Clinical and subclinical atherosclerosis in patients with systemic sclerosis: an observational, multicentre study of GIRRCS (Gruppo Italiano di Ricerca in Reumatologia Clinica e Sperimentale)

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Abstract

Objective

Conflicting results about clinical and subclinical atherosclerosis in systemic sclerosis (SSc) and the associated risk factors have been reported. Hence, we aimed to determine the prevalence of clinical and subclinical atherosclerosis in a large number of Italian SSc patients and the associated risk factors.

Methods

This study included 613 SSc patients from 11 Italian tertiary Rheumatologic Units. All patients underwent full history taking, clinical examination, and relevant laboratory and radiological investigations. Doppler ultrasonography (US) of the common carotid and upper and lower limbs was performed to measure carotid and femoral intima-media thickness (cIMT and fIMT), and carotid and peripheral atheroma plaques. Doppler US of the brachial artery was performed to measure flow-mediated dilatation (FMD).

Results

Patients were mostly women (91.4%) with a median age of 61 years (range, 20-100); a median disease duration of 14 years (range, 0–77) from the onset of the first non-Raynaud's phenomenon (RP); 9.3% had a history of clinical atherosclerosis (9 stable/unstable angina, 21 myocardial infarctions, 24 heart failure, 3 strokes, 8 transient ischaemic attack, 6 intermittent claudication, 10 atrial thrombo-embolism). In 37.1% of patients, subclinical atherosclerosis was detected, after excluding those with a history of clinical atherosclerosis. The prevalence of clinical and subclinical atherosclerosis was higher than that reported by the European Society of Cardiology and observational studies that enrolled Italian healthy individuals as a control group, respectively.

Conclusion

A higher prevalence of clinical and subclinical atherosclerosis was detected in SSc Italian patients and correlated with traditional and SSc-related risk factors.

Key words

systemic sclerosis, subclinical atherosclerosis, clinical atherosclerosis, flow-mediated vasodilation, traditional cardiovascular risk factors

Affiliations: page 1652.

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Introduction

Systemic sclerosis (SSc) is a rare autoimmune disease characterised by widespread microangiopathy, abnormal fibrosis of the skin and internal organs, and autoimmunity (1-3). In the last decades, a shift in mortality rates has been reported in some papers showing that the mortality rate linked to SSc-related complications is declining while a gradual increase in death due to atherosclerotic cardio- and cerebro-vascular complications is reported (4-6). Based on the data collected by the European League Against Rheumatism Scleroderma Trials and Research (EUSTAR) database, mortality due to clinical atherosclerosis in SSc was estimated to be 29%, therefore higher than in the general population (7). A cross-sectional analysis of a large United States hospitalisation database showed that atherosclerotic cardiovascular disease (CVD), as a primary discharge diagnosis was reported in 5.4% out of 308,452 SSc hospitalisations (8). Indeed, an Australian study on 850 patients (9) and an American study on 865 patients with SSc (10) demonstrated a higher incidence of clinical atherosclerosis than in healthy individuals and particularly of angina pectoris, myocardial infarction (MI), cerebral stroke, and peripheral vascular disease. A Canadian study instead quantified the cardiovascular risk of SSc patients in the first 5 years of the disease and compared it with that of the control population resulting in a 3.49 times greater risk of MI and a 2.35 times greater risk of stroke in SSc patients (11), confirming the importance of an earlier assessment and treatment of the underlying associated risk factors.

Other studies failed to confirm these reports (12, 13). Indeed, it has been shown that the prevalence of coronary artery disease documented by coronary angiography in SSc patients was similar to that expected in individuals without SSc (14). Although few studies investigated the prevalence of cerebrovascular disease in SSc patients by Doppler or angiographic evidence of carotid or vertebral stenosis, no significant difference was found between patients and healthy individuals (15). Furthermore, studies investigating different surrogate markers of subclinical atherosclerosis did not show any difference between patients and healthy individuals (16, 17) and well-controlled studies in a large cohort of SSc are not yet available.

At present, the methodologic heterogeneity of the published data hinders the comparison of these studies. In this undefined and controversial context, hence we report the increased prevalence of clinical and subclinical atherosclerosis, derived from an observation of the Gruppo Italiano di Ricerca in Reumatologia Clinica e Sperimentale (GIRRCS) SSc cohort and try to define the role played by the traditional pro-atherosclerotic risk factors in these patients, together with the possible impact of the pathogenic mechanisms of the disease itself, leading to the development of subclinical and clinical atherosclerosis.

Patients and methods

Study design, patients, and assessment of clinical and subclinical atherosclerosis

The study population included 613 SSc patients from 11 tertiary Rheumatologic Units, throughout the whole Italy with a high experience in the management of this disease. All patients fulfilled the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) 2013 classification criteria (18), and were consecutively enrolled from January 1, 2021, to February 15, 2023. The Ethics committee of the coordinator of the study approved the protocol (0029176/i) following the Good Clinical Practice Guidelines and the Declaration of Helsinki. Written informed consent was obtained from all the patients.

All patients were subjected to full history taking, clinical examination, and relevant laboratory and radiological investigations related to their SSc, clinical and subclinical atherosclerosis, and traditional cardiovascular risk factors were reported. The prevalence of clinical atherosclerosis, defined as the history of unstable/stable angina, MI, chronic heart failure, stroke, transient ischaemic attack, episodes of atrial thromboembolism, and peripheral claudication intermittent was compared

with those registered in the Italian general population by the European Society of Cardiology (19, 20).

