

## Original article

## Lung vascular changes as biomarkers of severity in systemic sclerosis–associated interstitial lung disease

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## Abstract

**Objectives.** It has recently become possible to assess lung vascular and parenchymal changes quantitatively in thoracic CT images using automated software tools. We investigated the vessel parameters of patients with SSc, quantified by CT imaging, and correlated them with interstitial lung disease (ILD) features.

**Methods.** SSc patients undergoing standard of care pulmonary function testing and CT evaluation were retrospectively evaluated. CT images were analysed for ILD patterns and total pulmonary vascular volume (PVV) extents with Imbio lung texture analysis. Vascular analysis (volumes, numbers and densities of vessels, separating arteries and veins) was performed with an in-house developed software. A threshold of 5% ILD extent was chosen to define the presence of ILD, and commonly used cut-offs of lung function were adopted.

**Results.** A total of 79 patients [52 women, 40 ILD, mean age 56.2 (s.d. 14.2) years, total ILD extent 9.5 (10.7)%, PVV/lung volume % 2.8%] were enrolled. Vascular parameters for total and separated PVV significantly correlated with functional parameters and ILD pattern extents. SSc-associated ILD (SSc-ILD) patients presented with an increased number and volume of arterial vessels, in particular those between 2 and 4 mm of diameter, and with a higher density of arteries and veins of <6 mm in diameter. Considering radiological and functional criteria concomitantly, as well as the descriptive trends from the longitudinal evaluations, the normalized PVVs, vessel numbers and densities increased progressively with the increase/worsening of ILD extent and functional impairment.

**Conclusion.** In SSc patients CT vessel parameters increase in parallel with ILD extent and functional impairment, and may represent a biomarker of SSc-ILD severity.

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**Key words:** systemic sclerosis, interstitial lung disease, lung vessels

### Rheumatology key messages

- Vascular volume increases with the severity of interstitial lung disease extent.
- Increasing number of small vessels, mainly arteries, occurs with pulmonary functional decline.
- Lung vessels may represent a target outcome measure in interstitial lung diseases.

## Introduction

SSc is a connective tissue disorder characterized by inflammation, vasculopathy and fibrotic processes [1, 2], progressively occurring in the lung either as interstitial lung disease (ILD) [3] and/or pulmonary arterial hypertension (PAH) [4]. Clinically, the cornerstones in evaluating lung involvement are CT and pulmonary function testing, particularly including forced vital capacity (FVC) and diffusing capacity for carbon monoxide (DLco). These are commonly used to assess the functional impairment and severity of the disease, and its progression and prognosis [5, 6]. An accurate measurement of pulmonary artery pressure with invasive right heart catheterization is limited to cases suspected of pulmonary hypertension (PH) [7, 8].

Recently, post-processing of CT images by means of dedicated software has shown promising results in the non-invasive investigation of pulmonary vessels [9–11]. In SSc-associated ILD (SSc-ILD) patients, this approach has identified a link between parenchymal and vascular changes and a reduced vascular volume in regions with higher ILD extent [12]. Further advancements in automated software programs have allowed not only the quantification of pulmonary vessels but also the separation between arteries and veins [10, 11]. While the separation of arteries and veins did not show a significant value in patients with idiopathic pulmonary fibrosis (IPF), this analysis opens a promising scenario in SSc patients affected by vasculopathy of the pulmonary vessels.

Following our previous results showing increased pulmonary vascular volume (PVV) in patients with SSc-ILD, we hypothesized that numbers of vessels increase in parallel with the worsening of ILD extent. Therefore, in this study we evaluated the characteristics of the pulmonary vasculature on CT images of SSc patients with an automated software program separating arteries from veins. Moreover, we quantified each component and identified the precise location of the vessel involvement in the lungs. Finally, the relationship between the functional impairment, the vessels parameters and the ILD features was analysed.

## Methods

This two-centre, retrospective, cross-sectional and longitudinal study was conducted in compliance with the

declaration of Helsinki and approved by the local Ethics Committees of the University of Florence (CEAVC protocol 12300\_oss) and the Catholic University of Sacred Heart in Rome (Comitato Etico Fondazione Policlinico Universitario “Agostino Gemelli”). All participants gave written informed consent in Florence, whereas it was waived for this retrospective evaluation in Rome. For this study, we selected SSc patients classified according to ACR/EULAR 2013 criteria [13], who underwent thoracic CT for the evaluation or detection of ILD and pulmonary function evaluation within 1 week from CT. Patients whose CT scan parameters did not fulfil the technical requirements needed for quantitative analysis were excluded.

### Clinical and functional evaluation

For each patient demographics, laboratory, clinical [diffuse or limited cutaneous scleroderma subsets, modified Rodnan skin score (mRSS) [14], right heart catheterization proven pre-capillary PH [4]], lung [FVC%, total lung capacity (TLC%), DLco%, DLco/alveolar volume (DLco/VA%)] and cardiac [estimated systolic pulmonary arterial pressure on echocardiography (sPAP)] functional parameters were collected, using the most recent data before the CT imaging.

