Endothelial dysfunction and subclinical atheromatosis in patients with systemic sclerosis

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ABSTRACT

Objective. *To assess subclinical vascular features in patients with systemic sclerosis (SSc) via carotid ultrasound, and flow-mediated vasodilation (FMD), as measures of cardiovascular risk (CVR).*

Methods. *This was a cross-sectional study of 70 patients diagnosed with SSc (diffuse or limited forms), on whom a vascular study protocol was performed to assess angiodynamic parameters measured by FMD in brachial artery and carotid ultrasound lesions: carotid intima-media thickness (CIMT) and carotid atheroma plaques (AP). Classical CVR factors were also assessed, as well as main features of SSc regarding skin and organic involvement, laboratory parameters, presence of autoantibodies and specific treatments.*

Results. *94% of patients were women with a mean age of 50.2±12.5 years. 84% had endothelial dysfunction (ED), being severe in 49%, statistically associated with glucocorticoid (GC) treatment (OR=8.78; CI=1.52-50.78; p=0.015). CIMT was pathological in 39%, 23% had AP (none had significative haemodymanic stenosis). Serum vitamin D concentration (25(OH)D3) showed a protective effect on CIMT (OR=0.94; CI=0.89-0.99; p=0.025). No differences between types of SSc were obtained; neither association between SSc features and classical CVR factors.*

Conclusions. *GC treatment has implications in CVR, despite in SSc GC doses administered are lower than in other autoimmune diseases (in our cohort even prednisone ≤10 mg daily was associated with ED). GC may be associated with an early vascular damage in these patients, which could lead to changes in FMD, ED and finally AP. On the other hand, optimum levels of 25(OH)D3 seemed to be beneficial against vascular damage.*

Introduction

Systemic sclerosis (SSc) is a systemic autoimmune disease of unknown aetiology and a very heterogenic clinical course. Prognosis is determined by the degree of skin involvement likewise organic compromise. It is currently amongst the connective tissue diseases with the highest mortality rates and it has an estimated survival of 66–82% at 10 years. 55% of deaths are directly related to the disease itself, being pulmonary involvement the main cause. According to EUSTAR registries, death causes unrelated to the disease represent 41% (1).

Cardiovascular disease (CVD) is the first cause of death amongst general population in developed countries (2), likewise in the main rheumatologic diseases (3). In recent years, there has been in-depth study into cardiovascular risk (CVR) in SSc, evidencing the coexistence of macrovascular damage (4-6). The development of ischaemic cardiopathy, CVD and chronic ischaemia of lower limbs has also been described leading to a hypothesis regarding the existence of possible hastened early onset arteriosclerosis (AS) in this pathology (7). Various studies have evidenced the subclinical presence of AS in SSc by means of carotid intima-media thickness (CIMT), flow-mediated vasodilation (FMD) in the brachial artery, and the presence of coronary and cerebral calcifications assessed in computed tomography. On most occasions this AS progresses silently; therefore early CVR detection by non-invasive imaging techniques and laboratory biomarkers is essential in CVD prevention and treatment (8). This is why, in this study we propose the assessment of CVR in SSc patients measured as per angiodynamic parameters (FMD) and ultrasound vascular lesions (CIMT and AP).

Patients and methods

In this cross-sectional study we evaluated a cohort of 70 adult patients diagnosed with SSc (ACR/EULAR 2013 criteria) (9), attended our Rheumatology Department between 2012 and 2016. CVR assessment was performed according to the established protocol between Rheumatology and Vascular Surgery Units in our centre. The study was developed pursuant to the Declaration of Helsinki principles and informed consent was obtained from all patients.

Clinical and laboratory data related to SSc were gathered during the study basal appointment: diffuse (dcSSc) or limited (lcSSc), organic and skin involvement evaluated by Modified Rodnan Skin Score, (mRSS), SSc-specific antibodies and other laboratory data (complete blood cell count, kidney and liver function, ESR, CRP, homocysteine and vitamin D levels).

Treatments received were also collected: immunosuppressants, antiplatelets, lipid-lowering and vasoactive drugs.

As to determination of CVR parameters, the main classic cardiovascular risk factors (CVRF) were collected: high blood pressure (HBP), diabetes mellitus (DM), dyslipidaemia, smoking, and personal and family CVR history; including SCORE risk CV (10).

Capillaroscopy was performed in most of the patients (94%).

