

Pulmonary arterial hypertension in limited cutaneous systemic sclerosis: a distinctive vasculopathy

MJ Overbeek^{*}, MC Vonk[§], A Boonstra^{*}, AE Voskuyl[†], A Vonk-Noordegraaf^{*}, EF Smit^{*},
BAC Dijkmans[†], PE Postmus^{*}, WJ Mooi[‡], Y Heijdra^{||}, K Grünberg[‡]

Departments of ^{*}Pulmonary Diseases, [†]Rheumatology, [‡]Pathology; VU University Medical Center, Amsterdam, The Netherlands

Departments of [§]Rheumatology, ^{||}Pulmonary Diseases; Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

Name and Address for Correspondence:

Maria J. Overbeek

Department of Pulmonary Diseases

VU University Medical Center

De Boelelaan 1117

P.O. Box 7057

1007 MB Amsterdam, The Netherlands

e-mail: mj.overbeek@VUmc.nl

tel: +31-20-4443453

fax: +31-20-4444328

Short title: Vasculopathy in SScPAH

Abstract

Systemic sclerosis-associated pulmonary arterial hypertension (SScPAH) has worse prognosis and response to PAH-therapy than idiopathic PAH (IPAH). These differences are unexplained yet, but differences in pulmonary vasculopathy might provide a clue. However, knowledge concerning histologic pulmonary vasculopathy in SScPAH is limited, in contrast to IPAH. Therefore, we explore patterns of vasculopathy in SScPAH compared with IPAH.

Parameters of vasculopathy were assessed of lung tissue of 8 PAH patients with limited cutaneous SSc and 11 IPAH patients, obtained at autopsy (n=15), explantation (n=3) and biopsy (n=1).

Pulmonary arterial/arteriolar intimal fibrosis was identified in all SScPAH and in 3/11 IPAH patients ($p=0.003$); fibrosis of pulmonary veins/venules was found in all SScPAH and in 3/11 IPAH patients ($p=0.003$). In 4 SScPAH patients fibrosis of veins/venules was focal and associated with capillary congestion as in pulmonary veno-occlusive disease (PVOD). Ten out of 11 IPAH patients had unequivocal evidence of plexogenic arteriopathy compared with none of the SScPAH patients ($p=0.001$).

SScPAH is characterised by small vessel intimal fibrosis, which is associated with a PVOD-like pattern in some cases. This might explain its different clinical behaviour from IPAH. Small vessel intimal fibrosis may provide clues to elucidation of differences in pathogenetic mechanisms between the groups.

Keywords: intimal fibrosis; plexiform lesion; pulmonary arterial hypertension; pulmonary veno-occlusive disease; systemic sclerosis

Introduction

Systemic sclerosis is an autoimmune disease characterised by the deposition of excessive amounts of extra-cellular matrix components, dysfunction of endothelium and an altered immune tolerance(1). Patients with systemic sclerosis (SSc) are at risk of developing pulmonary arterial hypertension (PAH), with estimated prevalences between 8 and 12 % (2;3), which leads to right heart failure and death.

According to the Third World Symposium on Pulmonary Arterial Hypertension in Venice, 2003, pulmonary hypertension (PH) in patients with SSc is classified in the PAH category, and subclassified in the category of PAH associated with collagen vascular diseases(4). This categorisation is based on similarities of clinical presentation and therapy response. However, differences in clinical behaviour between SSscPAH and other forms of PAH have been noted, and have not been explained so far. First, in various reports SSscPAH has a worse prognosis than idiopathic PAH (IPAH) (5;6). Second, patients with SSscPAH present with lower values of the transfer factor of the lung for carbon monoxide (TLCO) than do patients with IPAH(7;8). Third, although responses to current PAH therapy have been reported effective in SSscPAH(9;10), some have reported less favourable responses as compared with IPAH(11-13). These findings suggest that differences might at least in part be related to differences in associated vascular lesions. However, knowledge of SSscPAH vascular morphology has remained limited, inherent to the rareness of the disease (14-18).

In this study we further explore the histopathologic characteristics of pulmonary vasculopathy in a series of patients with clinically well-defined limited cutaneous SSc with documented PAH, and compare these to plexogenic arteriopathy in IPAH. IPAH is the most common representative of the 2003 WHO-classification PAH group (17), and has a well described and homogeneous vascular morphology, with some similar features that have been reported in SSscPAH as well, such as concentric laminar intimal fibrosis and plexiform

lesions(19-21). As clinical differences between the IPAH and SScPAH groups are well known, emerging differences in patterns of vascular lesions produced by these two entities may point to differences in pathogenesis and pathophysiology between the two.

