CLINICAL SCIENCE

Quantitative analysis of pulmonary vasculature in systemic sclerosis at spirometry-gated chest CT

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ABSTRACT

Objective To prospectively investigate whether differences in pulmonary vasculature exist in systemic sclerosis (SSc) and how they are distributed in patients with different pulmonary function.

Methods Seventy-four patients with SSc undergoing chest CT scan for interstitial lung disease (ILD) screening or follow-up were prospectively enrolled. A thorough clinical, laboratory and functional evaluation was performed the same day. Chest CT was spirometry gated at total lung capacity and images were analysed by two automated software programs to quantify emphysema, ILD patterns (ground-glass, reticular, honeycombing), and pulmonary vascular volume (PVV). Patients were divided in restricted (FVC% <80, DLco%<80), isolated DLco% reduction (iDLco: FVC%≥80, DLco%<80) and normals (FVC%≥80, DLco%≥80). Spearman ρ, Mann-Whitney tests and logistic regressions were used to assess for correlations, differences among groups and relationships between continuous variables.

Results Absolute and lung volume normalised PVV (PVV/LV) correlated inversely with functional parameters and positively with all ILD patterns (ρ=0.75 with ground glass, ρ=0.68 with reticular). PVV/LV was the only predictor of DLco at multivariate analysis (p=0.007). Meanwhile, the reticular pattern prevailed in peripheral regions and lower lung thirds, PVV/LV prevailed in central regions and middle lung thirds. iDLco group had a significantly higher PVV/LV (2.2%) than normal (1.6%), but lower than restricted ones (3.8%).

Conclusions Chest CT in SSc detects a progressive increase in PVV/LV as DLco decreases. Redistribution of perfusion to less affected lung regions rather than angiogenesis nearby fibrotic lung may explain the results. Further studies to ascertain whether the increase in PVV/LV reflects a real increase in blood volume are needed.

INTRODUCTION

Systemic sclerosis (SSc) is a multisystem connective tissue disease that may affect the lung with interstitial lung disease (ILD) and/or pulmonary arterial hypertension (PAH).1 To detect lung involvement, pulmonary function tests (including forced vital capacity (FVC) and diffusing capacity for carbon monoxide (DLco)) and ILD visual analysis on chest CT are the basis for determining baseline disease severity, prognosis and evolution.2,3

Recent technological advances in CT image post processing permitted non-invasive and fully automated quantitative analysis of pulmonary vasculature, without the use of contrast media and exposure to repeated ionising radiation during injection.4–7 Nonetheless, quantitative imaging requires standardised acquisition protocols and quality certified and validated image analysis methodology to ensure accuracy and precision of the quantitative information provided.8 Among the sources of variation in quantifying chest radiological features, lung volume at acquisition time is the most relevant. Few studies used spirometry-gated CT to account for this possible source of variation.9–12

The main objectives of the present study were to investigate (1) the correlations between pulmonary vascular and functional parameters, (2) whether differences in pulmonary vascular parameters exist in SSc and (3) how they are distributed among patients with different pulmonary functional parameters by using a non-invasive fully automated methodology on spirometry-gated chest CT scans.
Systemic sclerosis

MATERIALS AND METHODS

From January to December 2018, patients with SSc classified according to American College of Rheumatology/European League Against Rheumatism 2013\textsuperscript{13} undergoing chest CT for screening or follow-up of ILD were prospectively enrolled in the study. Patients with lung masses and those unable or unwilling to provide informed consent were excluded. All participants gave their written informed consent.

Clinical and Functional Evaluation

Demographic and clinical data, serology and laboratory, instrumental and functional data were collected. FVC/DLco ratio was derived.\textsuperscript{14} Patients self-assessment features (disability index—Scleroderma Health Assessment Questionnaire; Visual Analogic Scales (VAS); quality of life index as 5-level EQ-5D version EQ-5D-5L\textsuperscript{14}) were collected from medical charts, within a 3 months maximum range for modifiable variables.