In line with previous publications, an 80% predicted cut-off value for both FVC% and DLco% was used to differentiate normal (FVC%  $\geq$ 80), isolated DLco% reduction (iDLco, FVC%  $\geq$ 80 and DLco%  $<$ 80) or restricted (FVC%  $<$ 80) patients [15, 16]. Moreover, we arbitrarily chose a 5% total ILD extent (ILD\_EXT) cut-off to distinguish between patients with and without ILD, as recently proposed [17]. According to the combination of radiological and functional cut-offs, patients were divided into four clusters: ILD/restricted (ILD\_EXT  $\geq$ 5%, FVC%  $<$ 80), ILD/iDLco (ILD\_EXT  $\geq$ 5%, FVC%  $\geq$ 80 and DLco%  $<$ 80), non-ILD/iDLco (ILD\_EXT  $<$ 5%, FVC%  $\geq$ 80 and DLco%  $<$ 80) and non-ILD/normal (ILD\_EXT  $<$ 5%, FVC%  $\geq$ 80).

### Radiological evaluation

Scans were acquired on the same CT scanner in each Institution (in Florence: SIEMENS Sensation 16, Erlangen, Germany; in Rome: Philips Brilliance 64, Philips Medical Systems) with the following fixed protocol: 120 kV, slice thickness  $\leq$ 1 mm, 0.5 s rotation time, pitch 1.0. CT images were acquired and processed as

previously reported [12]. Images with standard reconstruction algorithm were post-processed using two automated software programs:

- i. Imbio lung texture analysis (LTA), based on CALIPER software (Mayo Clinic, Rochester, MN, USA), a CE-approved computational platform for the near-real-time characterization and quantification of lung parenchyma patterns on CT scans. Imbio LTA automatically quantified lung patterns, including normal lung, hyperlucencies, ground-glass opacities (GGO), reticular pattern (RET) and honeycombing pattern (HC). The sum of GGO, RET and HC constituted *ILD\_EXT*;
- ii. the lung vascular software developed at the Ludwig Boltzmann Institute for Lung Vascular Research (Graz, Austria), a novel quantitative tool for dedicated assessment of pulmonary vasculature with diameters between 2 and 10 mm, performing a fully automated separation of the pulmonary vascular tree into arteries and veins [10] (details in [Supplementary Data S1](#), available at *Rheumatology* online). The quantitative readouts included the number of vessel segments in total and in certain diameter bins, the vessel density [number of vessel segments per lung volume (LV)], the total volume of the detected vessels normalized to the LV, and the tortuosity of the vessel segments as assessed by DM method. Detection of vessels was independent of the detected lung parenchyma patterns. Validation of the artery/vein separation was performed in all scans by a thoracic radiologist (M.O.), as specified in [Supplementary Data S1](#) (available at *Rheumatology* online). Visual inspection yielded an estimation of the amount of erroneously labelled vessels; cases with wrong labelling >15% were excluded from the analysis of arteries and veins.

#### Data analysis

Statistical analysis was performed with SAS software version 9.3, considering a *P*-value <0.05 to be statistically significant. Absolute and relative frequencies were used to describe qualitative variables. Quantitative variables were presented as mean (s.d.). Spearman's correlation coefficient ( $\rho$ ) was used to correlate imaging with functional and clinical variables. To evaluate the distribution of continuous parameters within groups, normal distribution and homoscedasticity were tested using Shapiro–Wilk's test and Bartlett's test, respectively. To assess association between continuous parameters and groups of patients, ANOVA, Welch ANOVA or Kruskal–Wallis test were used according to the results of normal distribution and homoscedasticity test results. Only in cases where an association was statistically significant were pairwise comparisons performed between groups of patients using Tukey, Games–Howell or Dwass, Steel, Critchlow–Fligner test according to association test. To evaluate association between categorical variables,  $\chi^2$  or Fisher's test was used according to the frequencies of the table cells.