A preliminary protocol was designed for the vascular study, which established the CVR variables to be studied: carotid ultrasonography (US) was performed to determine the presence of carotid AP and measure CIMT (considered pathological if >0.9), and endothelial function was measured by FMD of post-ischaemic brachial artery, considered pathological <10% and severe if <5% (according to the protocol described by Correti *et al*. (11)). Both assessed with Samsung SonoAce R7® equipment. The vascular study was performed in all cases by a vascular surgeon experienced in these techniques and blind for patient clinical information. Study quality and reliability was assessed in all examinations pursuant to the recommendation of the Spanish Angiology and Vascular Surgery Society's Chapter on Non-Invasive Vascular Diagnosis (12).

Table I. Clinical characteristics of the participants.

Characteristic	n	Mean ± SD	Median (IQR)	Range
Age at onset of symptoms	70	41.8 ± 14.6	41 (33-51)	12-73
Age at diagnosis	70	47.2 ± 13.4	47 (39-58)	17-75
Age on inclusion	70	50.2 ± 12.5	50.5 (42.0-59.0)	19-76
Years since diagnosis	70	3.0 ± 4.4	1 (0-3)	0-20
Body mass index (BMI)	70	24.5 ± 5.2	23.5 (21.0-26.2)	18-50
Waist perimeter	60	82.6 ± 13.0	81.5 (73-90)	63-136
Hip parameter	60	101.4 ± 10.1	101.5 (95-105.5)	84-142
ESR	70	15.0 ± 14.4	11.0 (6.0-19.0)	1-61
CRP	70	2.6 ± 3.6	1.1 (0.9-3.3)	0-22
25(OH)D3	64	30.3 ± 17.4	24.0 (19.8-36.6)	10.7-118
Homocysteine	61	13.0 ± 17.4	10.0 (7.4-13.9)	1.5-143
Total cholesterol (TC)	67	192.5 ± 31.9	187 (166-218)	143-270
HDL cholesterol	55	72.4 ± 25.5	65 (56-83)	36-164
LDL cholesterol	55	102.4 ± 29.4	97 (82-117)	51-167
Triglycerides (TG)	60	92.2 ± 43.6	79 (59.5-108.5)	38-239
mRSS	**70**	**9.3 ± 7.0**	**8 (5-12)**	**0-42**

Patients were included by convenience sampling. Statistical descriptive analysis was made using mean and standard deviation for continuous variables and frequencies for qualitative variables. Association between different clinical variables and vascular involvement defined by the different result measures, was studied using bivariant and multivariant logistic regression models, with odds ratio (OR) calculation as the association measurement, with its confidence interval (CI 95%), taking *p*-value <0.05 as statistically significant. For the inclusion of continuous variables, linearity was checked in the logistic function via a linear trend test. Despite the lack of compliance with this assumption, all continuous variables were included in order to rise the statistical power. Statistical software was STATA (v. 14.0).

Results

Most of the patients were women 94%, with mean age 50.2±12.5 years and mean disease duration 3.0±4.4 years. Tables I and II show the clinical characteristics of patients included in the study and Table III shows the treatments received.

38.6% were dcSSc and 61.4% were lcSSc. Mean mRSS was 9.3±7.0. In the immunological study, ANAs were positive in 91.4% of the patients. The frequency of specific antibodies was variable: anti-centromere (ACA, 51.4%), anti-topoisomerase-1 (ATA1, 10%), anti-RNA polymerase III (4.2%), anti-fibrillarin (1.4%) and one patient with simultaneous presence of ACA plus AAT1. 31% of the sample had no SSc-specific antibodies.

50% received glucocorticoid (GC) at the time of inclusion, usually with doses lower than 10 mg a day (75%), and for a period longer than one year (65%). 51% were taking disease-modifying ant-irheumatic drugs at the present time, with methotrexate being the most common (34%) and for more than 1 year (76%). Other treatments: mycophenolate mofetil (MMF, 3%), azathioprine (11%), hydroxychloroquine (14%). 8.5% received cyclophosphamide and biological therapy was used as compassionate use with rituximab in three patients (RTX, 4%). There were fewer patients undergoing treatment with MMF than with RTX in our sample because MMF requires a medical inspection visa in the national health system for its dispensation, limiting its prescription in our private medical centre. Regarding treatment, 57% received vasoactive drugs: angiotensin II receptor blockers drugs (ARBs, 75%), followed by far by calcium channel blockers (CCBs, 10%) and angiotensin-converting-enzyme inhibitors (ACE inhibitors, 5%). 10% took an endothelin receptor antagonist (ERA, Bosentan), 4.2% received a phosphodiesterase type 5 inhibitor (PDE5 inhibitor, Sildenafil), and 2.8% combined therapy, ERA plus PDE5.