Radiological evaluation

Along with functional evaluation, patients underwent unenhanced chest CT in the same day. Chest CT scans were acquired on the same scanner (SIEMENS Sensation 16, Erlangen, Germany) with a fixed protocol: 120 kV, 0.5 s rotation time, pitch 1.0. Patients were instructed on how to perform the respiratory manoeuvre lying supine during the scan acquisition by the same pulmonologist (GC) who did also the functional evaluation. Patients were instructed to inspire as deeply as possible and to hold their breath under supervision of the curve at the portable spirometry (Micro Medical, VyAire Medical, California, USA). The curve was repeated at least three times to ensure consistency among volumes at maximal inspiration (total lung capacity (TLC)). If the TLC values were consistent, then chest CT scan was acquired at the desired volume. The portable spirometer was hanged on a fixed C-arm and connected through USB wire to the laptop with the spirometric software.

CT raw data were processed to create two sets of 1 mm thick axial slices: one using standard reconstruction algorithm (Convolution Kernel B35f) for quantitative analysis and one using a high-resolution reconstruction algorithm (Convolution Kernel B60f) for qualitative analysis.

CT images with standard reconstruction algorithm were post-processed using two automated software programs dedicated to lung diseases.

Imbio LTA (based on CALIPER algorithm) was used to identify and quantify lung patterns through texture analysis, including normal lung, hyperlucencies, ground-glass, reticular and honeycombing. The sum of ground-glass, reticular and honeycombing constituted the total ILD extent (ILD_Ext). Moreover, Imbio LTA identified and quantified the pulmonary vascular volume (PVV) in whole lungs and across lung vertical thirds.\textsuperscript{15} Its normalised value to lung volume (PVV/LV) was then derived.

Imbio LDA was used to quantify the low density areas (LDA), as both absolute and relative volumes, through densitometric analysis with the threshold at −950 HU that is considered consistent with emphysema extent.\textsuperscript{16} A variable called ‘true hyperlucency’ (TH) was derived from the difference between hyperlucencies and LDA.

Data analysis

Absolute and relative frequencies were used to describe qualitative variables. Mean and SD were used to describe quantitative variables. Spearman Rho correlation coefficient was used to compare radiological with functional and clinical variables.

Patients were divided according to FVC\% and DLco\% values in three groups using cut-offs reported in previous studies\textsuperscript{17,18}: restricted (FVC\%<80 and DLco\%<80), isolated DLco\% reduction (DLco, FVC\%≥80 and DLco\%<80) and normals (FVC\%≥80 and DLco\%≥80). An arbitrary cut-off at 70% for DLco was also considered for comparison. Differences between groups were assessed by Mann-Whitney test. Kolmogorov-Smirnoff and Shapiro-Wilk tests were used to test for variables distribution. Univariate and multivariate regression analyses were performed to test the relationship between DLco\%, PVV/LV and ILD patterns. The linear regression model with generalised estimating equation was used to test the relationship between ILD_Ext and PVV/LV across lung thirds. P<0.05 was deemed statistically significant. Data analysis was performed by using MedCalc V.16, SAS V.9.3 and Prism V.8.4.2 (GraphPad Software, LLC).

RESULTS

Seventy-four patients composed the final population (65/74 females—88\%, median time from first non-Raynaud’s phenomenon duration 7 years); 3/77 were excluded for the presence of infection or bronchiectases at CT. Clinical, functional and radiological characteristics of the population are shown in table 1 (continuous variables) and table 2 (categorical variables).

All patients showed a preserved right (tricuspid anular plane systolic excursion) and left cardiac (left ventricle ejection fraction) systolic functions, with mean systolic pulmonary arterial pressure (sPAP) within upper level of normal ranges. Thirty-five to 54\% of patients had iDLco, considering the cut-off of DLco reduction at 70 or 80\%, respectively. Emphysema extent (%LDA) was greater than 6\% in three patients and greater than 14\% in one patient; two patients with higher %LDA were part of the iDLCO group, whereas the other two were among normals.