## Results

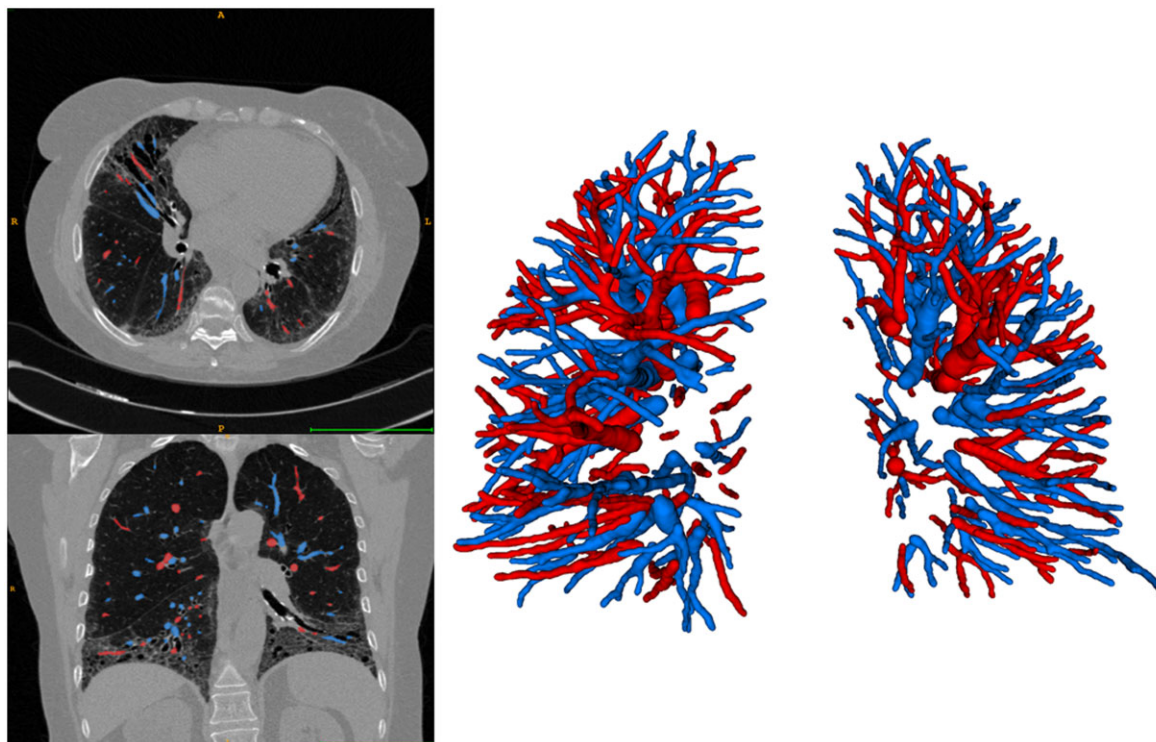
### Study population

We enrolled 105 patients with 120 sets of CT images for the analysis with Imbio LTA, of whom 21 represented follow-up evaluations. Five scans were excluded for failure in segmentation and/or mislabelling of arteries/veins >15%; 87/92 scans (including 13 follow-up evaluations) from 79 SSc patients were additionally analysed for the arteries/veins separation ([Supplementary Data S2](#), available at *Rheumatology* online, provides additional information on reasons for exclusion of CTs from the analysis). [Fig. 1](#) shows the separation between arteries and veins, as performed by the algorithm of lung vascular analysis. With a female predominance, patients had a high prevalence of diffuse cutaneous subset and anti-topoisomerase antibody positivity. Seven patients had also pre-capillary PH. They further had normal FVC% and TLC% [95.9 (22.3) and 91.9 (178), respectively], with a moderate impairment of DLco% [61.2 (19.9)] that turned out to be mild when adjusted for the VA [75.8 (23.1)] [18]. Mean *ILD\_EXT* was 9.5%, mostly represented by GGO. Further description of the cohort is listed in [Table 1](#). PVV/LV represented 2.8% of LV, with a mean of 269 vessels per litre. Arterial and venous components were similarly distributed in terms of volumes and tortuosity. Although the venous component had a lower number of vessels and density than the arterial counterpart, this difference was not statistically significant (see [supplementary Table S1](#), available at *Rheumatology* online).

### Correlations between vascular features, ILD patterns and clinical functional

[Supplementary Table S2](#) (available at *Rheumatology* online) reports the correlations between vascular features, ILD patterns and clinical-functional parameters. TLC%, FVC% and DLco were all inversely correlated to PVV/LV and vessel density and, to some extent, also to the number of vessels, considering both all vessels and the arterial and venous components separately. No significant correlation between sPAP and the vascular CT features was found (data not shown), whereas mRSS showed positive correlations with absolute and relative vascular volumes, number of vessels and vessel density for the total, and arterial and venous component.

Overall, *ILD\_EXT*, as well as GGO and RET, positively correlated with all vascular normalized volumes and densities. In addition, RET correlated with the number of vessels, and *ILD\_EXT* with number of separated vessels. Conversely, HC and hyperlucent extents negatively correlated with total and separate vascular features (total, arterial and venous absolute and normalized vessel volumes, vessel density and vessel numbers), as detailed in [supplementary Table S1](#) (available at *Rheumatology* online). Finally, the parameters describing vessel tortuosity correlated only with normal lung in a negative direction and positively with HC, for total and venous vessels. According to vessel size, most correlations were confirmed for the total, arterial and venous 2–4 mm diameter vessels only (see [supplementary Table S3](#), available at *Rheumatology* online).

**Fig. 1** CT scan of an SSc patient belonging to the ILD/restrictive subgroup

Transverse (top left) and coronal (bottom left) CT acquisition with vessels identification. The green scale bar at the bottom of the CT images is 10 cm long. Arteries are identified in blue and veins in red. On the right side, there is a 3D reconstruction of the arteries (red) and veins (blue). ILD: interstitial lung disease.

#### Vascular features in ILD vs non-ILD

According to the 5% ILD\_EXT cut-off, 40 (50.6%) patients were considered as complicated by ILD. As expected, SSc-ILD patients presented with a significant functional impairment, except for DLco/VA (see [Table 2](#) and [supplementary Table S4](#), available at *Rheumatology* online). A statistically significant difference was confirmed for all the normalized vessel volumes and vessel densities (for all vessels as well as separately for arteries and veins), possibly affected by the significantly lower LV in the SSc-ILD group. When considering absolute vessel volume and number of vessels, which are measures not affected by LV, the same results were confirmed for the arterial component only. In the SSc-ILD group, there was an increased number and volume of arterial vessels, in particular those between 2 and 4 mm of diameter, as well as a higher density of smaller vessels (<8 mm diameter) in both arterial and venous components. Vessel tortuosity was not different among groups.

