Advanced microvascular damage associated with occurrence of sarcopenia in systemic sclerosis patients: results from a retrospective cohort study

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ABSTRACT

Objective. *Systemic sclerosis (SSc) is characterised by microvascular inflammatory damage, loss of capillaries and progressive systemic fibrosis. Capillary rarefaction may precede sarcopenia, we therefore evaluated the body composition and occurrence of sarcopenia in SSc patients, in relation to the peripheral microcirculatory status, assessed and scored by nailfold videocapillaroscopy (NVC) patterns, including capillary number count and microangiopathy evolution score (MES).*

Methods. *Body composition and bone mineral density were assessed by Dual x-ray absorptiometry and a dedicated software (GE Lunar, USA) in 43 SSc patients (age 64.1 ± 11.2 yrs, 83.7% women) affected by limited or diffuse cutaneous (74.4%) according to the 2013 EULAR/ACR criteria and 43 age-matched healthy subjects (HS). Sarcopenia was checked as relative skeletal muscle index (RSMI). Clinical, laboratory, body composition and bone parameters were analysed according to the different NVC patterns and MES. Means were compared by the Student's t-test or by one way analysis of variance; medians were compared by the Kruskall Wallis test; and frequencies by the chi square test.*

Results. *Sarcopenia was found in 23.26% of SSc patients with a prevalence significantly higher than age matched HS (4.65%; p=0.03). Interestingly, SSc patients with "late" NVC pattern showed a significantly higher prevalence of sarcopenia (43.75%) compared to "early" (9.1%) and "active" (12.5%) NVC patterns (p<0.0002). In addition, capillary density was found significantly lower in sarcopenic versus non-sarcopenic patients (4.4±1.8 vs. 5.8±2.2, p<0.05). Finally, MES showed significantly most severe score in sarcopenic SSc patients (p<0.001): peripheral blood flow analised in a sample of sarcopenic SSc patients by Laser speckle contrast analysis (LASCA) showed lowest values (p<0.05). Total mass (TM), lean mass (LM), fat mass (FM) and bone mineral content (BMC) values were found significantly lower in sarcopenic SSc patients (p<0.0001, p<0.001, p=0.004, p=0.04, respectively).*

Conclusion. *SSc patients with sarcopenia and altered body composition were found affected by the most severe NVC pattern ("late"), a significantly reduced/altered number of capillaries and microvascular array (MES), suggesting a strong link between severity of local microvascular failure and associated muscle sufferance.*

Introduction

Systemic sclerosis (SSc) is a complex autoimmune connective tissue disease, characterised by self-amplifying microvascular damage, that is followed by autoimmune response and inflammation and finally characterised by increasing fibrosis (1). The progressive pathophysiology in SSc involve a plethora of immune cells, particularly M2 macrophages polarised to profibrotic phenotype and T-helper-2 cytokine that contributes to myofibroblast activation and deposition of extracellular matrix components able to induce fibrosis (2). Progressive loss of capillaries, fibrosis and ischaemia involve skin and internal organs and are associated with the clinical complications (1-3).

Assessing microvascular damage with nailfold videocapillaroscopy (NVC) it is able to identify the early differential diagnosis of SSc and to predict the clinical complications and severity of the disease (4-7).

Among a large spectrum of different organ involvement in SSc, recently, new

data have been reported about body composition abnormalities, in particular sarcopenia and micro/macroarchitectural changes in bone status (8-13).

Sarcopenia is defined as degenerative loss of skeletal muscle mass, quality, and strength generally associated with aging, but it has been associated in SSc with a more aggressive disease characterised by the diffuse subset, longer disease activity, higher modified Rodnan skin score (mRSS), higher C-reative protein (CRP) levels and erythrocyte sedimentation rate (ESR) levels, as well as positivity of Scl70 antibodies (8-11, 14).

Among the causes that can explain sarcopenia in SSc are included malnutrition, chronic inflammation, older age, comorbidities, endocrine factors and physical inactivity due to skin fibrosis and the progression of the disease (15).

Microvascular structure and function are key aspects of tissue and organ health and it has been suggested that capillary rarefaction may precede sarcopenia (16, 17).

In addition, considering that muscle and bone are not independent of each other, but exert their functions together as a single unit, due the anatomic position and endocrine common regulation, it is supposed that the same risk factors responsible for body composition abnormalities could influence both muscle and bone (12, 13).

The aim of this study was to evaluate, body composition in a cohort of SSc patients, focusing on sarcopenia and microcirculation status in relation to different patterns of nailfold microvascular damage evaluated and scored by NVC and the microangiopathy evolution score (MES) (18).

Methods

Study population

In this retrospective study a cohort of 43 consecutive SSc patients and 43 age- and sex-matched healthy subjects (HS) were recruited during routine clinical assessment at the Scleroderma Clinic of Rheumatology Division, University of Genova (Italy).

The diagnosis of SSc was based on the 2013 American College of Rheumatology (ACR)/European League against Rheumatology (EULAR) classification criteria (19).

All patients and the HS were aged over 18 years. The main exclusion criteria included a history of malignancies, the overlap with other autoimmune diseases (such as rheumatoid arthritis, Sjögren's syndrome, systemic lupus erythematosus and inflammatory myopathies) or with other possible causes of sarcopenia and osteoporosis (such as severe neurological, pulmonary, cardiac and endocrine diseases).

Patients treated with bisphosphonate or with supplementation on calcium and vitamin D were not excluded being the present a real-life investigation, however concomitant treatment were recorded. This study was conducted in accordance with the principles of Good Clinical Practices and the declaration of Helsinski. All patients provided at the right time, the mandatory signed informed consent form to enter the Scleroderma Clinic assistance. All the standard clinical investigations performed at the Scleroderma Clinic were approved by the local Ethical Board Committee (ECB).

Assessment of SSc patients

All SSc patients underwent clinical evaluation, laboratory and instrumental exams within a maximum 3-month period, as a part of the regular follow-up approved by SSc international guidelines, and local clinical practice (ECB) (20).