Correlations between clinical, functional and imaging parameters

Table 3 reports the correlations between vascular CT metrics and radiological patterns, functional and clinical-laboratory parameters. Vascular CT metrics (PVV and PVV/LV) showed an inverse correlation with functional parameters (FVC\%, FEV\%, TLC\% and DLco\%) and CT patterns of normal lung, emphysema (%LDA) and TH. Conversely, a strong positive correlation was found between vascular CT metrics and all ILD patterns, the highest with %ground glass (p=0.75). Among the clinical-laboratoristic parameters, PVV/LV correlated with those related to cardiovascular involvement (sPAP, N-terminal pro-brain natriuretic peptide, VAS digital ulcers) and to scores related to disease activity, disability and quality of life (VAS disease activity, Heart Assessment Questionnaire (HAQ), HAQ for walking, EQ-5D-5L).

Table 4 shows differences in vascular CT metrics according to clinical parameters. Patients with VEDOSS had a significantly lower PVV/LV, whereas those with ACA or ATA positivity, elevated ESR or CRP, a late scleroderma pattern at nailfold video capillaroscopy, and NYHA functional class ≥2 had a significantly higher PVV/LV. At multivariate regression analysis, PVV/LV was the only predictive parameter for DLco (slope: −11.47±4.08; 95\% CI: −19.62 to −3.31; p=0.007). At univariate analysis, both reticular and PVV/LV were predictive for DLco (p=0.028 and p=0.019, respectively).

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**ILD and vascular differences according to functional presentation and regional distribution**

By using 80 as cut-off of FVC%L, significant differences in ILD patterns were seen between patients with higher and lower FVC%L values (all p<0.05, Table 5). Differences in ILD patterns were observed between patients with higher and lower FVC%L values (all p<0.05, Table 5).

Table 6 shows differences in ILD patterns among the three functional groups (see online supplementary table 1 with 70% as cut-off for DLco%L for comparison). Overall MLA and specific ILD patterns (ground glass, reticular, honeycombing) extent by using a cut-off as lower as 70.

### Table 1 Clinical, functional and imaging characteristics of the 74 systemic sclerosis included patients

| Variable                        | Mean | SD  |
|---------------------------------|------|-----|
| BMI (kg/m²)                     | 24.6 | 4.5 |
| DLco %                          | 65.8 | 18.9 |
| DLco/AV                         | 80.0 | 21.3 |
| Kroghs                          | 3.1  | 0.9 |
| FVC %                           | 101.1| 24.8 |
| FVC/DLco                        | 1.6  | 0.7 |
| FEV1 %                          | 95.3 | 21.3 |
| FEV1/FVC (%)                    | 78.9 | 12.2 |
| IC %                            | 91.7 | 26.8 |
| TLC %                           | 94.5 | 18.3 |
| 6MWT (%)                        | 79.3 | 7.7 |
| SaO2 (%)                        | 96.2 | 3.8 |
| VAS Resp                        | 30.4 | 31.9 |
| mRSS                            | 6.1  | 7.5 |
| NYHA                            | 1.5  | 0.8 |
| sPAP (mm Hg)                    | 29.0 | 9.3 |
| TAPSE (mm)                      | 22.4 | 4.7 |
| LVEF (%)                        | 61.9 | 5.8 |
| RRI                             | 0.68 | 0.07 |
| Creatinine clearance (mg/dL/24 hours) | 92  | 18  |
| % Normal                        | 85.3 | 13.6 |
| % Ground glass                  | 5.5  | 10.4 |
| % Reticular                     | 1.5  | 2.3 |
| % Honeycombing                  | 0.1  | 0.3 |
| % TH                            | 7.1  | 11.0 |
| % LDA                           | 1.1  | 3.3 |
| MLA (HU)                        | −820.3| 126.5 |
| LV (ml)                         | 4587.0| 1148.3 |
| PVV (cm³)                       | 99.4 | 35.9 |
| PVV/LV (%)                      | 2.3  | 1.2 |
| PVV/LV RU central/peripheral (%)| 2.806| 1.107 |
| PVV/LV RM central/peripheral (%)| 3.607| 1.309 |
| PVV/LV RL central/peripheral (%)| 4.717| 3.724 |
| PVV/LV LU central/peripheral (%)| 2.505| 1.009 |
| PVV/LV LM central/peripheral (%)| 4.009| 1.813 |
| PVV/LV LL central/peripheral (%)| 4.523| 4.530 |