#### Radiological and functional clustering

Combining the presence/absence of ILD with the functional classification, four groups were generated including 77 patients: ILD/restricted (22 patients, 28.6%), ILD/iDLco (18 patients, 22.2%), non-ILD/iDLco (25 patients, 32.5%) and non-ILD/normal (12 patients, 15.6%). Two

patients were excluded from this analysis: one presented restrictive functional involvement without ILD on CT, the other presented ILD but with normal functional data.

These groups were characterized by a progressive increase in extent of ILD-CT patterns and functional impairment from non-ILD/normals to ILD/restricted ([Fig. 2](#) left panel, [Table 3](#), [supplementary Table S5](#), available at *Rheumatology* online). Among the groups there were significant differences for normalized vessel volume and vessel density, progressively increasing from the non-ILD/normals to the ILD/iDLco group, then reaching a plateau or slightly reducing for the ILD/restricted group. Although not statistically significant, a numerical increase in the vessel count, in particular of those between 2 and 4 mm of diameter, was observed for total and separated vessels ([Fig. 2](#) right panel, [Table 3](#), [supplementary Table S5](#), available at *Rheumatology* online). No difference between the two functional groups was found, either between patients with ILD (restricted vs iDLco) or between those without ILD (iDLco vs normals). The increase in vessels in the non-ILD/iDLco group was significantly lower than in patients with ILD. Again, no difference in vessel tortuosity was detected. When we repeated the same analyses excluding patients with pre-capillary PH diagnosed by right heart catheterization, our results were confirmed ([supplementary Tables S6 and S7](#), available at *Rheumatology* online).

**TABLE 1** Clinical-functional characteristics and CT-derived quantitative parenchymal features of the SSc patients enrolled ( $n = 79$ )

Variable	
Female sex	52 (65.8%)
Age (years)	56.2 (14.2)
Disease duration (years)	9.7 (9.3)
Diffuse cutaneous skin subset	43 (54.4%)
RHC-PH	7 (8.9%)
sPAP (mmHg)	28.9 (9.5)
ANA positivity	79 (100%)
ACA/ATA/ARA/other autoantibodies positivity	14 (17.7%)/48 (60.8%)/3 (3.8%)/14 (18.5%)
mRSS	8.6 (8.3)
FVC%	95.9 (22.3)
TLC%	91.9 (17.8)
DLco%	61.2 (19.9)
DLco/VA%	75.8 (23.1)
Lung volume (cm <sup>3</sup> )	4340 (988)
Normal lung (%)	85.6 (12.3)
Hyperlucency (%)	5.4 (11.1)
GGO (%)	7.5 (9.3)
RET (%)	1.9 (2.1)
HC (%)	0.1 (0.3)
ILD extent (%)	9.5 (10.7)

Data represented as mean (S.D.) or  $n$  (%) for categorical variables. RHC-PH: right heart catheterization-proven pre-capillary pulmonary hypertension; sPAP: systolic pulmonary arterial pressure on echocardiography; ATA: anti-Topoisomerase I antibodies; ARA: anti-RNA polymerase III antibodies; mRSS: modified Rodnan Skin score; FVC: forced vital capacity; TLC: total lung capacity; DLco: lung diffusion for carbon monoxide; VA: alveolar volume; GGO: ground-glass opacity; RET: reticular pattern; HC: honeycombing; ILD: interstitial lung disease.

### Longitudinal evaluation

Thirteen patients had a second CT scan suitable for Imbio LTA analysis, 10 of them being also suitable for the artery/vein artery/vein separation, as presented in Table 4. After a mean follow-up of 24 months, ILD\_EXT numerically increased in 6/13 cases, decreased in 6/13 cases and remained stable in 1/13 cases. Patients with increase in ILD\_EXT showed more frequent increase in total vessels number (5/6) and density (4/6), as well as FVC% decline (4/6). Conversely, patients whose ILD\_EXT reduced over time also had a decrease in total vessels number (4/6) and density (6/6), as well as in PVV/LV (5/6). When available, the change in arteries and veins parameters showed the same tendency as the total vessel evaluation; none of the cases developed any form of PH during the follow-up and vessel tortuosity remained overall comparable (data not presented).

### Discussion

Our data show that, in SSc patients, the vascular parameters quantified on CT significantly correlate with the

ILD\_EXT and ILD patterns. In SSc patients with an ILD\_EXT >5% at CT, a higher number and a higher density of all vessels with diameters <6 mm than in patients with lower ILD\_EXT was found. Similar trends were found when the change in ILD\_EXT was observed over time. However, it is important to note that in restricted patients the increase in lung vessel parameters reached a plateau.