Clinical and instrumental evaluation

Demographic and lifestyle data included: sex, age, disease duration (defined since the first non-Raynaud's phenomenon symptom that satisfy the 2013 American College of Rheumatology (ACR)/European League against Rheumatology (EULAR) criteria), age at diagnosis, smoking condition, alcohol consumption, prior fragility fractures, familiarity for femoral fractures, menopausal status, body weight and height (with relative body mass index – BMI), weight loss. No one was cachetic. The patients were classified as underweight, normal weight, overweight and obese according to World Health Organization (WHO) criteria (21).

Clinical parameters

Microvascular damage was assessed by NVC (see below). Skin involvement was assessed by modified Rodnan skin score (mRSS; range 0–51) to evaluate skin thickness. Peripheral vascular involvement assessment included the history of pitting scars, ulceration or gangrene, presence of Raynaud's phenomenon (RP).

Diagnosis of limited cutaneous (lcSSC) and diffuse cutaneous (dcSSC) systemic sclerosis was made according to the LeRoy classification (22).

Pulmonary involvement was defined by the evidence of high-resolution computed tomography interstitial lung and reporting the pulmonary function tests (PFT) with determination of forced vital capacity (FVC) and diffusion for carbon monoxide (DLCO/VA% and DLCOAdj%) and six minute walking test (6MWT) (23).

Cardiac involvement was evaluated by Doppler echocardiography study (with estimated PAPs) and ejection fraction (FE%), and with evaluation of conduction defect or presence of arrhythmia on the electrocardiogram (ECG).

Pulmonary arterial hypertension (PAH) was screened as proposed by the 2015 European Society of cardiology/European Respiratory Society guidelines and a non-invasive echographic assessment of increased systolic pulmonary arterial pressure (sPAP) was made with a cut-off of 38 mmHg (24-27).

Gastrointestinal (GI) involvement was defined as distal oesophageal hypomotility or aperistalsis documented by manometric study and/or presence of upper and lower GI tract symptoms (reflux, vomiting, early satiety, bloating, diarrhoea, constipation, diagnosis of malabsorptive syndrome or episodes of pseudo-obstruction).

Renal involvement was defined by elevated serum creatinine levels and/or elevated renal artery resistive index (RI) (28).

Muscoloskeletal involvement was defined as the history of myopathy, arthralgia, arthritis and joint conctractures (29). Medsger severity score was calculated for each organ (30).

Laboratory tests

Routine follow-up laboratory tests were performed, such as haemoglobin (Hb),

creatinine (crea), erythrocyte sedimentation rate (ESR), C reactive protein (CRP), creatine kinase (CK), bone alkaline phosphatase (BAP), calcium (Ca), phosphorus (Ph), 25-hydroxyvitamin D (25OH)D. Antinuclear antibodies (ANAs) were assessed using indirect immunofluorescence on Hep-2/liver cells (EUROPLUS ANA Mosaic FA 1510-1), with a 1:80 serum dilution as cut-off value. Extractable Nuclear Antigen antibodies (ENA) were assessed using ELISA (EUROASSAY Anti-ENA ProfilePlus ELISA IgG, EA 1590-1G).

Ongoing treatments

For each patient, treatment concerning the specific organ involvement were reported as well as data regarding ongoing therapy with proton pump inhibitors (PPI), low dose prednisone (PDN), calcium channel blockers (CCBs), ACE inhibitors (ACEi), angiotensin II receptor blockers (ARB), acetyl salicylic acid (ASA), iloprost, phosphodiesterase type 5 inhibitors (PD5i), endothelin receptor antagonists (ERA), conventional DMARDs (cDMARDs), bisphosphonates, denosumab and oral supplementation with D vitamin were collected (Supplementary Table S1).

Nailfold videocapillaroscopy, MES and LASCA

To evaluate the microvascular damage, SSc patients underwent a nailfold videocapillaroscopy (NVC) the same week of DXA evaluation. NVC was performed using an optical probe equipped with a x200 contact lens, connected to image analysis software (Videocap, DS Medica, Milan, Italy). The same physician performed all the examinations (CP). The fast track algorithm developed by Smith *et al*. was used to assess SSc microvascular damage, and all patients presented a typical "scleroderma pattern" (31). According to the classification proposed by Cutolo *et al*., SSc patients were classified following three patterns: "Early", "Active" and "Late" scleroderma patterns (5, 32).

The mean value of microangiopathy evolution score (MES: sum of three scores of loss of capillaries, disorganisation of the microvascular array and capillary ramifications) was calculated to assess the progression of the vascular damage (18).

Considering that capillary density is the most reliable NVC parameter and correlates with disease severity, we also manually counted the capillary number per linear mm, by using the NVC images for each SSc patient (5, 33).

Peripheral blood flow was analysed in a sample of sarcopenic SSc patients by Laser speckle contrast analysis (LASCA) at the level of both volar and dorsal fingertips (expressed as perfusion units PU) as reported in our previous validation manuscript (34).

Body composition measurements

All patients affected by systemic sclerosis and the healthy subjects underwent dual x-ray absorptiometry to obtain evaluation of body composition, bone quantity and bone quality (DXA scan, Lunar Prodigy, GE Lunar, Madison, WI, USA).

A dedicated software analysed, by non-invasive techniques, the whole-body composition and the different body composition of three major areas (arms, legs and trunk) describing total body mass (TM) (gr), total lean mass (LM) (gr), total fat mass (FM) (gr) and bone mineral content (BMC) (gr) for each area. Using Baumgartner's equation, we calculated the relative skeletal muscle index (RSMI) and, according to the European Working Group on Sarcopenia in Older People (EWGSOP) criteria, we classified patients as having sarcopenia or not; RSMI was derived from the ratio between appendicular skeletal lean mass and height squared and sarcopenia is defined with values < 5.5 kg/m^2 in women and <7.26 kg/m2 in men) (35, 36).

Bone mineral density

At the same time of body composition analysis, the DXA scan software also investigated the bone mineral density (BMD), expressed in g/m^2, of seven different areas (head, upper limbs, lower limbs, trunk, spine, ribs, pelvis) and the total BMD of the whole body.