Material and Methods

Case selection and review

Cases were identified from the departments of pulmonary diseases and rheumatology of the VU University medical center, Amsterdam and the departments of rheumatology and pulmonary diseases of the Radboud University Nijmegen medical center, Nijmegen, both in The Netherlands. The study was approved by the Institutional Review Board on Research Involving Human Subjects of the VU University Medical Center.

Patients were included if pulmonary arterial hypertension was diagnosed by means of right heart catheterisation (RHC) (mean resting pulmonary arterial pressure (mPpa) ≥ 25 mmHg and a pulmonary capillary wedge pressure < 15 mmHg). From two patients with IPAH we did not have RHC data, as one patient refused a heart catheterisation, and another patient had a RHC in another hospital, but the results were not retrieved. At echocardiography, both had an elevated systolic Ppa (100 mmHg and 75 mmHg respectively) without left atrial (LA) or left ventricular (LV) abnormalities. SSc cases were excluded if restrictive lung disease was present, as indicated by total lung capacity as percentage of predicted (TLC%) $< 70\%$, vital capacity (VC%) $< 70\%$ and/or severe fibrosis on HRCT scan. SSc subset classification(22;23), SSc disease duration and antibody profile were assessed.

For histologic investigation paraffin-embedded lung tissue samples from 8 SScPAH patients and 11 IPAH patients were examined. Lung tissue was obtained at autopsy (n=15), open lung biopsy (n=1) or at lung transplantation, which had in all cases been performed because of deterioration of PAH (n=3) (see Table 3). Histological sections were stained with haematoxylin and eosin (H&E) and Elastica von Gieson's (EvG). At least four blocks per patient were studied. Evaluation was done using a standard semi-quantitative scoring sheet. The scoring of vascular morphology was performed by a histopathologist experienced in assessing pulmonary vascular histopathology (KG). All scoring of cases was assessed within

a 2-week period, in a randomised order, and the observer was blinded to the clinical diagnosis. Review of all cases took place by both contributing pathologists, both blinded to the clinical information, and resulted in full agreement on specific vascular lesions (notably, PVOD-like pattern, concentric laminar intimal fibrosis, plexiform lesions).

Scoring of histomorphology

The lung tissue samples were evaluated for vasculopathy according to the consensus on assessing vasculopathy established at the Third World Symposium on pulmonary hypertension(24) and according to the descriptions by Wagenvoort and Mooi(20;25).

Media hyperplasia, intimal fibrosis and adventitial fibrosis were scored with reference to the type of vessel and microanatomical localization. The same was done for plexiform lesions, post-thrombotic lesions, concentric laminar intimal fibrosis and vasculitis. Plexiform lesions were identified as arterial lesions consisting of a plexus of slit-like channels enclosed within or in continuity with a dilated segment of the affected artery(20). Concentric laminar intimal fibrosis was identified on H&E stainings, based on an onionskin-type of arrangement; care was taken not to mingle this configuration with the development of a new muscle coat within a layer of intimal fibrosis just around the narrowed vessel lumen(20), or non-concentric lamellar intimal fibrosis by loose connective tissue. PVOD and capillary hemangiomatosis in our analysis was considered as one disease entity based on the clinical and morphologic similarities(26). Vasculitis/vascular inflammation was defined as transmural infiltration of leucocytes (specified for type).

In addition to scoring vascular pathology, pulmonary interstitial fibrosis was scored and graded as none, mild (some interstitial fibrosis), moderate (architectural changes) and severe (honeycombing). Pneumonitis was described as none, sparse interstitial aggregates, mild

diffuse interstitial infiltrates without fibrosis, moderate diffuse interstitial infiltrates, diffuse interstitial infiltrates and fibrosis and infiltrates confined to fibrotic areas.

Statistics

Mann-Whitney U test was performed to compare differences in hemodynamic and respiratory parameters differences between the groups. Results are reported as median (range). Fisher's exact test was used for comparison of vascular histopathological parameters between groups. A p value < 0.05 was considered statistically significant. All analyses were performed with SPSS (version 12.0; SPSS; Chicago, IL).