AV, alveolar volume; BMI, body mass index; DLco, diffusion lung capacity for carbon monoxide; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; HU, Hounsfield Units; IC, inspiratory capacity; LDA, per cent low density area; LL, left lower; LM, left middle; LU, left upper; LV, lung volume; LVEF, left ventricle ejection fraction; MLA, mean lung attenuation; mRSS, modified Rodnan Skin Score; 6MWT, 6 min walking test; NYHA, New York Heart Association; PVV, pulmonary vascular volume; VAS Resp, Visual Analogic Scale for Dyspnoea; RL, right lower; RM, right middle; RRI, Renal Resistivity Index; SaO2, arterial oxygen saturation; sPAP, systolic pulmonary arterial pressure; TAPSE, tricuspid anular plane systolic excursion; %TH, per cent of true hyperlucent; TLC, total lung capacity.

### Table 2 Clinical presentation of the population studied (categorical variables)

| Variable                                      | N (%)                          |
|-----------------------------------------------|--------------------------------|
| VEDOSS/diffuse/limited subset/sine scleroderma| 13/34/26/1 (17.6/45.9/35.1/1.4) |
| ANA/AACA/ATA/ARA                               | 74/20/31/5 (100/27.0/41.9/6.8) |
| Smoking history                                | 31 (41.9)                      |
| Upper/lower gastrointestinal involvement       | 42/21 (56.8/28.4)              |
| Digital ulcers                                 | 32 (42.3)                      |
| PAH                                           | 3 (4.0)                        |
| SRC                                           | 1 (1.4)                        |
| CRP >ULN                                      | 5 (6.8)                        |
| ESR >ULN                                      | 16 (21.6)                      |

Restricted //DLco/normals* 16/40/18 (21.6/54.0/24.3)

*Cut-offs considered at 80% for both forced vital capacity and diffusion lung capacity for carbon monoxide (DLco).

AC, antinontoremae antibodies; ANA, antinuclear antibodies; ARA, anti-RNA polymerase III antibodies; ATA, antitopoisomerase I antibodies; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; iDLco, isolated DLco reduction; PAH, pulmonary arterial hypertension; SRC, scleroderma renal crisis; ULN, upper limit of normal; VEDOSS, very early diagnosis of systemic sclerosis.

### Table 3 Correlations between vascular CT metrics and parenchymal patterns, functional and clinical-laboratory parameters

| PVV                  | PVV/LV                   |
|----------------------|--------------------------|
| rho                  | P value                  | rho                  | P value                  |
| LV                   | −0.12                    | −0.57                 | ≤0.001                 |
| %Normal              | −0.53                    | ≤0.001                | −0.60                 | ≤0.001                 |
| ILD_Ext              | 0.36                     | 0.30                  | −0.05                 | −0.05                  |
| %Ground glass        | 0.26                     | −0.40                 | ≤0.01                 |
| %Reticular           | 0.31                     | 0.45                  | −0.05                 | −0.01                  |
| %Honeycombing        | 0.28                     | −0.45                 | ≤0.01                 |
| %LDA                 | −0.30                    | −0.48                 | ≤0.01                 |
| MLA                  | 0.36                     | 0.34                  | ≤0.01                 |
| FVC%L                | −0.24                    | −0.40                 | ≤0.01                 |
| mRSS                 | 0.31                     | 0.23                  | −0.05                 | −0.01                  |
| %TH                  | −0.36                    | −0.62                 | ≤0.001                |
| %LDA                 | 0.30                     | −0.45                 | ≤0.01                 |
| MLA                  | 0.18                     | −0.29                 | ≤0.05                 |
| FVC%L                | 0.10                     | 0.45                  | ≥0.01                 |
| mRSS                 | 0.24                     | −0.19                 | −0.05                 | −0.01                  |
| %TH                  | 0.24                     | −0.14                 | −0.05                 | −0.01                  |
| %LDA                 | 0.12                     | 0.40                  | ≤0.01                 |
| MLA                  | 0.15                     | 0.44                  | ≤0.01                 |
| VAS dyspnoea         | 0.07                     | 0.19                  | −0.05                 | −0.01                  |
| VAS Raynaud          | −0.04                    | 0.15                  | −0.05                 | −0.01                  |
| VAS digital ulcers   | 0.23                     | 0.50                  | ≤0.001                |
| VAS disease activity | 0.11                     | 0.35                  | ≤0.01                 |
| EQ-5D-5L             | 0.19                     | 0.43                  | ≤0.01                 |