Furthermore, BMD for lumbar spine (L1-L4) and femoral neck, were classified as osteopenic (T score= -1.0 to -2.4 DS) or osteoporosic (OP) (T score <-2.5 DS) according to the T-score (37).

All scans were carried out on the same machine by the same operator (AC) and were analysed by the same dedicated physician (SP).

Statistical analysis

Means were compared by the Student's t-test or by one way analysis of variance; medians were compared by the Kruskall Wallis test; and frequencies by the chi square test. Correlations were calculated by the Pearson's method. A p-value <0.05 was considered significant. All the calculations were performed using Medcalc® v. 12.3 (Belgium) as statistical software.

Results

Clinical and laboratory data according to the different NVC patterns

There were no significant differences in the principal demographic data (age, disease duration, sex, height, BMI) between the whole cohort of SSc patients and the three groups of SSc patients based on their NVC pattern according to a recent study evaluating the different pattern progression over 12 years (Table I) (38).

Patients weight in "Late" SSc pattern group was significantly lower than in patients with "Early" and "Active" NVC patterns (p<0.05). We didn't observe any difference regarding the most important risk factors for osteoporosis (OP), such as prevalence of menopause, smoking condition, alcohol consumption, familiarity for hip fractures and previous OP-related fractures.

Regarding the organ involvement, there were differences between "Late" SSc subgroup and "Early"/"Active" SSc subgroups concerning the Medsger severity scale for skin (p=0.0001), peripheral vascular (p<0.0005), gastrointestinal (p=0.008) and muscle (p=0.003) involvement.

Indeed, patients with "Late" SSc pattern had higher prevalence of digital ulcers (p=0.0002), oesophageal involvement (p=0.009), proximal muscle weakness (p=0.003), an higher mRSS (p<0.01), and they were more frequently affected by the diffuse form (dcSSC) (p=0.04) than the limited one (lcSSC) (p<0.0005) (Table I).

No significant abnormalities were observed in the median values of the labo-

Table I. Demographic, past medical history and clinical characteristics of patients with SSc, according to the NVC patterns ("Early", "Active" and "Late").

	SSc (n=43)	SSc with "Early" NVC pattern (n=11)	SSc with "Active" NVC pattern (n=16)	SSc with "Late" NVC pattern (n=16)	*p*-value
Age, media ± SD, years	64.1 ± 11.2	68.6 ± 8.3	64.4 ± 11.2	60.8 ± 12.2	NS
Male, n (%)	7 (16.3)	1 (9)	4 (25)	2 (12.5)	NS
Female, n (%)	36 (83.7)	10 (91)	8 (16)	14 (87.5)	
Disease duration, media ± SD, years	10.23 ± 6.0	9.6 ± 4.9	9 ± 5.4	11.8 ± 7.6	NS
Weight, median (IQR), kg	62 (45-105)	71.1 ± 15.8	64.7 ± 12.5	58.6 ± 10.4	<0.05
Height, media ± SD, cm	163.1 ± 8.3	164 ± 7.1	165.8 ± 9.5	159.8 ± 6.9	NS
BMI, media ± SD, kg/m^2	24 ± 4.1	26.3 ± 4.5	23.5 ± 3.6	23 ± 4	NS
Normal weight, n (%)	24 (55.8)	5 (45.45)	9 (56.25)	10 (62.5)	NS
Underweight, n (%)	2 (4.65)	0 (0)	1 (6.25)	1 (6.25)	NS
Overweight, n (%)	13 (30.23)	3 (27.27)	6 (37.5)	4 (25)	NS
Obesity, n (%)	4 (9.30)	3 (27.27)	0 (0)	1 (6.25)	0.049
Menopause, n (%)	24 (66.7)	10 (100)	11 (91.7)	11 (78.6)	NS
Smoker[a], n (%)	4 (9.3)	1 (9.1)	3 (18.75)	0 (0)	NS
Alcohol consumption[b], n (%)	0 (0)	0 (0)	0 (0)	0 (0)	NS
Previous osteoporosis related fractures, n (%)	11 (25.6)	2 (18.2)	3 (18.75)	6 (37.5)	NS
Familiarity for hip fractures, n (%)	8 (18.6)	2 (18.2%	3 (18.75)	3 (18.75)	NS
ORGAN INVOLVEMENT					
Microcirculation					
History of RP, n (%)	39/43 (90.7)	11/11 (100)	13/16 (81.25)	15/16 (93.75)	NS
Capillary density, median (IQR), number of capillaries/mm	4.8 (2.2 – 9.7)	8.5 (6.6-9.7)	4.75 (2.7-7.6)	3.95 (2.2-5.5)	<0.000002
MES	6 (0 - 8)	1 (0-4)	5 (3-6)	6.5 (6-8)	<0.000001
Medsger score vascular	1 (0-3)	1 (1-1)	1 (0-3)	3 (0-3)	<0.0005
Skin					
Medsger score general	1 (0-4)	0 (0-1)	1 (0-4)	1 (0-2)	0.004
lcSSC, n (%)	11/43 (25.6)	0/11 (0)	4/16 (25)	7/16 (43.75)	0.04
dcSSC, n (%)	32/43 (74.4)	11/11 (100)	12/16 (75)	9/16 (56.25)	<0.0005
mRSS, media ± SD	10,7 ± 8.5	3.8 ± 3.4	8.9 ± 6.4	17.3 ± 8.4	<0.001
History of digital ulcers, n (%)	20/43 (46.5)	0/11 (0)	7/16 (43.75)	13/16 (81.25)	0.0002
Medsger score skin	1 (0-3)	1 (0-1)	1 (0-2)	2 (1-3)	<0.0001
Muscoloskeletal					
BMI, media ± SD, kg/m^2	24 ± 4.1	26.3 ± 4.5	23.5 ± 3.6	23 ± 5	NS
Muscoloskeletal involvement, n (%)	5 (11.6%)	1 (9.1%)	2 (12.5%)	2 (12.5%)	NS
Medsger score joint/tendon	0 (0-1)	0 (0-0)	0 (0-1)	0 (0-1)	NS
Medsger score muscle	0 (0-1)	0 (0-0)	0 (0-0)	0 (0-1)	0.003
Lung[c]					
DLCO, Adj % predicted	77.3 ± 21.5	88.4 ± 10	78.6 ± 24.7	65.5 ± 20.4	0.04
6MWT, media ± SD, mt walked	374.2 ± 80.9	366.9 ± 82.8	386.4 ± 96	NS	NS
Medsger score lung	1 (0-4)	1 (0-1)	1 (0-4)	1 (0-4)	NS
Heart					
Cardiac involvement, n (%)	6 (13.9%)	0 (0%)	2 (12.5%)	2 (12.5%)	NS
PAPs estimated	32 (18-57)	31 (25-45)	31 (18-45)	33 (22-57)	NS
FE% predicted		58.2 ± 11.4	56.7 ± 3.1	57.2 ± 3.1	NS
Medsger score heart	0 (0-4)	0 (0-1)	0 (0-4)	0 (0-4)	NS
Renal					
Renal involvement, n (%)	57.3 ± 3.1	0%	1 (%)	1 (%)	NS
RI	0.65 (0.54-1)	0.67 (0.6-0.84)	0.65 (0.54-1)	0.68 (0.56-1)	NS
Medsger score kidney	0 (0-2)	0 (0-0)	0 (0-2)	0 (0-1)	NS
Gastrointestinal					
Esophageal gastrointestinal involvement, n (%)	19 (44.2%)	1 (9.1%)	7 (43.75%)	11 (68.75%)	0.009
Medsger score gastrointestinal tract	0 (0-3)	0 (0-1)	0 (0-3)	1 (0-3)	0.008