Results

Demographics

Age was not significantly different between the groups (SScPAH 52 (range 32-60) vs. IPAH 47 (range 23-59) years, $p = 0.90$), nor was gender ($p = 0.63$). Survival did not differ between the groups (SScPAH 0.75 (range 0.08-7) vs. IPAH 2.75 (range 0.08-9) years, $p = 0.27$).

Characteristics of SSc patients are listed in Table 1. All SSc patients fulfilled the preliminary ACR classification criteria for SSc and were classified as limited cutaneous SSc (LcSSc) (22;23). Mean disease duration from first non-Raynaud symptom of SSc to the time of PAH diagnosis was 10 years (median 8, range 0.5-34 years). Of the autopsied patients, 14 had died of right ventricular failure, and one SScPAH patient had died of hypovolumic shock due to iatrogenic bleeding (case 6). Three SSc patients demonstrated pericardial effusion at PAH diagnosis, compared with none of the IPAH patients. Macroscopic cardiac abnormalities at autopsy consisted mainly of RV hypertrophy and dilatation. One of the 3 patients who underwent lung transplantation died post-operatively. In 1 SScPAH patient and 1 IPAH patient, macroscopic pulmonary emboli in the large vessels were found at post mortem examination. From 5 of the SScPAH patients and 5 of the IPAH patients, data on reversibility (27) were acquired; none of the SScPAH and 3 of the 5 IPAH patients responded upon vasodilator therapy. At time of death, most of SScPAH and IPAH patients were being treated with prostacycline therapy (table 2).

Clinical cardiopulmonary parameters

Haemodynamic and pulmonary function parameters are listed in Table 2. Values of cardiac index and pulmonary vascular resistance did not significantly differ, whereas mean Ppa

values tended to be lower in the SScPAH group as compared to IPAH. No correlations with SSc characteristics such as autoantibody profile and haemodynamic values were found.

Vasculopathy in SScPAH

Parameters of vascular morphology for individual cases are outlined in Table 3. Each single SScPAH case demonstrated a spectrum of vasculopathic parameters. Several characteristic features were found in the majority of the SScPAH patients.

Some degree of intimal fibrosis was found in the arterial tree at all levels in all patients. Notably, in all SScPAH cases, intimal fibrosis involving the small vessels at the level of alveolar parenchyma was observed (Figure 1 A, B and C). It should be noted that pulmonary arterioles and venules cannot be distinguished by the morphology of their walls: both consist of a layer of endothelium resting directly on a single elastic lamina. In many such small vessels, no connection to a larger, morphologically recognizable artery or vein was evident(20). The pattern of intimal fibrosis of the small vessels dominated the overall picture of vascular morphology in 4 cases. In 4 SScPAH cases, the small vessel intimal fibrosis was associated with a pattern of pulmonary veno-occlusive disease (PVOD) (Figure 2A, B and C). This pattern includes patchy intense capillary congestion in the alveolar parenchyma, and obliterative intimal fibrosis of small veins and venules. In 3 of these cases deposition of iron salts on elastin fibers as well as haemosiderin-laden macrophages could be observed, also fitting in with capillary congestion and occult alveolar haemorrhage (see figure 2B and C) (28). In one case, a giant cell reaction to iron-encrusted elastin fibers was seen (figure 2B). In 3 of the 4 cases, this PVOD-like pattern was quite prominent, and hence designated the predominant pattern. It should be noted that such PVOD-like pattern is found in the setting of a heterogeneous picture including extensive intimal fibrosis, setting this picture apart from idiopathic PVOD. Intimal fibrosis of venules was observed in 7 of 8 SScPAH cases. These

cases lacked the more diffuse congestion, interstitial fibrosis and fibrotic thickening of the walls of the large veins, indicative of congestive vasculopathy as seen for example in left heart failure or mitral valve insufficiency. In addition, there was no evidence of left-sided heart disease in the medical records including the autopsy report.

Careful scrutiny of all available slides of the SScPAH patient group failed to reveal a single lesion as convincing example of a plexiform lesion. The one lesion most closely resembling it is shown in figure 3A. Some dilatation lesions and slit-like channels are identified in this lesion, but it lacked the crowded endothelium with dark nuclei lining characterising plexiform lesions. It seems likely that the lesion represents a remnant of an organised thrombus.

