be due to different study populations, risk profiles and measurement procedures or non-compliance to FMD recommendations and guidelines. Celemajer *et al.* (1992) [20] described a non-invasive technique to measure endothelial function and defined guidelines and recommendations [4, 11, 21].

Controversial results have been published regarding FMD in SSc patients, favouring reduction of FMD and non-endothelial dependent nitroglycerine mediated dilatation (NMD) [22-24], reduction only of FMD [1415], reduction of FMD and preserved NMD [25] and similar FMD and NMD [2627].

In SSc patients, ultrasonography of digital arteries shows small artery lumen, reduced pulsation, thickened artery walls, low vessel compliance [18] and increased RI [16]. Vessel fibrosis and calcification reduce vascular compliance and affect NO signalling by limiting vascular stretch due to arterial stiffness [15]. Different disease profiles and mostly disease duration may reflect different responses to shear stress stimulus.

We assessed endothelial-dependent macrovascular functional impairment in PRP and SRP patients. Baseline artery diameter was similar between the groups. FMD and absolute difference in baseline diameter/post-ischemic artery diameters were significantly lower in patients with SSc than patients with active DU *(figure 1)*. Patients without DU still had a preserved response to shear stress, as no significant differences were found when compared to the PRP and control groups. Late pattern had significantly reduced FMD. Our SSc patients were mostly lcSSc, just as in other reports with similar values of FMD in SSc patients [26, 27]. The patients of Lekakis *et al.* [22] and Cypiene *et al.* [23] demonstrated that dcSSC patients have lower FMD values. In our study, most of the dcSSc patients had DU and no significant difference was found when comparing lcSSc and dcSSc patients regarding FMD. More investigation with a larger series of patients is needed to analyse FMD in SSc disease subsets.

Basal PSV was decreased in RP patients compared to the control group. Post-occlusive PSV and EDV were significantly reduced in SSc DU patients, while RI was considerably increased in the SSc DU patient group. We found no relationship of PSV, EDV and RI with progression of microvascular damage in NVC. The lack of compensatory increase in blood flow to the ischemic stimulus may be due to endothelial dysfunction, reduced compliance, impaired distensability or increased arterial stiffness [28-30]. Increased arterial stiffness has been related to vascular fibrosis, leading to decreased elastic proprieties of large conduit arteries [31] and to diffuse inflammation involving arterioles and elastic arteries [26]. An increase in peripheral resistance due to microvascular damage might also contribute to impaired FMD.

In accordance to Takahashi *et al.* [15] our study suggests that FMD might be a useful marker of severity of obliterative vasculopathy in SSc patients with DU, as shown by the lower FMD value, decreased PSV and EDV and higher RI. Few studies evaluating endothelial dysfunction and microangiopathy as clinical markers for increased risk for DU have been reported. In our study, the main highlights were: 1) DU SSc patients had more avascular areas, disorganization of capillary arrays and neoangiogenesis capillaries than the non-DU group; 2) a high number of giant capillaries and haemorrhages with moderate capillary drop-off was the most frequent pattern in the non-DU group; 3) no patients with DU had early scleroderma patterns; 4) MES had lower scores in the non-DU group (89.7% had scores <3) while in the DU group, severe alterations in capillary morphology and distribution were present; 5) patients with late pattern had significantly lower FMD, in accordance with Le *et al.* [32] and Rollando *et al.* [14]. Our findings point to a trend towards association between macrovascular endothelial dysfunction diseases and damage of microvascular structure in patients with DU. In agreement with Le *et al.* [32], micro and macrovascular function might still be partially preserved in early phases of vascular diseases.

Positive Allen has been associated to RP and scleroderma [33]. Occlusion of the ulnar artery in SSc patients as predictive of DU has been reported [34], probably due to a lack of compensatory flow of the radial/ulnar artery and incomplete palmar arch. In our study, patients with DU had a high prevalence of positive Allen test compared to other groups, suggesting macrovascular disease in these patients. This simple test might be a useful tool in the evaluation of patients with increased risk for DU.

We failed to identify an association of ACA and anti-Scl70 with DU. A trend towards the association of positive Anti-Ro52/TRIM21 and DU was verified. These antibodies can be detected across a number of autoimmune diseases with significant prevalence but the clinical relevance remains controversial. They are often detected in patients with scleroderma but are not diagnostically specific [35, 36]. They may be overlapped with other main scleroderma related antibodies, like anti-centromere, anti-topoisomerase I, anti-RNA polymerase III, or anti-Pm/Scl antibodies and there is some evidence of an association with interstitial lung disease and overlap syndrome [37]. More studies of larger numbers of SSc patients with DU cohorts are needed to identify possible autoantibodies as a possible risk for DU.

Conclusions

In our study, findings favour FMD and NVC as important tools in the early identification of patients at risk of developing DU. Lower values of FMD, late NVC scleroderma pattern and worsening of capillaroscopic scleroderma pattern are red flags towards DU occurrence.

More studies, mainly longitudinal, are needed to investigate these vascular markers as long-term risk predictors, their possible role in the evaluation of disease evolution and as markers for target therapy. ■

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