Subclinical atherosclerosis was defined as the presence of carotid and peripheral atheroma plaques and/or cIMT and fIMT more than 0.9mm of diameter by performing Doppler US of the common carotid and upper and lower limbs. Doppler US of the brachial artery was performed to measure FMD, a validated non-invasive technique of endothelial dysfunction based on nitric oxide release (NO), as an alternative early vascular marker of subclinical atherosclerosis (21, 22). Centres strictly followed the FMD guidelines for preparation and assessment, involving appropriately trained sonographers to reduce variability. Patients sat with their forearm prone on a table for at least 10 minutes. A sphygmomanometer cuff was placed distally to the artery, and the ultrasound probe was positioned 3-5 cm above the wrist. Brachial artery diameter was measured at baseline. The cuff was inflated above systolic pressure, occluding the artery for 5 minutes. After deflation, the diameter was measured every 30 seconds for 5 consecutive times using a high-resolution ultrasound device. Normal values were defined as FMD >10%. (23, 24). The prevalence of subclinical atherosclerosis was compared with data reported in Italian healthy individuals based on the available literature**,** comparable for age, sex, and traditional cardiovascular risk factors (25-27).

Traditional cardiovascular risk factors assessed in our study included a family history of clinical atherosclerosis, smoking, serum levels of total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides, fasting blood glucose, diagnosis of high blood pressure, type II diabetes, metabolic syndrome (MS), body mass index (BMI). Hypercholesterolaemia was defined considering the value of cholesterol and the limit less than 200mg/dl, and/or treatment with medications lowering the blood cholesterol levels (28, 29). Hypertriglyceridaemia was defined considering the value of triglycerides and the limit less than 130 mg/dl, and/or treatment with medications lowering the blood triglyceride levels (30). Metabolic syndrome and type II diabetes were defined according to standard criteria and/or treatment with anti-diabetic (31, 32). Clinical, serological, and radiological disease-related features included disease duration from the onset of the first non-RP, disease subset according to LeRoy criteria (33), anti-nuclear antibodies (ANA) and SSc-specific autoantibodies, puffy hands, ischaemic digital ulcers, pitting scars, telangiectasias, subcutaneous calcinosis, tendon friction rubs, scleroderma renal crisis (SRC), pulmonary function tests (PFTs) with carbon monoxide diffusion capacity (DL_{CO}), electrocardiogram (ECG), echocardiographic features, modified Rodnan skin score (mRSS), interstitial lung disease (ILD) assessed by high resolution computed tomography (HRCT), pulmonary arterial hypertension (PAH) measured by echocardiography. Patients with estimated systolic pulmonary arterial pressure (sPAP) >40mmHg are at higher risk of presenting PH with the majority of SSc studies investigating PAH screening used sPAP as a suitable parameter for this goal (34). Moreover, when right heart catheterisation (RHC), the "gold standard" technique to diagnose a PAH, was performed, the results were also reported. The European Scleroderma Study Group (EScSG) activity index was used to assess disease activity [35,36]. Disease severity was assessed by the core set of variables proposed by Medsger *et al*. (37). The current therapy was also recorded.

Table I. Demographic and immunological features of the enrolled SSc patients.

Patients (total number)	613
Gender (female/male)	91.4%/8.6%
Age (years)	
Mean±SD	59.53%±13.93
Median (range)	61 (20-100)
Disease duration from RP (years)	
Mean±SD	15.5%±13.03
Median (range)	16 (0-68)
Disease duration from non-RP (years)	
Mean±SD	12.51%±11.67
Median (range)	14 (0-77)
Subset (limited/diffuse)	72% (171/611)/28% (440/611)
ANA (positive/negative)	83.4% (503/603)/16.6% (100/603)
SSc autoantibodies (positive/negative)	82% (482/588)/18% (106/588)
ACA (positive)	47.1% (277/588)
ATA (positive)	31% (190/612)
Anti-RNA Pol III (ARA) (positive)	2.6% (16/613)
ACA/ATA/ARA (triple negative)	18% (106/588)

ACA: anticentromere antibodies; ANA: antinuclear antibodies; ARA: anti-RNA polymerase III; ATA: anti-topoisomerase I antibodies.

Statistical analysis

Data were collected and analysed using the statistical package SPSS version 20 SPSS (Inc., Chicago, IL, USA). We modelled 2 statistical analyses, adjusted for gender and age, by performing logistic regression, to evaluate, the possible association of each identified covariate on clinical atherosclerosis occurrence, and in the second analysis to evaluate the possible association of the same set of covariates on subclinical atherosclerosis occurrence. Covariates were selected from 2 main areas: traditional cardiovascular risk factors and SSc-related features. A significant threshold was set at $p<0.05$.

Results

Baseline characteristics of the evaluated SSc patients

The GIRRCS cohort study population comprised 613 SSc patients. The demographic and disease features of the patients are shown in Table I and II. SSc patients were mostly women (91.4%) with a median of 61 years (range: 20–100). The disease duration ranged from 0 to 77 years with a median of 10 years; 442 (72%) out of patients were affected by the limited cutaneous SSc (lcSSc) and 171 (28%) by the diffuse cutaneous SSc (dcSSc); 86 (19.5%) out of patients had active disease; 503 (83.4%)

Table II. Clinical features of the enrolled SSc patients.