AV, alveolar volume; BMI, body mass index; DLco, diffusion lung capacity for carbon monoxide; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; HU, Hounsfield Units; IC, inspiratory capacity; LDA, per cent low density area; LL, left lower area; LM, left middle; LU, left upper; LV, lung volume; LVEF, left ventricle ejection fraction; MLA, mean lung attenuation; mRSS, modified Rodnan Skin Score; 6MWT, 6 min walking test; NYHA, New York Heart Association; PVV, pulmonary vascular volume; VAS Resp, Visual Analogic Scale for Dyspnoea; RL, right lower; RM, right middle; RRI, Renal Resistivity Index; SaO2, arterial oxygen saturation; sPAP, systolic pulmonary arterial pressure; TAPSE, tricuspid anular plane systolic excursion; %TH, per cent of true hyperlucent; TLC, total lung capacity; VAS, Visual Analogic Scale.
were significantly higher in restricted than in iDLco and normal, while no differences in ILD patterns were found between the last two groups. No differences were found between groups in terms of LDA and TH. Conversely, all three groups had significant differences in lung volume as quantified on spirometry-

| Table 4 | Distribution of vascular metrics at CT according to clinical, laboratory and instrumental features in patients with systemic sclerosis |
|--------------------------------------|----|----|----|--------------------------------------|----|----|----|
| PVV (cm³) | PVV/LV (%) |
| Mean | SD | P value | Mean | SD | P value |
| VEDOSS criteria |
| Yes | 86.86 | 37.18 | – | 1.69 | 0.68 | 0.009 |
| No | 102.23 | 36.20 | – | 2.48 | 1.38 | – |
| CRP elevation (>ULN) |
| Yes | 125.63 | 34.76 | – | 4.02 | 2.06 | 0.001 |
| No | 95.44 | 35.30 | – | 2.14 | 1.10 | – |
| ESR elevation (>ULN) |
| Yes | 124.05 | 46.77 | 0.001 | 3.47 | 2.02 | <0.001 |
| No | 90.80 | 29.06 | – | 1.97 | 0.79 | – |
| ATA positivity |
| Yes | 106.48 | 42.27 | – | 2.50 | 1.57 | 0.001 |
| No | 93.13 | 28.93 | – | 2.23 | 1.04 | – |
| ACA positivity |
| Yes | 87.18 | 28.78 | – | 1.85 | 0.59 | 0.009 |
| No | 103.05 | 37.20 | – | 2.48 | 1.37 | – |
| Diffuse cutaneous subset |
| Yes | 108.11 | 38.62 | – | 2.65 | 1.57 | – |
| No | 92.02 | 32.97 | – | 2.11 | 1.00 | – |
| NYHA functional class |
| ≥2 | 106.84 | 39.69 | – | 2.76 | 1.61 | 0.007 |
| <2 | 92.53 | 30.72 | – | 1.94 | 0.72 | – |
| NVC scleroderma pattern |
| Late | 120.22 | 46.19 | 0.032 | 3.13 | 1.88 | 0.013 |
| Others | 95.99 | 30.81 | – | 2.12 | 1.00 | – |

Data are expressed as mean (±SD). Significant differences (p<0.05) are reported as:

* Between groups restricted and iDLco.
+ Between groups restricted and normals.
† Between groups iDLco and normals.