Classic CVRF distribution was: 4% DM, 7% obesity, 11% smokers, 28% ex-smokers, 13% HBP and 28% dyslipidaemia. Regarding CVD history, only 2 patients previously suffered coronary

Table II. Clinical characteristics of the participants.

Characteristic	n	(%)
Woman (n=70)	66	(94.3%)
Family history of CVR (n=69)	12	(17.4%)
Obesity [BMI >30.0] (n=70)	5	(7.1%)
HBP (n=70)	9	(12.9%)
DM (n=70)	3	(4.3%)
Smoking (n=70)		
Ex-smoker	20	(28.6%)
Smoker	8	(11.4%)
Prior ischaemic stroke (n=70)	2	(2.9%)
Ischaemic heart disease (n=70)	2	(2.9%)
Thrombotic phenomena (n=70)		
Pulmonary thromboembolism	4	(5.7%)
Deep vein thrombosis in lower limbs	2	(2.9%)
Others	1	(1.4%)
Hyperhomocysteinemia (n=70)	4	(5.7%)
SSc Specific antibodies (n=70)		
ACA	36	(51.4%)
AAT	7	(10.0%)
ARP III	2	(2.9%)
Fibrillarin	1	(1.4%)
Echocardiogram (n=70)		
Valvulopathy	3	(4.3%)
LV hypertrophy	1	(1.4%)
Diastolic dysfunction	1	(1.4%)
More than one finding	3	(4.3%)
Pulmonary affectation [PAH or ILD] (n=70)	14	(20%)
Ischaemic ulcers (n=70)	23	(32.9%)
Capillaroscopy pattern (n=66)		
Normal	19	(19.7%)
Non-specific	3	(4.6%)
Active	19	(28.8%)
Early	18	(27.3%)
Late	13	(19.7%)
SSc subtype (n=70)		
dcSSc	27	(38.6%)
lcSSc	43	(61.4%)

ischaemia (2.8%), 2 patients stroke (2.8%) and 4 thrombosis (5.7%).

Suboptimal levels of 25(OH)D3 were observed in over half the sample (59%): insufficiency (<30ng/mL) was detected in 32% of the sample, and deficit (<20 ng/mL) in 27% of patients. The average serum concentrations of homocysteine were 13.0 µmol/L (*DS*±17.4), with raised values in 30% of patients.

Capillaroscopy pattern distribution was: normal (19.7%), non-specific (4.6%), active (28.8%), early (27.3%) and late (19.7%).

84% of the sample presented ED (FMD <10%), and severe in 49% of patients (FMD <5%). Treatment with glucocorticoids (GC) present in 75% of patients, and 65% actively in the last year was significantly associated in the bivariant analysis with the presence of ED (OR=6.12; CI=1.44–25.92; p=0.014), likewise in the multivariant model (OR=8.78; CI=1.52–50.78; p=0.015).

CIMT presented pathological values in 39% of patients with a mean value of 0.64±0.15 mm, this pathological value was inversely associated to 25(OH) D3 concentration in the multivariant analysis.

Subclinical AS affected 41.4% of patients (23% of the sample had carotid AP in US, which were bilateral in 40%) without implying significant haemodynamic stenosis in any of them. The presence of AP in the bivariant analysis was associated with an increase in the mRSS score (OR=1.09; CI=1.00-1.19; p=0.046). Both the presence of pathological CIMT and/or AP were more common in lcSSc (55.8%) than in dcSSc (41.6%) but not statistically significant.

Disease duration, clinical features of SSc including capilaroscopy patterns or specific treatments (except GC) were not associated with CVR in our study.

Discussion

The most relevant result in this study was the high percentage of patients with ED (84% of patients), and comparing these with other studies, we found heterogeneous results regarding FMD reduction in the brachial artery (16-22) and its relation with the development of CVD (19). Au *et al*. in a systematic revision and meta-analysis (including 12 CIMT and 7 brachial artery FMD studies) reported increased AS risk in SSc patients, detecting a CIMT increase and FMD decrease compared to controls in healthy patients (23). Another important point regarding this result was the association between ED and GC treatment, even though low doses of GC are used in this disease, it is likewise proven to have implications in CVR. These results are consistent to Bartoli *et al*. (24), in whose study, a relation between FMD and GC dose was found. The effect of GC treatment in SSc has been assessed in several studies, mostly in CIMT and AP, *e.g*. Vettori *et al*. concluded that higher cumulative doses of GC were associated with higher CIMT values (29) and Frerix *et al*. confirmed a relation between the cumulative doses of GC and the presence of AP in patients with SSc (30). Fewer studies have analysed ED and GC treatment, but macrovascular damage probably occurs early due to early structural changes and GC treatment, which causes dyslipoproteinaemia may lead to ED, and finally AS and AP, as occurs in other autoimmune diseases in which prolonged GC treatment has been associated with accelerated AS.