For several years, lung vasculature has been of interest in the pulmonary fibrosis research field [19]. Recent advances in technology have allowed an in-depth *in vivo* assessment of the pulmonary vasculature, providing qualitative and quantitative parameters. The vessel volume quantification at CT has a prognostic role in IPF and RA-related ILD, being a significant predictor of FVC decline and mortality [20–22]. In addition, a significant negative correlation of normalized vessel volume, vessel density and vessel heterogeneity parameters with FVC and DLco in IPF patients has been shown previously [9]. This is in line with our results in SSc that show an increase in the vascular components along with the levels of progressive and severe functional impairment. In addition to FVC and DLco, our analyses also showed a weak but statistically significant correlation for DLco/VA. Despite the predominance of vasculopathy in SSc, we did not identify any significant differences in parameters expressing vessel tortuosity in patients with PH as well as between patients with an evident ILD ( $\geq 5\%$  of total LV) and those without it. Previously, Helmberger *et al.* identified vessel tortuosity as a CT marker discriminating patients with PAH, using a relatively small sample of pre-capillary PH patients with mixed aetiologies, partially reflecting our current pre-capillary PH subgroup [23]. In line with the literature [4], a minority of patients in our study had pre-capillary PH ( $n = 7$ ), and evidence may support the role of vessel tortuosity as a biomarker of pure vasculopathy (as in the context of idiopathic and CTDs-related PH) and not of the vascular changes associated to the parenchymal lung involvement in pulmonary fibrosis (i.e. SSc-ILD and IPF). However, the low number of patients remains a limitation, which did not allow a powerful statistical analysis.

In SSc patients with ILD\_EXT  $\geq 5\%$ , a significantly higher vessel volume was found, in particular in smaller arteries below 4 mm of diameter. In the pathological slices derived from explanted or autoptoc lungs from SSc-ILD, different parameters of capillary proliferation were significantly increased in patients with usual interstitial pneumonia in comparison with those with non-specific interstitial pneumonia [24]. These data are in line with our CT evaluation, which found a significant correlation between all kind of vessel numbers and the extent of HC (typical of usual interstitial pneumonia pattern), with only a weak or absent correlation with the GGO (more prominent in non-specific interstitial pneumonia pattern). In addition, the same capillary proliferation parameters were also significantly increased in patients complicated by PAH vs non-PAH cases [24].

**TABLE 2** Distribution of parenchymal and vascular parameters among systemic sclerosis patients with and without interstitial lung disease

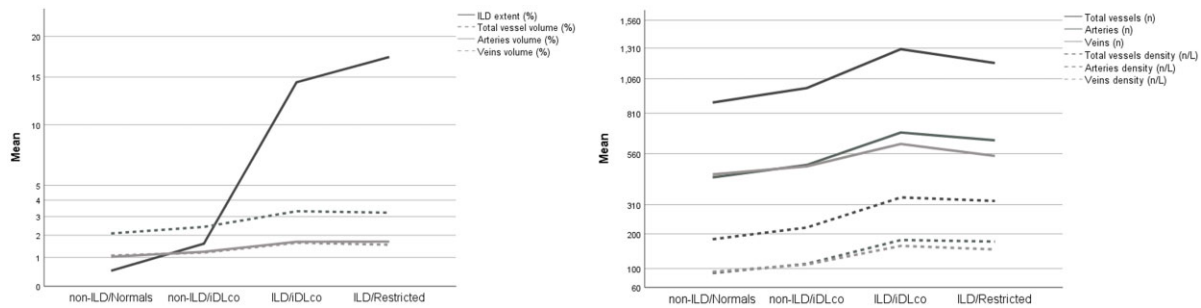
Number of patients (for analysis of arteries/veins)	ILD	Non-ILD	P-value
	n = 40 (37)	n = 39	
	Mean (s.d.)	Mean (s.d.)	
FVC (%p)	86 (23)	105 (18)	<0.001
DLCO (%p)	55 (18)	67 (21)	0.013
LV (cm <sup>3</sup> )	3861 (761)	4686 (1023)	<0.001
ILD extent (%)	17.3 (9.8)	1.3 (1.6)	<0.001
All vessels			
Volume (cm <sup>3</sup> )	122 (41)	109 (24)	—
PVV/LV (%)	3.22 (1.06)	2.39 (0.60)	<0.001
Number of vessels	1,214 (758)	973 (349)	—
Vessels density (n/l)	323 (208)	218 (99)	0.008
Density of 2–4 mm diameter vessels	277 (196)	185 (94)	0.012
Density of 4–6 mm diameter vessels	35 (12)	26 (6)	<0.001
Density of 6–8 mm diameter vessels	8 (4)	6 (2)	0.006
Density of 8–10 mm diameter vessels	1.8 (1.4)	1.3 (0.8)	—
Vessel tortuosity (DM method)	1.029 (0.004)	1.028 (0.003)	—
Arteries			
Volume (cm <sup>3</sup> )	64 (21)	55 (13)	0.028
PVV/LV (%)	1.67 (0.53)	1.19 (0.29)	<0.001
Number of vessels	650 (423)	485 (167)	0.032
Number of 2–4 mm diameter vessels	561 (405)	410 (161)	0.040
Vessels density (n/l)	173 (115)	108 (48)	0.003
Density of 2–4 mm diameter vessels	150 (92)	93 (46)	0.005
Density of 4–6 mm diameter vessels	18 (5)	13 (3)	<0.001
Density of 6–8 mm diameter vessels	4 (2)	3 (1)	0.001
Density of 8–10 mm diameter vessels	1.1 (1.1)	0.7 (0.6)	—
Vessel tortuosity (DM method)	1.030 (0.004)	1.030 (0.004)	—
Veins			
Volume (cm <sup>3</sup> )	59 (22)	55 (13)	—
PVV/LV (%)	1.56 (0.58)	1.19 (0.34)	0.001
Number of vessels	564 (346)	488 (189)	—
Vessels density (n/l)	150 (96)	109 (53)	0.027
Density of 2–4 mm diameter vessels	128 (88)	92 (49)	0.035
Density of 4–6 mm diameter vessels	18 (8)	13 (4)	0.003
Density of 6–8 mm diameter vessels	4 (2)	3 (2)	—
Density of 8–10 mm diameter vessels	0.8 (0.7)	0.6 (0.5)	—
Vessel tortuosity (DM method)	1.029 (0.004)	1.028 (0.003)	—