Puffy fingers	47.9% (292/610)
Ischaemic digital ulcers	38.7% (236/610)
Pitting scars	30.3% (179/590)
Telangiectasias	49.3% (288/584)
Calcinosis	12.9% (767/588)
Tendon friction rubs	11.2% (68/609)
EScSG (active)	19.5% (86/440)
NVC	
Aspecific abnormalities	13.5% (73/539)
Early pattern	24.3% (131/539)
Active pattern	38.2% (2067539)
Late pattern	23.9% (129/539)
SRC	1.5% (9/611)
FVC%	
≥80%	84.7% (430/509)
70-79%	8.1% (41/509)
50-69	7.1% (36/509)
<50%	0.2% (1/509)
O2 therapy	0%
FEV/FVC<80%	25.6% (113)
DLCO%	
≥80%	44.6% (209)
70-79%	17.3% (81)
50-69%	27.3% (128)
<50%	10.9% (51)
O_2 therapy	0%
TLC%	
≥80%	76.1% (287/377)
70-79%	11.9% (45/377)
50-69%	9.0% (34/377)
<50%	2.9% (11/377)
O2 therapy	0%
ECG	
Normal	70.8% (354/500)
Conduction abnormalities	15.2% (76/500)
Arrhythmias	7.0% (35/500)
RV hypertrophy	1.2% (6/500)
Other	5.8% (29/500)
EF<55%	2.5% (14/562)
Diastolic dysfunction	29.0% (164/565)
PAPs>40MMHG	7.3% (39/535)
Pericardial effusion	13.9% (81/581)
mRSS>14	17.5% (87/496)
ILD	46.9% (272/580)
PAH (RHC)	14.7% (75/511)
VES>30mmHg	25.8% (134/519)
CRP>0.5mg/dl	32.8% (172/525)
CYC	0% (0)
MMF	15.4% (91/590)
AZA	6.3% (37/590)
MTX	9.2% (54/590)
HCQ	14.2% (84/590)
GC	30.3% (179/590)
COLCHICINE	1.5% (9/590)
IVIG	0%
RTX	2.5% (15/590)
TCZ	1.55 (9/590)
Antifibrotics	0.5% (3/590)
CCB	58.5% (345/590)
Other peripheral vasodilators	7.8% (46/590)
Sildenafil	3.4% (20/590)
Tadalafil	0.7% (4/590)
Riociguat	0.5% (3/590)
Bosentan	23.6% (139/590)
Macitentan	2.0% (12/590)
Ambrisentan	0.3% (2/590)
Iloprost	38% (2247590)
Alprostadil	0.2% (1/590)
Antiaggergant	54.9% (324/590)
Anticoagulant	3.2% (197590)
ACEI	13.1% (77/590)
ARBS	7.8% (46/590)
B-blockers	8.6% (51/590)
Diuretics	11.4% (67/590)
Statins	17.5% (103/590)

CRP: C reactive protein; DLCO: diffusing capacity for carbon monoxide; ECG: electrocardiogram; EF: ejection fraction; ESR: erythrocyte sedimentation rate; EScSG: European Scleroderma Study Group activity index; FEV1/FVC: forced expiratory volume in the first second to forced vital capacity; FVC: forced vital capacity; ILD: interstitial lung disease; mRSS: modified skin score; NVC: nailfold videocapillaroscopy; PAH: pulmonary arterial hypertension; PAPs: estimated pulmonary arterial pressure by echocardiography; RP: Raynaud's phenomenon; SRC: scleroderma renal crisis; TLC: total lung capacity.

Table III. Clinical atherosclerotic events of the enrolled patients.

Clinical atherosclerosis, % (number/total number)	9.3% (55/593)
Stable/unstable angina	1.5% (9/582)
Acute myocardial infarction	3.6% (21/582)
Chronic heart failure	4.0% (24/594)
Cerebral stroke	0.5% (3/580)
Transient ischaemic attack	1.4% (8/580)
Peripheral claudication intermittent	1.0% (6/580)
Atrial thrombosis/embolus	1.7% (10/585)

Table IV. Specific prevalence rate ratios of clinical and subclinical atherosclerosis in the SSc GIRRCS cohort.

Age (years)	SSc pts, total number (valid)	Clinical atherosclerosis, number	Specific prevalence rate (*100.000 people)
20-30	20	0	0
31-40	33	0	0
41-50	87	4	4.597
51-60	148	8	5405
61-70	155	11	7.096
71-80	122	21	17.213
81-90	26	10	38.461
91-100	2	1	50.000
20-100	593	55	9.274
Age (years)	**SSc pts, total number (valid)**	**Subclinical atherosclerosis, number**	**Specific prevalence rate (*100.000 people)**
20-30	10	0	0
31-40	19	4	21.052
41-50	53	20	37.735
51-60	77	29	37.662
61-70	81	43	53.086
71-80	65	18	27.692
81-90	14	5	37.514
91-100	2	1	50.000
20-100	323	120	37.151

Pts: patients.