[a]Smoker: active; [b]Alcohol consumption: more than 3 U/day; [c]Pulmonary involvement: ILD on HR-CT.
BMI: body mass index; OP: osteoporosis; lcSSC: limited cutaneous systemic sclerosis;. dcSSc: diffuse cutaneous systemic sclerosis; mRSS: modified Rodnan skin score; RP: Raynaud's phenomenon; MES: microangiopathy evolution score; FVC: forced vital capacity; DLCO/VA: diffusive capacity of carbon monoxide divided by alveolar volume; DLCOAdj: diffuse capacity of carbon monoxide adjusted; 6MWT: 6 minutes walking test; sPAP: systolic pulmonary arterial pressure; FE: ejection fraction; RI: renal artery resistive index.

Table II. Comparison of body composition parameters between SSc patients and healthy subjects according to the microvascular involvement (NVC patterns) and body area.

	SSc patients (n=43)	Pt with "Early" NVC pattern (n=11)	Pt with "Active" NVC pattern (n=16)	Pt with "Late" NVC pattern (n=16)	Healthy subjects (n=43)	*p*-value
Age, media ± SD years	64.1 ± 11.2	68.6 ± 8.3	64.4 ± 11.2	60.8 ± 12.2	62.2 ± 11.7	0.29
Prevalence of sarcopenia, n (%)	10 (23.26)	1 (9.1)	2 (12.5)	7 (43.75)	2 (4.65)	0.002
RSMI,** median (IQR), kg/m^2	5.9 (5.4 -8.5)	6.7(5.5-8.5)	5.8(4.5-7.6)	5.3 (3.8-8.8)	6.2 (3.8-6.6)	0.003
Upper limbs						
TM, media ± SD, gr	7055 ± 1924	8164 ± 1832	7401 ± 1942	5947 ± 1422	7020 ± 1397	0.004
LM, median (IQR), gr	3802 (1525-7019)	4250 (3267-6518)	4150 (2232-7019)	3133 (1525-5150)	3902 (1971-7200)	0.002
FM, media ± SD, gr	2736 ± 1120	3444 ± 1138	2581 ± 1167	2405 ± 882.2	2990 ± 871.4	0.03
BMC, median (IQR), gr	259 (167-493)	306 (198-442)	284 (198-493)	228 (169-394)	257 (167-391)	NS
Lower limbs						
TM, media ± SD, gr	21602 ± 4463	23515 ± 4230	21555 ± 4016	20335 ± 4830	21413 ± 3084	NS
LM, median (IQR), gr	12770 (7341-19747)	13381 (10605-19747)	13092 (9315- 18988)	11297 (7341-18895)	12610 (9392-19050)	NS
FM, media ± SD, gr	7784 ± 2312	8674 ± 2414	7108 ± 2008	7835 ± 2445)	8739 ± 1927	NS
BMC, median (IQR), gr	701 (495-1257)	786 (522-972)	721 (495-1257)	635 (507-1015)	697 (505-1114)	NS
Trunk						
TM, media ± SD, gr	30800 (21613-55127)	31400 (2700-55127)	31150 (22381-52600)	27350 (21613-45037)	31509 (20641-48981)	NS
LM, median (IQR), gr	18127 (14358-28439)	20350 (16205-24760)	18302 (14358- 28439)	17119 (14462-25568)	18300 (12682- 26185)	NS
FM, media ± SD, gr	12297 ± 5345	16283 ± 6645	11725 ± 5156	10814 ± 4438	14343 ± 4802	NS
BMC, median (IQR), gr	557 (357-987)	621 (468-922)	581 (377-987)	536 (357-788)	565 (290-953)	NS

RSMI: relative skeletal muscle index; TM: total mass; LM: lean mass; FT: fat mass; BMC: bone mineral content.
**The values included male and female SSc patients with relative altered values of RSMI accorning to their gender.

ratory tests, neither differences between the three subgroups according to the different NVC patterns (Suppl. Table S2).
No significant difference was detected among treatment in the present cohort of patients. It was only observed that patients in "Late" NVC pattern subgroup *versus* earliest NVC patterns, received mycophenolate mofetil (MMF) and endothelin receptor antagonist (ERA) in a significant higher % (p=0.038 and p=0.002, respectively), possibly due to their higher disease severity. (Suppl. Table S1).