Patient numbers in brackets represent eligibility for arterial-veins separation analysis. Significant differences for P-value < 0.05. FVC: forced vital capacity; DLco: lung diffusion of carbon monoxide; ILD: interstitial lung disease; PVV: pulmonary vessel volume; LV: lung volume, %p: percent predicted.

As stated above, our study included a small PH sample, which we could not analyse as a separate cohort. As an indirect surrogate marker, we could not find any association between vascular CT markers and sPAP on echocardiography, which could also be explained by the non-sufficient sensitivity of this echocardiographic measure in defining patients with PAH [7]. Despite these limitations, our results may support the hypothesis that vessel recruitment and vessel dilatation occurs in SSc and likely in other fibrosing lung diseases. While capillary proliferation may represent an angiogenic effort, typically

of hypoxic conditions but invisible at CT due to spatial resolution, the increased number of small vessels >2mm detectable on CT may represent the combination of vessel recruitment and dilatation, still secondary to a hypoxic condition. In patients with SSc-ILD, our data show a significant increase of the number of arterial vessels of 2–4 mm in diameter which was not seen for any size of venous vessels. Indeed, these results support the nature of the vascular changes as being partially independent from the LV itself, which could be a confounding factor when considering normalized vessel volumes alone [12].

**Fig. 2** Distribution of vascular features among the radiological and functional groups of interstitial lung disease



Left panel includes volumes of total vessels, arteries and veins. Right panel shows number and density of total vessels, arteries and veins. ILD: interstitial lung disease; iDLco: isolated diffusing capacity for carbon monoxide.

**TABLE 3** Distribution of parenchymal and vascular parameters among the four radiological and functional clusters of SSc patients

Number of patients (for analysis of arteries/veins)	ILD/restricted n = 22 (20)	ILD/iDLco n = 18 (18)	Non-ILD/iDLco n = 25 (24)	Non-ILD/ normals n = 12 (12)	ANOVA (P)
	Mean (s.d.)	Mean (s.d.)	Mean (s.d.)	Mean (s.d.)	
FVC (%p)	66 (10)	103 (14)	106 (14)	112 (15)	<0.001
DLCO (%p)	58 (18)	52 (15)	56 (15)	89 (9)	<0.001
LV (cm <sup>3</sup> )	3662 (821)	4069 (528)	4651 (812)	5202 (1301)	<0.001
ILD extent (%)	19.3 (10.9)	14.4 (8.2)	1.8 (2.0)	0.5 (0.7)	<0.001
<b>All vessels</b>					
Volume (cm <sup>3</sup> )	118 (43)	128 (38)	110 (24)	105 (25)	—
PVV/LV (%)	3.2 (1.2)	3.3 (0.9)	2.4 (0.5)	2.1 (0.5)	<0.001
Number of vessels	1184 (791)	1301 (716)	989 (368)	884 (241)	—
Vessels density (n/l)	326 (221)	341 (196)	221 (88)	182 (77)	0.013
Density of 2–4 mm diameter vessels (n/l)	279 (204)	294 (188)	188 (84)	152 (72)	0.021
Density of 4–6 mm diameter vessels (n/l)	35 (14)	36 (10)	26 (6)	24 (6)	<0.001
Density of 6–8 mm diameter vessels (n/l)	8 (4)	8 (3)	6 (2)	5 (2)	0.005
Density of 8–10 mm diameter vessels (n/l)	2.2 (1.6)	1.6 (1.1)	1.3 (0.6)	1.1 (0.9)	0.016
Vessel tortuosity (DM method)	1.030 (0.003)	1.030 (0.004)	1.029 (0.003)	1.030 (0.004)	—
<b>Arteries</b>					
Volume (cm <sup>3</sup> )	62 (23)	66 (18)	56 (13)	52 (15)	—
PVV/LV (%)	1.7 (0.6)	1.7 (0.4)	1.2 (0.3)	1.0 (0.3)	<0.001
Number of vessels	638 (432)	685 (406)	498 (180)	434 (125)	—
Vessels density (n/l)	174 (118)	179 (110)	112 (44)	90 (40)	0.006
Density of 2–4 mm diameter vessels (n/l)	151 (112)	156 (106)	95 (42)	76 (38)	0.013
Density of 4–6 mm diameter vessels (n/l)	18 (6)	18 (4)	13 (3)	11 (3)	<0.001
Density of 6–8 mm diameter vessels (n/l)	4 (2)	4 (2)	3 (1)	2 (1)	0.003
Density of 8–10 mm diameter vessels (n/l)	1.5 (1.2)	0.7 (0.9)	0.8 (0.6)	0.5 (0.6)	0.013
Vessel tortuosity (DM method)	1.030 (0.003)	1.030 (0.004)	1.030 (0.003)	1.030 (0.004)	—
<b>Veins</b>					
Volume	57 (23)	64 (21)	54 (13)	54 (13)	—
PVV/LV (%)	1.5 (0.6)	1.6 (0.5)	1.2 (0.3)	1.1 (0.3)	0.002
Number of vessels	547 (373)	616 (324)	491 (194)	449 (122)	—
Vessels density (n/l)	151 (106)	161 (90)	109 (46)	92 (38)	0.037
Density of 2–4 mm diameter vessels (n/l)	128 (95)	138 (84)	93 (43)	76 (35)	0.048
Density of 4–6 mm diameter vessels (n/l)	17 (9)	18 (7)	13 (3)	13 (4)	0.009
Density of 6–8 mm diameter vessels (n/l)	4 (3)	4 (2)	3 (1)	3 (2)	—
Density of 8–10 mm diameter vessels (n/l)	0.8 (0.8)	0.9 (0.7)	0.5 (0.4)	0.5 (0.4)	—
Vessel tortuosity (DM method)	1.029 (0.004)	1.029 (0.005)	1.028 (0.003)	1.029 (0.002)	—