of patients were positive for ANA antibodies; 482 (82%) of patients were positive for SSc-related autoantibodies: 277 (47.1%) were positive for anticentromere (ACA), 190 (31%) for antitopoisomerase I (ATA), and 16 (2.6%) for anti-RNA polymerase III (Pol III) antibodies. 292 (47.9%) of patients had puffy fingers, 236 (38.7%) had ischaemic digital ulcers, 179 (30.3%) had pitting scars, 288 (49.3%) had telangiectasias, 68 (12.9%) had tendon friction rubs and 76 (11.2%) had subcutaneous calcinosis. 73 (13.5%) of patients had aspecific capillaroscopic abnormalities, 131 (24.3%) had an early scleroderma pattern, 206 (38.2%) had an active, and 129 (23.9%) had a late pattern (37); 9 (1.5%) had SRC, 272 (46.9%) had ILD on HRCT and 75 (14.7%) had PAH on RHC; 146 (29,2%) had ECG abnormalities: 76 (15,2%) aspecific conduction abnormalities, 35 (7%) arrhythmias, 6 (1,2%) ECG sign of right ventricular hypertrophy and 29 (5,8%) other ECG abnormalities. The most prescribed immunosuppressive drug was mycophenolate (15.4%) while rituximab was the most prescribed bDMARD (2.5%); antifibrotic therapy (nintedanib) was prescribed in 3 patients (0.5%); 345 (58.5%) of patients were under treatment with calcium channels blockers and 46 (7.8%) with other peripheral vasodilators; Phosphodiesterase 5 inhibitors were prescribed in 24 (4,6%) of patients; anti-endothelin-1 inhibitors in 153 (25,9%) of patients; prostacyclin analog iloprost in 224 (38%) of patients; antiplatelet therapy in 324 (54.9%) and proton pump inhibitors in 280 (47.5%) of patients.

Prevalence of clinical atherosclerosis and related risk factors

Fifty-five patients (9.3%) had a history

Table V. Clinical atherosclerosis univariate and multivariate analyses.

Univariate analyses	OR	SE	*p*	CI 95%
Gender	0.641	0.433	0.305	0.274-1.498
Age	1.083	0.014	**0.000**	1.053-1.112
RP onset	1.010	0.020	0.631	0.971-1.050
Non-RP onset	1.025	0.020	0.218	0.9861.065
Subset (diffuse)	1.279	0.030	0.417	0.706-2.316
ANA (positive)	3.808	0.605	**0.027**	1.164-12.456
SSc Abs (positive)	0.916	0.370	0.814	0.443-1.894
ACA (positive)	0.090	0.294	0.562	0.667-2.108
ATA (positive)	0.750	0.323	0.373	0.398-1.413
RNA Pol III (positive)	0.646	1.043	0.675	0.084-4.983
ACA/ATA/Pol III (triple negative)	1.091	0.370	0.814	0.528-2.256
Puffy fingers	1.466	0.285	0.180	0.838-2.564
Ischaemic digital ulcers	2.848	0.292	**0.000**	1.068-5.044
Pitting scars	1.492	0.295	0.175	0.837-2.659
Telangiectasias	1.196	0.287	0.532	0.682-2.099
Calcinosis	1.184	0.405	0.677	0.535-2.617
Tendon friction rubs	1.031	0.455	0.946	0.423-2.514
SRC	2.863	0.815	0.197	0.580-14.031
FVC%	1.792	0.214	**0.006**	1.179-2.724
FVC/FEV1%	2.286	0.358	0.021	1.131-4.610
DLCO%	1.758	0.161	**0.000**	1.282-2.410
TLC%	1.277	0.218	0.263	0.832-1.958
EF%<55	6.712	0.591	**0.001**	2.109-21.357
Diastolic dysfunction	3.964	0.304	**0.000**	2.185-7.192
PAPs>40mmHg	3.476	0.416	**0.003**	1.539-7.851
Pericardial effusion (PE)	3.469	0.332	**0.000**	1.810-6.649
mRSS>14	1.424	0.381	0.354	0.675-3.006
ILD	1.785	0.296	0.050	0.999-3.190
PAH (RHC)	3.077	0.351	**0.001**	1.547-6.121
Family history of CVEs	1.354	0.336	0.368	0.700-2.618
Smoking habits (current or past)	1.382	0.318	0.309	0.741-2.580
Cholesterol>200mg/dl	0.910	0.366	0.796	0.444-1.865
Triglycerides>130mg/dl	1.127	0.400	0.765	0.515-2.467
Hyperglycaemia	4.311	0.522	**0.005**	1.548-12.002
ESR>30mmHg	2.412	2.330	**0.008**	1.263-4.608
CRP>0.5mg/dl	1.652	0.327	0.125	0.870-3.137
High blood pressure	5.412	0.303	**0.000**	2.987-9.805
Type II diabetes	3.181	0.391	**0.003**	1.479-6.843
Metabolic syndrome	1.623	0.465	0.298	0.652-4.040
EScSG (active)	3.418	0.375	**0.001**	1.638-7.134
General	1.708	0.316	0.090	0.919-3.172
Peripheral vascular	1.450	0.177	**0.036**	1.025-2.050
Skin	1.540	0.191	**0.023**	1.060-2.238
Joint/tendon	0.930	0.327	0.825	0.490-1.766
Muscles	1.805	0.330	0.073	0.946-3.443
GI	0.965	0.291	0.902	0.546-1.706
Lung	1.426	0.160	**0.027**	1.042-1.952
Heart	1.163	0.278	0.588	0.674-2.006
Kidney	1.795	0.412	0.156	0.800-4.029
Multivariate analyses	**OR**	**SE**	***p***	**CI 95%**
Gender	0.774	0.618	0.678	0.230-2.600
Age	1.055	0.017	**0.002**	1.020-1.091
Ischaemic digital ulcers	4.990	0.430	**0.000**	2.149-11.583
EScSG	2.600	0.411	**0.020**	1.161-5.821
High blood pressure	2.819	0.418	**0.013**	1.241-6.399
Type II diabetes mellitus	1.173	0.586	0.328	0.562-5.591