Occurrence of sarcopenia and altered body composition in SSc patients vs. healthy subjects

Significantly higher prevalence of sarcopenia was found in SSc patients *versus* age-matched subjects: 23.26% *versus* 4.65%, p=0.03, respectively.
Regarding the analysis of body composition no significant differences were observed in the distribution of fat mass (FM p=0.40), lean mass (LM p=0.97) and bone mineral content (BMC p=0.33) both in the evaluation of the whole-body and in the analysis of upper limbs (FM p=0.08, LM p=0.47, BMC p=0.51), lower limbs (FM p=0.04, LM p=0.69, BMC p=0.82) in SSc patients compared to controls. (data not shown).
In the trunk of SSc patients, FM was found significantly lower than in control group (p=0.036), but no statistical differences was noted regarding LM p=0.99 and BMC p=0.83. Interestingly, even if the prevalence of sarcopenia was higher in SSc patients than in healthy subjects, no differences were reported in RSMI median values in two groups (p=0.97) (data not shown).
Concerning the analysis of bone status, the prevalence of femoral OP was found higher only in SSc patients with BMD values and T-score index at femoral neck lower than in the healthy control group (p= 0.038 and p=0.047) (data not shown).
The analysis of bone mass in different body areas showed no significant differences in the median BMD values at the level of head (p=0.06), upper limbs (p=0.22), trunk (p=0.79), ribs (p=0.69), and lower limbs (p=0.44).

Comparison of body composition in SSc patients according to the NVC pattern vs. healthy subjects

Interestingly, SSc patients presenting with the "Late" NVC pattern showed higher prevalence of sarcopenia, compared to patients with "Early" or "Active" NVC patterns (p=0.003) (Table II). The values included male and female SSc patients with relative altered values of RSMI according to their gender.
In addition, SSc patients who showed the most advanced NVC patterns, had a lower LM (p=0.04) and FM (p=0.017) for the whole body; of note, by analysing the body composition of each individual anatomical region it was found that patients with "Late" NVC pattern showed lower LM (p=0.002), FM (p=0.029) and FM (p=0.004) at the upper limbs, but only reduction of FM on the trunk (p=0.016) and lower limbs (p=0.052).
Concerning the bone status, there was a more significant incidence of femoral OP (p = 0.016) in SSc patients with "Late" NVC pattern by a decrease of T-score (p=0.007) and BMD of femoral neck (p=0.027) *versus* the other NVC patterns.
In addition, the incidence of vertebral OP in patients with "Late" NVC pattern appear to be greater than in the "Early" and "Active" (p=0.018), whereas no significant differences were observed on L1-L4 T-score and L1-L4 BMD (Table III).

Features of sarcopenic vs. non-sarcopenic SSc patients

As reported in details in Table III, sar-

copenic SSc patients showed a significant loss of capillaries (p<0.05), and altered capillary array (MES, p=00.001), as well as significant lower values TM (p= 0.0001), LM (p<0.001): FM (p=0.004) and BMC (p=0.04) *versus* non-sarcopenic patients.

Nine of the 37 SSc patients that were analysed by LASCA were found sarcopenic (24.3%) and their LASCA values were found to be significantly lower than non-sarcopenic patients.

No significant difference was detected concerning the organ involvement between sarcopenic and non-sarcopenic SSc patients (Medsger severity score (6.5 (0–8) *vs*. 5 (0–8), respectively) (data not shown). However, a statistical higher incidence of digital ulcers (p<0.005) and decreased DLCO/VA values was detected in sarcopenic *versus* non-sarcopenic SSc patients (59.4 ± 22.2 *vs*. 78.8 ± 17.8, p=0.009).

Regarding bone status sarcopenic SSc patients showed a lower statistical significative BMD in the trunk, upper and lower limbs; the average values of the BMD on the pelvis and ribs are lower without reaching statistical significance (data not shown).

No significant differences were observed in relation to the ongoing treatments and the autoantibody serum profiles.

Table III: Comparison of capillary number, MES and body composition between SSc patients according to the presence of sarcopenia.

	Sarcopenic SSc patients	Non-sarcopenic SSc patients	*p*-value
Age, media ± SD, years	60.4 ± 12.1	65.2 ± 10.8	NS
RSMI, median (IQR), kg/m^2	5.1 (3.8-5.5)	6.6 (5.4-8.8)	<0.001
NVC parameters			
Capillary number	4.4±1.8	5.8±2.2	<0.05
MES	6.6 (6-8)	5 (3-6)	<0.001
LASCA BP fingertips volar (PU)	42.65 (27.2-116)	83.20 (40.6-240)	<0.05
LASCA BP fingertips dorsal (PU)	37.95 (26.3-67.2)	69.16 (58.11-136.2)	<0.05
Whole body composition			
TM median (IQR), gr	51200 (43347-63300)	66900 (48599-104874)	<0.0001
LM media ± SD	32567 ± 3280	41291 ± 7669	<0.001
FM median (IQR), gr	17271 (12776-23830)	23649 (9871-48543)	0.004
BMC media ± SD	1803 ± 310	2152 ± 488	0.04
Upper limbs			
TM, media ± SD	5137 ± 1107	7637 ± 1738	<0.001
LM, media ± SD	2962 ± 676	4419 ± 1212	<0.001
FM, median (IQR), gr	1873 (834-2700)	2682 (962-5997)	0.004
BMC, media ± SD	230.7 ± 48.7	296.8 ± 80.6	<0.01
Lower limbs			
TM, media ± SD	17481 ± 3399	22852 ± 3998	<0.001
LM, media ± SD	10344 ± 1914	14025 ± 2890	<0.001
FM, media ± SD	6548 ± 1734	8153 ± 2356	NS
BMC, media ± SD	640.8 ± 127.2	769.3 ± 203.2	NS
Trunk			
TM, median (IQR), gr	25850 (21613-31000)	31700 (22381-55127)	0.0002
LM, median (IQR), gr	16795 (14358-18828)	18500 (15468-28439)	0.003
FM, median (IQR), gr	7933 (5819-12297)	12867 (3556-30367)	0.002
BMC, media ± SD	489.4 ± 73.4	629.2 ± 167.8	0.015

RSMI: relative skeletal muscle index; TM: total mass; LM: lean mass; FT: fat mass; BMC: bone mineral content; OP: osteoporosis; TBS: trabecular bone score; MES: microangiopathy evolution score; PU: perfusion units.