DLco, diffusion lung capacity for carbon monoxide; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; %LDA, per cent low-density area; LV, lung volume; MLA, mean lung attenuation; PVV, pulmonary vascular volume; RV, residual volume; %TH, per cent true hyperlucent; TLC, total lung capacity; VC, vital capacity.

were significantly higher in restricted than in iDLco and normal, while no differences in ILD patterns were found between the last two groups. No differences were found between groups in terms of LDA and TH. Conversely, all three groups had significant differences in lung volume as quantified on spirometry-gated CT (LV) and in vascular volumes (PVV and PVV/LV).

Table 5 | Differences in quantitative CT parameters at diverse cut-off percentages of forced vital capacity (FVC) and diffusion capacity for carbon monoxide (DLco) |
|--------------------------------------|----|----|----|--------------------------------------|----|----|----|
| FVC <80% | FVC≥80% |
| Mean | SD | P value | Mean | SD | P value |
| %Normal | 82.7 (9.6) | 86.2 (14.7) | – | 86.6 (12.7) | 80.8 (15.8) | – |
| %Ground glass | 10.3 (8.9) | 2.4 (3.9) | – | 5.0 (6.7) | 3.9 (6.9) | – |
| %Reticular | 2.9 (2.9) | 0.8 (1.3) | – | 1.6 (2.1) | 0.7 (0.9) | – |
| %Honeycomb | 0.4 (0.6) | 0.1 (0.1) | – | 0.2 (0.3) | 0.1 (0.1) | – |
| %TH | 3.6 (6.8) | 8.9 (12.1) | – | 5.4 (8.8) | 14.1 (15.4) | 0.050 |
| %LDA | 1.2 (8.8) | 5.5 (6.8) | – | 4.1 (6.1) | 4.9 (6.9) | – |
| MLA (HU) | −793.6 (35.8) | −849.7 (30.3) | – | −833.2 (36.2) | −843.3 (46.7) | – |
| PVV | 125.6 (39.1) | 90.9 (26.9) | – | 101.9 (34.8) | 84.7 (19.4) | 0.016 |
| PVV/LV | 3.8 (1.6) | 2.0 (0.7) | – | 2.5 (1.3) | 1.7 (0.6) | 0.002 |

Data are expressed as mean (±SD). P values are reported only if significant (p<0.05).

%LDA, per cent low-density area; LV, lung volume; MLA, mean lung attenuation; PVV, pulmonary vascular volume; TH, per cent true hyperlucent; TLC, total lung capacity.
located in the middle third. Meanwhile, the reticular pattern was significantly higher in the peripheral regions, the PVV/LV was significantly higher in the central regions (both p<0.001) and this was true for each lung third. A smaller association between PVV/LV and ILD_Ext was found in the lower third in respect to the other two-thirds (interaction slope: −0.0006; 95% CI −0.001 to −0.0001; p=0.013; see online supplementary figure 2). It follows that an increase in ILD_Ext in the lower third was coupled by a smaller increase in PVV/LV than in the upper and middle thirds.

DISCUSSION
Our data clearly demonstrate that patients with SSc have different patterns of pulmonary vasculature changes, as shown by a non-invasive fully automated analysis of spirometry-gated chest CT scans. In particular, PVV was significantly increased in patients with DLco% reduction. The increase in PVV was even higher in patients presenting with a restrictive pattern at spirometry. Furthermore, an increase in ILD_Ext in the lower third was coupled by a smaller increase in PVV/LV than in the upper and middle thirds.

In clinical practice, it is well known that patients with SSc, in relation to the stage of the disease, may have a heterogeneous spectrum of gas exchange and pulmonary function patterns, including normal, isolated DLco reduction, restrictive and obstructive patterns. In the current study, we found 35%–54% patients with iDLco according to the cut-off of DLco reduction used, around the upper limit of the range reported in literature (2%–40%, according to the cut-off used). In agreement with a previous study, no significant emphysema at CT was found in our patients cohort, as quantitatively assessed. Thus, the reduction in DLco detected may be ascribed to the vascular changes that characterise the disease. Vascular changes underlying DLco reduction have been documented at autopsy studies.