ACA: anticentromere antibodies; ANA: antinuclear antibodies; CRP: C reactive protein; DLCO: Diffusing capacity for carbon monoxide; ECG: electrocardiogram; EF: ejection fraction; EScSG: European Scleroderma Study Group activity index; ESR: erythrocyte sedimentation rate; FVC: forced vital capacity; ILD: interstitial lung disease; mRSS: modified skin score; PAH: pulmonary arterial hypertension; RP: Raynaud's phenomenon; SSc Abs: systemic sclerosis specific autoantibodies; TLC: total lung capacity; PAPs: pulmonary arterial pressure.

of clinical atherosclerosis (9 patients with stable/unstable angina, 21 patients with MI, 24 with heart failure, 3 with stroke, 8 patients with TIA, 6 claudication intermittent, 10 patients with atrial thrombo-embolism). Some of the patients experienced more than one clinical atherosclerotic event (12 patients experienced 2 events and 3 patients experienced 3 events). In our study, the prevalence of clinical atherosclerotic events in SSc patients is slightly higher than the prevalence reported in the European Society of Cardiology (19, 20) used as a control population (Table III and IV).

Univariate analysis showed an association of clinical atherosclerotic events with both traditional cardiovascular risk factors and SSc-specific features. As far as traditional cardiovascular risk factors are included, a significant association was observed with hyperglycaemia (OR: 4.3110; 95% CI: 1.548-12.002; $p<0.005$), high blood pressure (OR: 5.412; 95% CI: 2.987-9.805; $p<0.000$); type II diabetes (OR: 3.181; 95% CI: 1.479-6.843; $p<0.003$); when SSc-specific features were considered we observed that: ANA positivity (OR: 3.808; 95% CI: 1.164-12.456; $p<0.027$); ischaemic digital ulcers (OR: 2.848; 95% CI: 1.068-5.044; $p<0.000$); FVC<80% of % predicted (OR: 1.792; 95% CI: 1.179-2.724; $p<0.006$); DLCO<80% of % predicted (OR: 1.758; 95% CI: 1.282-2.410; $p<0.000$); ejection fraction (EF)<55% (OR: 6.712; 95% CI: 2.109-21.357; $p<0.001$); left ventricular diastolic dysfunction of (OR: 3.964; 95% CI: 2.185-7.192; $p<0.000$); PAPs>40mmHg (OR: 3.476; 95% CI: 1.539-7.851; $p<0.003$); pericardial effusion (OR: 3.469; 95% CI: 1.810-6.649; $p<0.000$); PAH on RHC (OR: 3.077; 95% CI: 1.547-6.121; $p<0.001$); EScSG≥3 (active disease) (OR: 3.418; 95% CI: 1.638-7.134; $p<0.001$); erythrocyte sedimentation rate (ESR) >30mmHg (OR: 2.412; 95% CI: 1.263-4.608; $p<0.008$), peripheral vascular severity score (OR: 1.450; 95% CI: 1.025-2.050; $p<0.036$); skin severity score (OR: 1.540; 95% CI: 1.060-2.238; $p<0.023$); lung severity score (OR: 1.426; 95% CI: 1.042-1.952; $p<0.027$), were significantly associated with the cardiovascular

events. After multivariate analysis, high blood pressure (OR: 2.819; 95% CI: 1.241-6.399; $p<0.013$), age (OR: 1.055; 95% CI: 1.020-1.091; $p<0.002$), ischaemic digital ulcers (OR: 4.990; 95% CI: 2.149-11.583; $p<0.000$), high activity of the disease assessed by EScSG (OR: 2.600; 95% CI: 1.161-5.821; $p<0.020$) were confirmed to be independent variables significantly associated with clinical atherosclerosis. There was a significant main effect of capillaroscopic specific abnormalities on clinical atherosclerosis ($p<0.005$) with a significant difference between the early and late pattern and active and late pattern with clinical atherosclerosis ($p<0.003$ and $p<0.045$, respectively). Fibrosis-related features including mRSS, tendon friction rubs, and the presence of ILD were not statistically associated with clinical atherosclerosis. Disease duration and subset, SSc-specific autoantibodies, puffy fingers, pitting scars, telangiectasias, calcinosis, ECG abnormalities, family history of CVD, smoking, high cholesterol, triglycerides, MS, and BMI were not statistically associated with the history of clinical atherosclerosis (Table V).

Prevalence of subclinical atherosclerosis and related risk factors

119 patients (37.1%) without a history of atherosclerotic events showed an increased prevalence of subclinical atherosclerosis when compared with the prevalence reported in other observational studies that enrolled Italian healthy individuals as a control group (25-27) (Table IV).