Discussion

The present study integrates very recent evidences showing abnormalities in body composition and in particular a significant increased occurrence of sarcopenia in SSc patients (other study range 20–42%, present study 23–26%) (8-11).

Notably, the study showed that the occurrence of sarcopenia in SSc is significantly correlated with the severity of the microvascular damage, showing a reduced capillary density (number of capillaries per linear mm) and decreased peripheral blood flow (LASCA), in sarcopenic *versus* non-sarcopenic SSc patients, suggesting important pathophysiological links with decreased muscle function.

Interestingly, sarcopenia, as well as body composition abnormalities, showed a higher prevalence in SSc patients characterised by the "Late" NVC pattern of microangiopathy (43.75%), together with a significantly higher progression of the microvascular array alteration as detected by the MES scoring.

Generally, besides the density of the capillary network, also the distribution of capillaries is crucial for adequate muscle oxygenation and function, and sarcopenic SSc patients with "Late" NCV pattern and advanced MES showed the most significant loss of capillaries and altered distribution (16, 17). While capillaries are important for oxygen delivery, the link between fibre size and capillary supply is also reflected by the similar time course of hypertrophy and angiogenesis, and the cross-talk between capillaries and satellite cells and systems (16, 17). In fact, in SSc, as in other autoimmune connective tissue diseases, the efficiency of the microvascular systems supports also the immune and endocrine system function (39).

Capillary rarefaction may contribute to sarcopenia and functional impairment in older adults, even if rarefaction during ageing does not occur at random like in SSc, but maintains the distribution of capillaries to preserve the potential for intramuscular oxygenation (40).

In the present study, the sarcopenic patients were the youngest among the SSc population studied and all were characterised by the advanced "Late" NVC pattern, further supporting the sclerodermic microangiopathy as an important risk factor for the muscle failure/sufference.

In fact, a sample of sarcopenic patients with "Late" NVC pattern showed a significantly reduced peripheral blood flow at the upper limbs, as evaluated by the LASCA analysis that has been already shown to correlate with microangiopathy severity in SSc (41).

A further indirect signal of microvascular failure (skin ischaemia) in sarco-

penic SSc patients, was the significantly higher incidence of digital ulcers in upper limbs, in concomitance with the "Late" NVC pattern (81%, p=0.0002).
On the other hand, fibrosis is a prevalent histopathologic feature in muscle biopsies of SSc patients with muscle disease together with microangiopathy (16, 42). As detected, fibrosing myopathy, or fibrosis predominance on muscle histopathology, is associated with a unique clinical phenotype in SSc patients and participate in sarcopenia (16).
Interestingly, and for the first time, the present study revealed differences in body composition at different body areas according to the different NVC patterns.
Particularly, patients with "Late" NVC pattern showed a significant reduction of fat mass in upper and lower limbs and trunk, but a significant reduction of lean mass only in the upper limbs.
Important location of muscle sufference at the upper limbs, was confirmed by Corallo *et al*. who described a very significant reduction (p<0.0001) of the hand grip strength (HGS) in their sarcopenic *versus* non-sarcopenic SSc patients (9). A recent study showed that arm cranking seems to be the successful mode of exercise for SSc patients to improve the microvascular endothelial function of upper limbs as compared to cycling (p<0.05) (43).
As mentioned, the prevalence of sarcopenia was confirmed significantly higher in SSc patients (range 23–26%) compared to the age-matched healthy subjects (4.5%): notably the literature suggests that the prevalence of sarcopenia in 60- to 70-year-olds is in the range of 5–13% (44, 45).
Therefore, by considering that the mean age of sarcopenic patients in the present study was 60 years, the severity of the progressive capillary rarefaction that characterises the SSc pathophysiology, is the more plausible concomitant risk factor for the occurrence of sarcopenia in SSc than simple ageing.
No significant differences were observed in the distribution of fat and lean mass in the whole body and in different area of body (upper limbs, lower limbs and trunk) in SSc patients compared to healthy subjects in line with a previous report from Corrado *et al*. (46). On the contrary, Marighela *et al*. showed a lower lean and fat mass in SSc patients *versus* the control group, and results were confirmed also by Souza *et al*. (10, 47).
The sarcopenic SSc patients showed in the present study a lower BMD more evident in presence of advanced miscrovascular damage. Few studies have evaluated BMD in SSc patients and a previous study reported that low weight and reduced lean mass were associated with a decrease in lumbar and femoral BMD in SSc women probably due to the chronic tissue inflammation and fibrosis, but also to the progressive and generalised microvascular damage (46). Recently, the literature data suggest a new interpretation of the role of at least some SSc-specific antibodies (ENAs) to explain different organ involvement and complication of the disease, having a profibrotic role in tissue remodelling. This could induce reconsidering specific antibodies as a target for early use of immunosuppressive drugs to achieve better disease control (48).
In the present study no significant differences were observed in terms of sarcopenia prevalence in relation to the autoantibody serum profiles probably due to the limited number of subjects analysed.
Some limitations of the present study might include the small patient sample, but we decided to analyse patients attending the Scleroderma Clinic since 2018 because at that time a standard detailed clinical screening was started. A further limitation, already presented by Corallo *et al*. in their study, is the lack of widely accepted criteria in terms of cut-off values for sarcopenia in chronic diseases such as SSc (as reference values), since all the cut-off values are intended for elderly healthy patients (9, 49).
In conclusion, a significant number of SSc patients seem to be affected by sarcopenia and reduced total body mass, at the level of trunk, lower limbs and significantly at the upper limbs, with a concomitant altered bone mass.
The significant association found in sarcopenic SSc patients, between the reported body composition alterations and the advanced SSc microvascular damage, identified by the "Late" NVC pattern and the MES/LASCA values, recommend that microvascular parameters should be considered as biomarkers of the disease progression and severity in SSc, as well as in connective tissue diseases (50-52).
Finally, all parameters related to body composition were found within sarcopenic SSc patients significantly more altered in upper limbs *versus* lower limbs and trunk, further suggesting a strong link between severity of local microvascular failure and associated muscle sufference at least in SSc.