Patient numbers in brackets represent eligibility for artery/veins separation analysis. Significant differences for *P-value* < 0.05. FVC: forced vital capacity; DLco: lung diffusion of carbon oxide; ILD: interstitial lung disease; PVV: pulmonary vessel volume; LV: lung volume; %p: percent predicted.

**TABLE 4** Change over time of parenchymal, functional and vascular parameters in a subgroup of patients with longitudinal CT evaluations

Code	Sex	Age (years)	Subset	Disease duration (years)	Follow up duration (months)	ILD_EXT (%)			FVC (%)			Dlco (%)			LV (ml)			PVV/LV (%)			All vessels (n)			All vessels density (n/l)		
						BL	FUP	Trend	BL	FUP	Trend	BL	FUP	Trend	BL	FUP	Trend	BL	FUP	Trend	BL	FUP	Trend	BL	FUP	Trend
SSC02	Female	38	Diffuse	5	15	16	18	↑	64	63	↓	35	38	↑	3.613	3.764	↑	4.8	4.4	↓	1982	2051	↑	549	545	↓
SSC05	Female	44	Diffuse	5	36	26	3	↓	87	100	↑	69	50	↓	3.687	4.243	↑	4.9	3.9	↓	1866	1831	↓	506	432	↓
SSC07	Female	26	Diffuse	4	25	3	17	↑	70	68	↓	54	69	↑	3.361	2.744	↓	4.1	3.8	↓	1840	1271	↓	547	463	↓
SSC11	Female	57	Limited	5	42	17	0	↓	93	105	↑	27	44	↑	4.328	4.629	↑	4.0	3.3	↓	1859	1948	↑	430	421	↓
SSC14	Female	46	Diffuse	7	17	8	39	↑	98	85	↓	59	26	↓	3.438	3.595	↑	3.5	3.5	↓	991	1402	↑	288	390	↑
SSC19	Female	58	Limited	16	40	10	9	↓	78	78	=	54	59	↑	4.159	3.453	↓	4.4	3.1	↓	1472	976	↓	354	283	↓
SSC20	Female	45	Limited	19	23	2	9	↑	90	97	↑	78	50	↓	3.841	4.397	↑	3.7	3.9	↑	1240	1576	↑	323	358	↑
SSC21	Female	47	Diffuse	7	8	1	1	=	96	98	↑	50	67	↑	4.911	4.211	↓	2.2	2.9	↑	1034	1316	↑	211	313	↑
SSC23	Female	58	Diffuse	2	34	5	3	↓	123	119	↓	80	43	↓	3.492	4.480	↑	3.1	2.4	↓	990	962	↓	283	215	↓
SSC25	Female	66	Diffuse	16	10	43	13	↓	65	59	↓	48	36	↓	2.823	2.797	↓	3.5	3.7	↑	915	891	↓	324	319	↓
SSC26	Female	47	Diffuse	7	35	3	37	↑	73	71	↓	61	31	↓	3.694	2.646	↓	2.9	4.3	↑	1034	1202	↑	280	454	↑
SSC29	Female	70	Diffuse	3	11	19	3	↓	93	116	↑	26	47	↑	3.910	3.840	↓	3.6	2.2	↓	1948	459	↓	498	120	↓
SSC38	Female	52	Diffuse	12	16	0	23	↑	95	95	=	28	34	↑	4.304	3.808	↓	3.5	3.8	↑	1897	2081	↑	441	546	↑

BL: baseline; FUP: follow-up; FVC: forced vital capacity; DLco: lung diffusion of carbon monoxide; ILD: interstitial lung disease; PVV: pulmonary vessel volume; LV: lung volume.