Univariate analysis confirmed a significant association of subclinical atherosclerosis with both traditional cardiovascular risk factors and SSc-specific features. Traditional cardiovascular risk factors included a family history of CVD (OR: 2.796; 95% CI: 1.609-4.856; $p<0.000$); smoking (OR: 2.797; 95% CI: 1.725-4.535; $p<0.000$); MS (OR: 8.443; 95% CI: 3.091-23.064; $p<0.000$) while SSc-specific features included disease duration from first non-RP (OR: 1.019: 95% CI: 1.002-1.038; $p<0.033$); the presence of ischaemic digital ulcers (OR: 1.676; 95% CI: 1.018-2.761; $p<0.042$); DLCO<80% of the predicted value (OR: 1.513; 95% CI: 1.176-1.945; $p<0.001$); C-reactive protein (CRP)>0.05 mg/dl (OR: 2.267; 95% CI: 1.352-3.800; $p<0.002$); EScSG (active disease) (OR: 1.892; 95% CI: 1.041-3.441; $p<0.037$); heart domain of the Medsger severity score (OR: 1.781; 95% CI: 1.172-2.708; $p<0.007$). There was a significant main effect of ECG abnormalities (arrhythmias) and BMI (overweight) on subclinical atherosclerosis ($p<0.015$ and $p<0.001$, respectively). After multivariate analysis, smoking (OR: 2.491; 95% CI: 1.407-4.412; $p<0.002$); MS (OR: 6.805; 95% CI: 2.208-20.974; $p<0.001$), family history of CVD (OR: 1.021; 95 % CI: 1.498-5.149; $p<0.001$), ischaemic digital ulcers (OR: 2.107; 95% CI: 1.141-3.890; $p<0.017$) and disease duration from the non-RP (OR: 1.029; 95 % CI: 1.007-1.051; $p<0.009$) were confirmed to be independent variables significantly associated with subclinical atherosclerosis (Table VI). Fibrosis-related features included mRSS, tendon friction rubs, and the presence of ILD were not statistically associated with subclinical atherosclerosis. Age, disease subset, SSc-specific autoantibodies, puffy fingers, pitting scars, telangiectasias, calcinosis, SRC, FVC, TLC, EF%, diastolic dysfunction, PAPs>40mmHg, pericardial effusion, PAH, capillaroscopic abnormalities, cholesterol, triglycerides, ESR were not statistically significant associated with subclinical atherosclerosis (Table VI). Lastly, univariate analysis confirmed an association of subclinical atherosclerosis with different plaque locations and/or cIMT and/or FMD. In particular, univariate analysis showed an association of subclinical atherosclerosis with cIMT (OR:26.437; 95% CI: 7.542-96.672; $p<0.000$), carotid plaque (OR: 23.818; 95% CI: 12.443-45.591; $p<0.000$), femoral plaque (OR: 11.356; 95% CI: 3.719-34.677; $p<0.000$), and FMD (OR: 6.733; 95% CI: 2.894-15.667; $p<0.000$). fIMT was not associated with subclinical atherosclerosis (OR: 3.143; 95% CI: 0.788-12.529; P<0.105). After multivariate analysis, cIMT (OR: 7,710; 95% CI: 1.474-40.323; P<0.016), was confirmed to be an independent variable significantly associated with subclinical atherosclerosis.

Discussion

Our observational, multicentric study, enrolling several hundred of SSc patients, included in GIRRCS Italian cohort, showed a slightly significant increase in the prevalence of clinical (9.3%) and subclinical atherosclerosis (37.1%) in these patients when compared with available controls (19, 20, 25-27). In addition, we showed that both traditional cardiovascular risk factors and SSc-specific features were independently associated with these features, suggesting a synergistic role in the development of cardiovascular complications. Of note, the presence of ischaemic digital ulcers was independently associated with both clinical and subclinical atherosclerosis, confirming the importance of endothelial and vascular damage in the pathogenesis of atherosclerosis. Concerning traditional cardiovascular risk factors systemic hypertension was independently associated with clinical atherosclerosis while a family history of CVD, smoking, and MS were independently associated with subclinical atherosclerosis.

Our study aligns with large retrospective cohort studies and nationwide registries worldwide, confirming an increased prevalence of clinical atherosclerosis in SSc patients (9-11, 39, 40). In SSc, structural macrovascular damage progresses with the worsening of microvascular damage (41). Ulnar artery occlusion has been reported to be strongly predictive of future digital ulcers (42, 43), as well as nailfold capillary abnormalities (pattern late) (44-49). We found that ischaemic digital ulcers were independently associated with both clinical and subclinical atherosclerosis. Furthermore, we found that active and late capillaroscopic patterns were significantly associated with both clinical and subclinical atherosclerosis. Vascular repair is mediated through angiogenesis and vasculogenesis. In SSc, low endothelial progenitor cells count predict digital ulcers, cardiac, and vascular issues in SSc (50-53).

In SSc, inflammation is expected in early stages or active disease. One of the domains of EScSG index is ESR, an inflammatory marker, that was found, in the general population, to be an inde-

Table VI. Subclinical atherosclerosis univariate and multivariate analyses.