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References

1. CUTOLO M, SOLDANO S, SMITH V: Pathophysiology of systemic sclerosis: current understanding and new insights. *Expert Rev Clin Immunol* 2019; 15: 753-64.
2. SOLDANO S, PIZZORNI C, PAOLINO S *et al*.: Alternatively activated (M2) macrophage phenotype is inducible by endothelin-1 in cultured human macrophages. *PLoS One* 2017; 12: e0175238.
3. BARSOTTI S, ORLANDI M, CODULLO V *et al*.: One year in review 2019: systemic sclerosis. *Clin Exp Rheumatol* 2019; 37 (Suppl. 119): S3-14.
4. SMITH V, DISTLER O, CUTOLO M: Might Nailfold Capillaroscopy Be a "Proxy" for Lung Involvement in Connective Tissue Diseases? *J Rheumatol* 2019; 46: 1061-63.
5. CUTOLO M, HERRICK AL, DISTLER O *et al*.: Nailfold videocapillaroscopic features and other clinical risk factors for digital ulcers in systemic sclerosis: a multicenter, prospective cohort study. *Arthritis Rheumatol* 2016; 68: 2527-39.
6. AVOUAC J, LEPRI G, SMITH V *et al*.: Sequential nailfold videocapillaroscopy examinations have responsiveness to detect organ progression in Systemic sclerosis. *Semin Arthritis Rheum* 2017; 47: 86-94.
7. CUTOLO M, PAOLINO S, SMITH V: Nailfold capillaroscopy in rheumatology: ready for the daily use but with care in terminology. *Clin Rheumatol* 2019; 38: 2293-7.
8. CAIMMI C, CARAMASCHI P, VENTURINI A *et al*.: Malnutrition and sarcopenia in a large cohort of patients with systemic sclerosis. *Clin Rheumatol* 2018; 37: 987-97.
9. CORALLO C, FIORAVANTI A, TENTI S, PECETTI G, NUTI R, GIORDANO N: Sarcopenia in systemic sclerosis: the impact of nutritional, clinical and laboratory features. *Rheumatol Int* 2019; 39: 1767-75.
10. MARIGHELA TF, DE GENARO PS, PINHEIRO MM, VERA L SZEJNFELD VL, KAYSER C:

Risk factor for body composition abnormalities in systemic sclerosis. *Clin Rheumatol* 2013; 32: 1037-44.
11. SIEGERT E, MARCH C, OTTEN L *et al*.: Prevalence of sarcopenia in systemic sclerosis: assessing body composition and functional disability in patients with systemic sclerosis. *Nutrition* 2018; 55-56: 51-5.
12. THIETART S, LOUATI K, GATFOSSE M *et al*.: Overview of osteo-articular involvement in systemic sclerosis: Specific risk factors, clinic-sonographic evaluation, and comparison with healthy women from the French OFELY cohort. *Best Pract Res Clin Rheumatol* 2018; 32: 591-604.
13. CAIMMI C, CARAMASCHI P, BARAUSSE G *et al*.: Bone metabolism in a Large cohort of patients with systemic sclerosis. *Calcif Tissue Int* 2016; 99: 23-9.
14. ALEXANDRE TDA S, DUARTE YA, SANTOS JL, WONG R, LEBRÃO ML: Sarcopenia according to the European Working Group on Sarcopenia in Older People (EWGSOP) versus dynapenia as a risk factor for mortality in the elderly. *J Nutr Health Aging* 2014; 18: 751-6
15. CRUZ-JENTOFT AJ, SAYER AA: Sarcopenia. *Lancet* 2019; 393: 2636-46.
16. PAYNE GW, BEARDEN SE: The microcirculation of skeletal muscle in aging. *Microcirculation* 2006; 13: 275-7.
17. HENDRICKSE P, DEGENS H: The role of the microcirculation in muscle function and plasticity. *Muscle Res Cell Motil* 2019; 40: 127-140.
18. SULLI A, SECCHI ME, PIZZORNI C, CUTOLO M: Scoring the nailfold microvascular changes during the capillaroscopic analysis in systemic sclerosis patients. *Ann Rheum Dis* 2008; 67: 885-7.
19. VAN DEN HOOGEN F, KHANNA D, FRANSEN J *et al*.: 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League against Rheumatism collaborative initiative. *Arthritis Rheum* 2013; 65: 2737-47.
20. SMITH V, SCIRÈ CA, TALARICO R *et al*.: Systemic sclerosis: state of the art on clinical practice guidelines. *RMD Open* 2018; 4: e000782-9.
21. PETERSON CM, THOMAS DM, BLACKBURN GL, HEYMSFIELD SB: Universal equation for estimating ideal body weight and body weight at any BMI. *Am J Clin Nutr* 2016; 103: 1197-203.
22. LEROY EC, BLACK C, FLEISCHMAJER R *et al*.: Scleroderma (systemic sclerosis) classification, subsets and pathogenesis. *J Rheumatol* 1998; 15: 202-5.
23. FRAUENFELDER T, WINKLEHNER A, NGUYEN TD *et al*.: Screening for interstitial lung disease in systemic sclerosis: performance of high-resolution CT with limited number of slices: a prospective study. *Ann Rheum Dis* 2014; 73: 2069-73.
24. GALLIÈ N, HUMBERT M, VACHIERY JL *et al*.: 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulomonary hypertension. *Eur Heart J* 2016; 37: 67-119.
25. VANDECASTEELE E, DRIEGHE B, MELSENS K *et al*.: Screening for pulmonary arterial hypertension in an unselected prospective systemic sclerosis cohort. *Eur Respir J* 2017; 49: 1602275.
26. PARASURAMAN S, WALKER S, LOUDON BL *et al*.: Assessment of pulmonary artery pressure by echocardiography: a comprehensive review. *Int J Cardiol Hear Vasc* 2016; 12: 45-51.
27. GIORDANO N, CORALLO C, CHIRICO C *et al*.: Pulmonary arterial hypertension in systemic sclerosis: Diagnosis and treatment according to the European society of cardiology and European respiratory society 2015 guidelines. *J Scleroderma Relat Disord* 2019; 4: 35-42.
28. VAN DEN HOOGEN F, KHANNA D, FRANSEN J *et al*.: 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League against Rheumatism collaborative initiative. *Arthritis Rheum* 2013; 65: 2737-47.
29. LÓRÁND V, CZIRJÁK L, MINIER T: Musculoskeletal involvement in systemic sclerosis. *Presse Med* 2014; 43: e315-e328.
30. MEDSGER TA, BOMBARDIERI S, CZIRJAK L, SCORZA R, DELLA ROSSA A, BENCIVELLI W: Assessment of disease severity and prognosis. *Clin Exp Rheumatol* 2003; 21 (Suppl. 29): S42.
31. SMITH V, VANHAECKE A, HERRICK AL *et al*.: EULAR Study Group on Microcirculation in Rheumatic Diseases. Fast track algorithm: How to differentiate a "scleroderma pattern" from a "non-scleroderma pattern". *Autoimmun Rev* 2019; 18: 102394
32. CUTOLO M, SULLI A, PIZZORNI C, ACCARDO S: Nailfold videocapillaroscopy assessment of microvascular damage in systemic sclerosis. *J Rheumatol* 2000; 27: 155-60.
33. WILDT M, HESSELSTRAND R, SCHEJA A, AKESSON A: Capillary density in patients with systemic sclerosis, as determined by microscopy counts and compared with computer-based analysis. *Clin Exp Rheumatol* 1999; 17: 219-22.
34. RUARO B, SULLI A, ALESSANDRI E, PIZZORNI C, FERRARI G, CUTOLO M: Laser speckle contrast analysis: a new method to evaluate peripheral blood perfusion in systemic sclerosis patients. *Ann Rheum Dis* 2014; 73: 1181-5.
35. BAUMGARTNER RN, KOEHLER KM, GALLAGHER D *et al*.: Epidemiology of sarcopenia among the elderly in New Mexico. *Am J Epidemiol* 1998; 147: 755-63.
36. CRUZ-JENTOFT AJ, BAEYENS JP, BAUER JM *et al*.: Sarcopenia: European consensus on definition and diagnosis: report of the European Working Group on Sarcopenia in Older People. *Age Ageing* 2010; 39: 412-23.
37. LEWIECKI EM: Review of guidelines for bone mineral density testing and treatment of osteoporosis. *Curr Osteoporosis Rep* 2005; 3: 75-83.
38. SULLI A, PAOLINO S, PIZZORNI C *et al*.: Progression of nailfold capillaroscopic patterns and correlation with organ involvement in systemic sclerosis: a 12 year study. *Rheumatology* (Oxford) 2020; 59: 1051-8.
39. MASI AT, BIJLSMA JW, CHIKANZA IC, PITZALIS C, CUTOLO M: Neuroendocrine, immunologic, and microvascular systems interactions in rheumatoid arthritis: physiopathogenetic and therapeutic perspectives. *Semin Arthritis Rheum* 1999; 29: 65-81.
40. BARNOUIN Y, MCPHEE JS, BUTLER-BROWNE G *et al*.: Coupling between skeletal muscle fiber size and capillarization is maintained during healthy aging. *J Cachexia Sarcopenia Muscle* 2017; 8: 647-59.
41. RUARO B, SULLI A, PIZZORNI C, PAOLINO S, SMITH V, CUTOLO M: Correlations between skin blood perfusion values and nailfold capillaroscopy scores in systemic sclerosis patients. *Microvasc Res* 2016; 105: 119-24.
42. CORALLO C, CUTOLO M, VOLPI N *et al*.: Histopathological findings in systemic sclerosis-related myopathy: fibrosis and microangiopathy with lack of cellular inflammation. *Ther Adv Musculoskelet Dis* 2017; 9: 3-10.
43. MITROPOULOS A, GUMBER A, CRANK H, AKIL M, KLONIZAKIS M: The effects of upper and lower limb exercise on the microvascular reactivity in limited cutaneous systemic sclerosis patients. *Arthritis Res Ther* 2018; 20: 112.
44. WAND C, BAI L: Sarcopenia in the elderly: basic and clinical issues. *Geriatr Gerontol Int* 2012; 12: 388-96.
45. MELDRON LJ, KHOSLA S, CROWSON CS *et al*.: Epidemiology of sarcopenia. *J Am Geriatr Soc* 2000; 48: 625-30.
46. CORRADO A, COLIA R, MELE A *et al*.: Relationship between body mass composition, bone mineral density, skin fibrosis and 25(OH) vitamin D serum levels in systemic sclerosis. *PLoS One* 2015; 10: e0137912.
47. SOUZA RB, BORGES CT, TAKAYAMA L *et al*.: Systemic sclerosis and bone loss: the role of the disease and body composition. *Scand J Rheumatol* 2006: 35: 384-7.
48. CORALLO C, CHELESCHI S, CUTOLO M *et al*.: Antibodies against specific extractable nuclear antigens (ENAs) as diagnostic and prognostic tools and inducers of a profibrotic phenotype in cultured human skin fibroblasts: are they functional? *Arthritis Res Ther* 2019 Jun 24; 21: 152.
49. MCLEAN RR, KIEL DP: Developing consensus criteria for sarcopenia: an update. *J Bone Miner Res* 2015; 30: 588-92.
50. BOULON C, AIOUAZ S, BLAISE S *et al*.: Correlation between capillaroscopic classifications and severity in systemic sclerosis: results from SCLEROCAP study at inclusion. *Clin Exp Rheumatol* 2019; 37 (Suppl. 119): S63-8.
51. PAOLINO S, FERRARI G, PIZZORNI C *et al*.: Long-term follow-up of nailfold videocapillaroscopic microvascular parameters in mixed connective tissue disease versus systemic sclerosis patients: a retrospective cohort study. *Clin Exp Rheumatol* 2019; 37 (Suppl. 119): S102-7.
52. SCHONENBERG-MEINEMA D, MELSENS K, NASSAR-SHEIKH RASHID A *et al*.: Capillaroscopy in childhood-onset systemic lupus erythematosus: a first systematic review. *Clin Exp Rheumatol* 2020; 38: 350-4.