Figure 1  Three-dimensional volume rendering of pulmonary vasculature in three patients with SSc representative for each group of functional presentation. Pulmonary vessel volume increases progressively from normals to isolated diffusing capacity for carbon monoxide (iDLco) and restricted groups. Images were generated by using Horos V.3.3.5 on Imbio LTA data.

Figure 2  Distribution of pulmonary vascular volume (PVV) normalised on lung volume (LV) and interstitial lung disease (ILD) patterns across lung thirds (A) and between central and peripheral regions (B). The increase in reticular pattern towards lung bases is not coupled by a parallel increase in PVV/LV, with PVV/LV mainly located in the middle third. Note the significant differences in distribution of PVV/LV and reticular pattern between central and peripheral regions. ILD patterns and PVV are normalised for the volume of the corresponding third. ground-glass/lung volume (GG/LV) is not shown in panel B to emphasise the contraposition between reticular and PPV/LV.
In 1978, Young and Mark found intimal and medial hyperplasia affecting pulmonary arteries of all sizes, with arteries of different diameter affected to a comparable degree. Most patients (82%) had moderate to marked arterial disease. On lung biopsies, despite differences in fibrosis severity, the vascular distribution of the lesions in idiopathic pulmonary fibrosis (IPF) and SSC is very similar and characterised by ablation of vessels in fibrotic lung portions and increased distance between vessels and airspaces. However, biopsies results refer only to the limited amount of removed fibrotic tissue, whereas correlations between structure and function are necessarily compelled by regional variations in disease activity and severity.

Recent technological advances in CT image post processing have led to the development of non-invasive and fully automated quantitative imaging tools to analyse the whole pulmonary vasculature without contrast media and repeated ionising exposure during injection. In a recent study, it has been shown that in patients with SSC, DLco is associated with CT changes of pulmonary vascular morphology. In particular, DLco reduction was associated with biomarkers quantifying the dilatation of proximal vessels and the number of capillaries. Another advanced tool (CALIPER) enabled the quantification of PVV in IPF, showing significant negative correlation with DLco. In the present study, we used the same tool to quantify PVV in patients with SSC to better define differences in pulmonary vasculature in relation to different gas exchange and functional presentation. We found PVV to be increased both in iDLco and restricted groups in comparison to the normal group. The observed increase in PVV confirms, even if of lower degree, that observed in IPF in a previous study (PVV/LV = 5%). It has been hypothesised that the PVV signal in IPF could represent a misclassified reticular pattern. Our results are not supportive of this hypothesis. Indeed, the different distribution of PVV/LV and reticular pattern across lung thirds, with the former mainly located in the middle third and in central regions and the latter in the lower third and in peripheral regions, corresponds to our knowledge of distribution of ILD patterns in patients with SSC at qualitative analysis of chest CT scans and with those studies demonstrating the unaffected mechanisms of redistribution of perfusion to less affected thirds. Furthermore, in patients with iDLco, we observed a significant increase in PVV with respect to normal, despite a not significant increase in reticular pattern.