Univariate analyses	OR	SE	*p*	CI 95%
Gender	0.767	0.401	0.508	0.350-1.682
Age	1.014	0.009	0.099	0.997-1.031
RP onset	1.015	0.009	0.079	0.998-1.033
Non-RP onset	1.019	0.009	**0.033**	1.002-1.038
Subset (diffuse)	1.180	0.265	0.532	0.702-1.983
ANA (positive)	2.675	0.297	**0.001**	1.494-4.790
SSc Abs (positive)	1.009	0.322	0.978	0.536-1.897
ACA (positive)	0.792	0.234	0.321	0.501-1.255
ATA (positive)	1.179	0.239	0.492	0.737-1.885
RNA Pol III (ARA) (positive)	5.198	1.161	0.156	0.535-50.552
ACA/ATA/ARA (triple negative)	0.991	0.322	0.978	0.527-1.864
Puffy fingers	0.881	0.239	0.596	0.551-1.408
Ischaemic digital ulcers	1.676	0.255	**0.042**	1.018-2.761
Pitting scars	1.067	0.246	0.791	0.659-1.727
Telangiectasias	1.472	0.233	0.097	0.933-2.323
Calcinosis	0.973	0.314	0.931	0.525-1.802
Tendon friction rubs	1.339	0.354	0.410	0.669-2.680
SRC	0.277	1.086	0.237	0.033-2.328
FVC%	1.103	0.219	0.656	0.717-1.695
FVC/FEV1%	1.361	0.340	0.365	0.698-2.653
DLCO%	1.513	0.128	**0.001**	1.176-1.945
TLC%	1.382	0.208	0.120	0.920-2.076
EF%<55	1.030	0.740	0.968	0.241-4.397
Diastolic dysfunction	1.444	0.258	0.154	0.871-2.395
PAPS>40mmHg	1.266	0.440	0.592	0.534-2.999
Pericardial effusion	0.892	0.342	0.739	0.456-1.745
mRSS>14	1.027	0.365	0.941	0502-2.102
ILD	1.568	0.234	0.055	0.990-2.481
PAH (RHC)	1.017	0.402	0.967	0.462-2.237
Family history of CVD	2.796	0.282	**0.000**	1.609-4.856
Smoking habits (current or past)	2.797	0.247	**0.000**	1.725-4.535
Total cholesterol>200mg/dl	0.906	0.282	0.906	0.521-1.576
Triglycerides>130mg/dl	1.280	0.318	0.437	0.687-2.385
Glycaemia	1.493	0.501	0.423	0.560-3.987
ESR>30mmHg	1.402	0.289	0.242	0.796-2.470
CRP>0.5mg/dl	2.267	0.264	**0.002**	1.352-3.800
High blood pressure	2.142	0.256	0.003	1.296-3.540
Type II diabetes	1.662	0.391	0.194	0.772-3.578
Metabolic syndrome	8.443	0.513	**0.000**	3.091-23.064
EScSG (active)	1.892	0.305	**0.037**	1.041-3.441
General	1.165	0.243	0.530	0.723-1.887
Peripheral vascular	0.957	0.124	0.725	0.750-1.221
Skin	0.922	0.144	0.574	0.695-1.223
Joint/tendon	0.922	0.205	0.691	0.617-1.378
Muscles	0.697	0.319	0.257	0.373-1.301
GI	1.204	0.322	0.322	0.834-1.738
Lung	1.161	0.119	0.212	0.919-1.466
Heart	1.781	0.214	**0.007**	1.172-2.708
Kidney	0.706	0.465	0.706	0.260-1.919
Multivariate analyses	**OR**	**SE**	***p***	**CI 95%**
Gender	1.253	0.503	0.654	0.467-3.358
Age	1.011	0.011	0.303	0.9901.032
Ischaemic digital ulcers	2.107	0.313	**0.017**	1.141-3.890
Disease duration from non-RP	1.029	0.011	**0.009**	1.007-1.051
Family history of CVD	1.021	0.315	**0.001**	1.498-5.149
Smoking habits	2.491	0.292	**0.002**	1.407-4.412
Metabolic syndrome	6.805	0.574	**0.001**	2.208-20.974

ACA: anticentromere antibodies; ANA: antinuclear antibodies; ARA: anti-polymrase III antibodies; CRP: C reactive protein; CVD: cardiovascular diseases; DLCO: Diffusing capacity for carbon monoxide; ECG: elettrocardiogram; EF: ejection fraction; EScSG: European Scleroderma Study Group activity index; ESR: erythrocyte sedimentation rate; FVC: Forced vital capacity; GI: gastrointestinal tract; ILD: interstitial lung disease; mRSS: modified skin score; PAH: pulmonary arterial hypertension; PAPs: pulmonary arterial pressure; RP: Raynaud's phenomenon; TLC: total lung capacity

pendent risk factor of coronary atherosclerosis and also a predictor of heart disease mortality (54-55). In our study, an ESR >30 mm/hr was significantly associated with clinical atherosclerosis. CRP, another inflammatory marker, predicts cardiovascular risk and is linked to increased coronary calcification (56). High CRP levels, present in 25% of SSc patients, especially early in the disease, correlate with disease activity, severity, poor pulmonary function, shorter survival, and cutaneous and musculoskeletal manifestations (57, 58) In our study, CRP was significantly associated only with subclinical atherosclerosis (59).