When the CT evaluation was combined with the results of the functional tests, the population was divided into four groups according to the significant differences detected in vessels parameters. These differences were also numerically visible in a theoretical progressive pulmonary involvement, starting from the non-ILD/normal group to the ILD/iDLco group. The phenomenon of the 'increasing vascular component' was not seen when the ILD/iDLco was compared with the ILD/restricted group. This observation is in line with previous results from an SSc cohort with iDLCO but without ILD: there, CT parameters of vascular morphology were among the independent predictors of DLco [25]. An interesting 'plateau' effect was observed between the two groups of ILD (ILD/restricted and ILD-iDLco) with stable vascular volumes and numbers of vessels, paralleled by a significant reduction of LV and FVC%, as well as a significant increase in ILD\_EXT.

Our results suggest that vascular changes may not only develop in parallel with parenchymal lung alterations but may also precede them. Considering non-ILD/normals as a starting phase, when the first functional impairment appears (non-ILD/iDLco patients), an increase of the vascular parameters but not in the ILD\_EXT was observed. Thus, it may represent the initial vascular injury in the lung parenchyma. Then, when ILD becomes radiologically detectable (ILD/iDLco), the increase in ILD\_EXT and its patterns is paralleled by the increase of the vascular tree. Finally, the further increase of ILD\_EXT leaves no more space for vascular increase in both volume and number, with possible phenomena of saturated vessel recruitment and/or loss of angiogenesis, thus indicating a significant restrictive impairment (ILD/restricted). This hypothesis finds theoretical support in the cases with available longitudinal assessments. In patients whose ILD\_EXT increased over time, we observed a more frequent increase in most vascular parameters, in particular number and density of total vessels, which conversely decreased more frequently in the cases who showed a reduction of ILD\_EXT. This may also support the possible reversibility of vascular changes, in parallel with an improvement in the ILD parenchymal changes, possibly as an effect of therapeutic interventions.

Our study has some limitations. First, the small sample size, further reduced when clustering patients according to radiologic/functional impairment or presence of PAH. Second, the cross-sectional nature of the disease cannot represent the real evolution of lung involvement in SSc patients and the few longitudinal cases did not allow us to perform a proper statistical analysis. However, these results should encourage further studies on the radiological assessment of lung vasculature to ascertain whether quantitative imaging biomarkers may predict the onset of an evident ILD or disease progression, as already proposed in IPF. Third, there were no differences of tortuosity between ILD and non-ILD patients. This could be a technical artefact rather than a real finding, due to the lack of investigation of those smallest vessels with diameters below the spatial resolution of the CT

scans processed. Fourth, the two centres presented differences in terms of prevalence of ILD in their cohorts (67% in Rome, 44% in Florence), with also slight differences in slice thickness ranging from 0.75–1.00 mm in Florence to 0.80–1.00 mm in Rome. Although this might represent a bias in our study, we believe that combining the two cohorts has potentially increased the heterogeneity of our study population and made it more similar to the prevalence reported in the literature [25], as well as improved the reproducibility of the results to a broader spectrum of CT acquisition protocols. Moreover, the prevalence of the diffuse cutaneous subset may appear higher compared with the prevalence reported in the literature; this may be explained by the enrolment of patients with high suspicion for ILD onset of progression, which is more frequent in the diffuse subset and is still in line with the distribution of subsets between the SSc-ILD cohort, such as the recently published SENSICIS™ trial testing nintedanib versus placebo in SSc-ILD [17]. Further, the software for parenchymal and for vessel analysis were applied independently of each other. Thus some voxels may have been labelled as one of the parenchymal patterns as well as vessel. The restriction to clearly identifiable vessels with diameters 2–10 mm and the visual inspection of the vessel segmentation make us confident that this has only a minor influence on the results. Finally, we did not adjust our analysis for possible factors impacting on lung functionality, such as smoking, which also has not been shown to be a risk factor for SSc-ILD progression [26].

In conclusion, we show that in SSc patients the number and the density of lung vessels, quantified by CT, significantly correlate with ILD\_EXT and its main patterns. Moreover, SSc patients with an ILD\_EXT >5% showed a higher number and density of smaller size arterial and venous vessels. The increase of lung vessels reaches a plateau or even decreases in patients with a clear functional restriction, with vessel number and density representing candidate biomarkers of ILD change over time. Future longitudinal multicentre studies on a larger population are warranted to further understand the role of lung vasculopathy in SSc-ILD and to elucidate the role of imaging biomarkers predicting the early phases of ILD and its progression.

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## Data availability statement

The data underlying this article will be shared on reasonable request to the corresponding author.

## Supplementary data

[Supplementary data](#) are available at *Rheumatology* online.

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