In our patients, the increase in PVV paralleled by the decrease in DLco could be explained by the redistribution of perfusion to well-ventilated lung regions or a concomitant aberrant angiogenesis, as demonstrated in IPF. Unlike IPF, the mechanisms of redistribution of perfusion are unimpaired in patients with SSC, as demonstrated both functionally and radiologically on magnetic resonance imaging. However, DLco may not be preserved when reduction in patency of some pulmonary vessels coexist with a compensatory dilatation of the unaffected vasculature. Disproportionate reduction in membrane diffusivity (DM) relative to capillary blood volume (Vc) may occur due to modifications of vascular geometry. Similarly to IPF, the increase in PVV could also be due to a concomitant aberrant angiogenesis. Indeed, patients with SSC are more prone to angiogenesis than control subjects as they have higher levels of circulating angiogenic factors (eg, vascular endothelial growth factor), cutaneous vessel formation, and an increased rate of endothelial cell proliferation in biopsied lung portions. Nonetheless, the increased rate of endothelial proliferation found in lung biopsies of patients with SSC would not be necessarily caused by new vessel formation, but instead it could be an expression of increased vascular turnover as a response to an ongoing insult. Thus, the increased rate of endothelial proliferation may not exclude a mere redistribution of lung perfusion coupled with an accelerated vascular turnover following the chronic vascular insult. The CT-derived parameter PVV cannot be deterministic to solve this issue. PVV is not a specific indicator of new vessels formation and it cannot distinguish the arterial wall from its content and, consequently, arterial wall thickening from increased blood volume. However, the analysis of regional distribution of PVV/LV and reticular pattern could help in the understanding of this pathophysiological aspect. The opposed distribution of PVV/LV and reticular pattern between central and peripheral regions across lung thirds may support the hypothesis of redistribution of blood flow in the regions and thirds less affected by ILD, where vessels can still maintain their ability to be recruited. The smaller association found between PVV/LV and ILD Ex in the lower third in respect to the other two thirds may also support this hypothesis.

In IPF, PVV is a predictive biomarker of mortality and its prognostic role in SSC needs further investigation in longitudinal studies, especially to better characterise CT subgroups with different prognosis within the iDLco group, as previously observed in a clinical study. Similarly to data reported in the literature, this group is characterised by an increase in the FVC/DLco ratio in comparison to both restricted and normal groups. Although an FVC/DLco ratio greater than 1.4 was found to be the best predictor for isolated PAH, only some patients with iDLco develop PAH and the remaining have a better prognosis than those presenting with a restrictive functional pattern. Thorough evaluation of PVV in a larger iDLco group may help stratifying subgroups with different prognosis. Nonetheless, DLco (silent in 30% of cases with lung fibrosis) has a lower sensitivity than diffusing lung capacity for nitric oxide (DLno) in detecting diffusion limitation. This may hamper our ability to define the group of patients with iDLco, being many of them possibly defined as ‘normal’. Future studies using DLno instead of DLco to assess diffusing capacity might improve the definition of the functional presentation of patients with SSC and therefore their prognosis.

Our study has some limitations. First, the cross-sectional nature precluded the opportunity of assessing longitudinal changes in PVV among groups of patients and its prognostic role. A longitudinal analysis might provide important information to better understand the clinical relevance of vascular changes characteristics in patients with SSC. Second, PVV cannot distinguish pulmonary arteries from veins. A more accurate investigation with further software development would be a relevant step forward in the pathophysiological study of vascular changes in SSC. Third, we did not enrol all patients with SSC presenting to our institution, but only those undergoing a chest CT scan for the evaluation or detection of ILD. This approach could have excluded patients with silent initial pulmonary vascular changes. Finally, spirometry gating at CT is time consuming and far to be implemented in the high flux of CT examinations of the routine radiological practice. The spirometry-gated approach was adopted in the present study for research purposes to avoid bias in quantification of lung patterns of disease. Moreover, no relevant improvement in correlation values was observed in respect to a previous study performed without gating.

In conclusion, our results have disclosed that patients with SSC have an increased PVV/LV, as assessed on CT scan, paralleled by a decrease in DLco. This increase in PVV/LV was even more pronounced when DLco reduction was coupled with FVC decrease. The PVV/LV was the only independent predictor of DLco at the multivariate analysis and its distribution was

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different from that of reticular pattern. The associations found across lung thirds between PVV/LV and ILD extent may support the hypothesis of a redistribution of pulmonary flow in thirds less affected by ILD. Further studies in larger multicentric cohorts are warranted to confirm these results and to assess the individual contribution of arterial and venous pulmonary vessels to the pathophysiology of SSC vascular involvement. Further advances in this field may help in stratifying patients to tailor different therapeutic approaches according to the severity and the distribution of pulmonary vascular changes.

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**Data availability statement**
Data are available upon reasonable request. All data relevant to the study are included in the article or uploaded as supplementary data. Data are available from the Ethics Committee for researchers who meet the criteria for access to confidential data. Please refer to the corresponding author.

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