As expected, our analysis showed that age is an independent risk factor for clinical atherosclerosis. Nevertheless, in our SSc patients, we found increased evidence of clinical atherosclerosis, suggesting that specific factors related to the disease may act in a synergistic role to induce increased atherosclerosis. This is also observed in many other autoimmune/autoinflammatory conditions (60-61). Regarding the impact of disease duration on clinical atherosclerosis, we did not find any statistical association while disease duration was associated with subclinical atherosclerosis in our patients, further confirming our hypothesis on the early role of the inflammation or low-grade inflammation on endothelial damage.

Notably, we found that SSc-specific features related to tissue fibrosis such as mRSS, tendon friction rubs, and the presence of ILD were not associated with the development of clinical atherosclerosis.

Although atherosclerosis shares some pathways with fibrotic disease, including histopathological features (62, 63), distinct processes such as LDL and cholesterol accumulation in the arterial walls, and foam cell formation differentiate them. On the contrary, in fibrotic disease, activated macrophages focus on tissue repair and fibrosis (2, 64).

Atherosclerosis may be induced by autoimmune processes including humoral factors and cells (65). Conflicting results are reported in the available literature about the role of specific SSc antibodies and subclinical atherosclerosis. The presence of ACA is associated

with decreased levels of HDL (66) and ischaemic arterial events and plaques are more common in these patients (67, 68) while ischaemic events were rare in the ATA-positive group. On the contrary, a previous study showed that the severity of large vessel macrovascular disease was independent of the clinical subset and that ACA positivity was weakly associated with atherosclerosis (12). In our cohort, the majority of patients were positive for ACA but we did not find any statistical association between autoantibody status and clinical and subclinical atherosclerosis. A study conducted on 46 SSc women showed that carotid plaques were significantly associated with the serum levels of IL-2, IL-6, CRP, ICAM-1, and others (69-72).

As far as the prevalence of traditional cardiovascular risk factors is concerned, data are contradictory. We found that systemic hypertension is only associated with clinical atherosclerosis while smoking and MS with subclinical atherosclerosis (Table VII). In other studies, the prevalence of traditional cardiovascular risk factors was found to be either higher (73), similar [10], or reduced in SSc patients (9, 10, 68, 74-76) when compared with the general population, thereby making difficult any comparability.

Concerning subclinical atherosclerosis, we showed an increased cIMT and fIMT, a higher presence of carotid and peripheral atheroma plaques, and an increased reduction of FMD. The higher incidence of subclinical atherosclerosis in SSc patients worldwide was highlighted by two meta-analyses (77-78), although its potential clinical implications remain unclear, and well-controlled studies in large cohorts are not yet available.

In our cohort, ischaemic digital ulcers and active disease as well as traditional cardiovascular risk factors, including smoking and MS, were associated with subclinical atherosclerosis. A previous study showed that older age, elevated ESR, and PAH were independently associated with subclinical atherosclerosis (79). Furthermore, an Italian study on 39 SSc patients showed that patients with left ventricular diastolic dysfunction and/or with the limited cutaneous form seem to represent a subset at higher risk of developing atherosclerosis (27).

Table VII. Traditional cardiovascular risk factors evaluation.

Family history of CVD % (number/total number)	28.8% (144/500)
Smoking habitus	35.0% (207/591)
Total cholesterol, mg/dl	
Total cholesterol>200mg/dl	39.6% (156/394)
Triglycerides, mg/dl	
Triglycerides >130mg/dl	29.6% (112/379)
Blood glucose, mg/dl	
Blood glucose>110mg/dl	8.1% (26/320)
High blood pressure	31.7% (190/600)
Type II diabetes	7.65% (45/596)
Metabolic syndrome	8.4% (48/570)
BMI	
Normal	74.4% (435/585)
Overweight	18.1% (106/585)
Obese	7.5% (44/585)

BMI: body mass index; CVD: cardiovascular disease.

Smoking causes atherosclerosis via oxidative stress and inflammation, leading to endothelial damage (80). Smoking history was significantly associated with worsening of RP and ischaemic digital ulcers (81, 82). Our study too, confirmed the relationship between smoke and subclinical atherosclerosis.

Few studies addressed the role of MS in SSc. It has been shown that disease severity was higher in SSc patients with MS (83) and associated with clinical atherosclerosis despite its low prevalence (84). Some non-traditional cardiovascular risk factors contributing to the development of MS in SSc include immune activation, systemic inflammation, vascular dysfunction, oxidative stress, fibrosis, and steroid therapy (85).

In cross-sectional observational data, evaluation of the outcome to exposure association is often complicated by non-random medication use, thus observational studies assessing treatments are at risk of incorrectly concluding and drug treatment represents a confounding factor. Based on this, we did not analyse the effects of various treatments on clinical and subclinical atherosclerosis, thus avoiding "confounding by indication for treatment" bias (72, 86, 87).

In conclusion, to the best of our knowledge, this is the largest first observational, multicentre, study showing an increased prevalence of clinical and subclinical atherosclerosis in SSc patients, from different geographic areas throughout Italy. Although the precise aetiology of atherosclerosis in SSc is still unknown, we may hypothesise that both traditional cardiovascular risk factors and SSc-related features could be responsible for this. This implies that global assessment and management of both the cardiovascular risk profile and the disease itself is necessary to slow down the onset and progression of atherosclerosis in these patients. We are aware that prospective, larger studies are needed to confirm these associations in scleroderma.

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