Regarding pathological CIMT, our study is the first to relate optimum 25(OH) D3 levels meant a protective effect over this vascular affectation parameter. The mechanism whereby 25(OH)D3 protects some people from CV events has not been elucidated yet. Several mechanisms have been proposed including negative regulation of renin to reduce blood pressure thereby improving vascular distensibility, a reduction in the production of contraction factors derived from the endothelium,

Table III. Treatments received.

	n (%)
Corticoids (n=70)	
Never	26 (37.1%)
Previously	9 (12.9%)
Currently	35 (50.0%)
Corticoid dose (n=44)	
0-10 mg	33 (75.0%)
10-30 mg	10 (22.7%)
>30 mg	1 (2.3%)
Corticoid treatment duration (n=44)	
<1 mth	6 (13.6%)
1-12 mths	10 (22.7%)
>12 mths	28 (63.6%)
DMARD (n=70)	
Never	32 (45.7%)
Previously	2 (2.9%)
Currently	36 (51.4%)
DMARD type (n=38)	
Methotrexate	13 (34.2%)
Azathioprine	6 (15.8%)
Hydroxychloroquine	6 (15.8%)
Cyclophosphamide	5 (13.2%)
Combined therapy	8 (21.0%)
DMARD treatment duration (n=38)	
<1 mth	2 (5.3%)
1-12 mths	7 (18.4%)
>12 mths	29 (76.3%)
Biological therapy (n=70)	
Never	67 (95.7%)
Currently	3 (4.3%)
Biological type (n=3)	
Rituximab	3 (100%)
Biological treatments > 1 year (n=3)	3 (100%)

increase in the VEGF expression and a drop in the parathormone levels (31). Some studies have observed an association between 25(OH)D3 deficit and disease or phenotype activity. Vacca *et al*. have associated these levels with disease activity, presence of pulmonary disease (PAH and ILD), ESR and CRP levels, likewise the presence of positive auto-antibodies (32). Caramaschi *et al*. have associated it with the disease duration levels of DLCO, PAP, CRP, ESR, and capillaroscopy pattern (33).

In terms of SSc subtypes, lcSSc and dcSSc, our results coincide with other studies where no differences were found in CIMT and disease subtypes (27-28).

Increased CIMT is a morbi-mortality risk marker of cardiovascular origin, enabling AS to be measured and monitored in asymptomatic individuals, as subrogated markers of a future coronary disease, stroke and general death among the general population and inflammatory rheumatologic diseases (13-15). Bartoli *et al*., Soltesz *et al*. and the recent study of Sedky Abdou *et al*. have shown a significant increase in CIMT in SSc patients compared to controls in healthy patients, which denotes thickening and stiffness of the arterial wall in SSc (24-26).

Lastly, our results coincide with those reported by other authors, which say that the increased risk of CVD in SSc cannot be explained solely by the presence of classic CVRF. Furthermore, several studies have related a lower prevalence of CVRF in SSc compared to healthy population (34-35).

The main limitation of this study is the small sample size (n=70), which in itself can be explained by the low disease prevalence, between 3 and 24 cases/100,000 people (36).

The cross-sectional design only enables references to association but not causality, thus limiting the value of the results, particularly in variables with high variability. The absence of a control cohort prevents contrasting of the classic CVRF and different CVR biomarker values as determining factors in AS.

In patients receiving regular vasodilator treatment (ARBs, CCBs, ACE inhibitors) wash-out was neither performed prior to study not suspended since these were patients with severe Raynaud's phenomenon. Andersen *et al*. allowed the use of ACE inhibitors in their study without observing any changes between treated and untreated patients (37). Rajagopalan *et al*. maintained vasodilator drugs provided they had not started them in the 4 months prior to the study (22).

Patients with raised CVR (previous CVD or kidney disease, uncontrolled HBP, or treatment with statins) were not excluded since these patients were deemed part of routine clinical practice.

Conclusions

GC treatment has implications in CVR, despite the fact that doses of GC administered in SSc are lower than in other autoimmune diseases, in our cohort even doses lower than prednisone 10 mg a day was associated with ED (which was present on 84% of patients, and severe in 49%). GC may be associated with an early vascular damage in these patients, which could lead to changes in FMD, ED and finally AP.

Furthermore, optimum levels of 25(OH) D3 seemed to be beneficial against vascular damage. Therefore, it is essential to perform cardiovascular risk screening in our SSc patients.

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