Other vascular lesions in SScPAH cases included concentric laminar intimal fibrosis, observed in 3 of the 8 SScPAH cases (Figure 4A and B). Concentric laminar intimal fibrosis was observed in the bronchiolar axial arteries and in one case also in the small parenchymal vessels. In one SScPAH case, this feature dominated other vasculopathic lesions. Loose concentric arterial intimal fibrosis was seen in 7 of 8 SScPAH cases, in all of these cases in the small vessels, in 5 patients in veins and venules and in 3 patients in the bronchial and bronchiolar muscular arteries. In none of the patients a pattern typically of hypoxic vasculopathy (20) was observed.

Vasculopathy in SScPAH vs. IPAH

When comparing the above-described features of the SScPAH cases with those characterising IPAH cases, some distinctions could be made. First, in general the intra-individual variety in SScPAH sections contrasted with the more homogenous aspect of vasculopathic features in IPAH sections, the vast majority of the latter demonstrating a picture of plexogenic arteriopathy. The absence of plexiform lesions in SScPAH also distinguished

this group from the IPAH group, which in 10 of 11 cases revealed plexiform lesions ($p = 0.001$) (Figure 3B). None of the IPAH patients showed concentric laminar intimal fibrosis. Four IPAH cases did show diffuse hyperaemia. In two of these cases, this could be related to vasodilatation, as they had been treated with prostacycline. Second, the involvement of intimal fibrosis in the small vessels in SSscPAH appeared to be a diacritical feature of the two groups as this was only found in 3 of 11 IPAH patients ($p = 0.003$). Again, in contrast with SSscPAH, in none of these a PVOD-like pattern could be detected ($p = 0.02$). When analysis was confined to SSscPAH patients who did not demonstrate PVOD-like lesions, differences between these four patients and the PAH group remained significant concerning the absence of plexiform lesions and the higher prevalence of small vessel intimal fibrosis ($p = 0.004$ and $p = 0.03$, respectively). Pulmonary venules and interlobular veins revealed intimal fibrosis in all SSscPAH cases, compared with 3 of 10 IPAH patients, which was a significant difference ($p = 0.02$). Finally, loose intimal fibrosis appeared a distinct characteristic, as it was observed in only a minority of IPAH (3 of 11) patients ($p=0.02$)

Similarities in vasculopathy between SSscPAH and IPAH

All PAH cases demonstrated some degree of intimal fibrosis of the axial arteries at bronchiolar level. Medial hypertrophy of axial pulmonary arteries was observed in 7 of the 8 SSscPAH cases and in 10 of 11 IPAH cases. Some degree of eccentric intimal fibrosis, indicative of post-thrombotic remodelling was found in 7 of 8 SSscPAH cases and in all IPAH cases. One SSscPAH and one IPAH case demonstrated collander lesions; a recanalized thrombus, thereby also a reflection of post-thrombotic remodelling. In these two patients macroscopic thrombo-emboli were found at autopsy.

In 4 SSscPAH and 2 IPAH cases we found vascular transmural infiltration of lymphocytes without evidence of destruction of the elastic laminae of fibrinoid changes of the vessel wall.

In 3 of the SScPAH patients and 1 IPAH patient we observed this at the level of the small vessels. In 1 SScPAH case and 1 IPAH case the lymphocytic infiltrate appeared to be concentrated around the alveolar capillaries without evidence of haemorrhage as a sign of capillaritis. In 2 SScPAH cases, vasculitis was present at venular level. In addition, in IPAH, mononuclear infiltrates were also detected in and around some plexiform lesions.

Non-vascular morphologic histological patterns in SScPAH and IPAH

Areas of fibrosis were observed in 2 of 8 SScPAH cases, consisting of mild interstitial fibrosis in one patient and areas of fibrotic architectural changes and some honeycombing in the other patient. In most slides and in all cases, areas without fibrosis allowed for evaluating patterns of vasculopathy. Pulmonary volumes, fibrosis on HRCT and peripheral oxygen saturation did not indicate pulmonary fibrosis as underlying cause of PH. Seven of 8 SScPAH cases and 6 of 11 IPAH cases showed some degree of pneumonitis. SScPAH cases demonstrated a pattern of diffuse interstitial infiltrates and a pattern of widening of the alveolar septa due to diffuse interstitial lymphocytic infiltrates, consistent with focal NSIP pattern. In the two patients with areas of fibrosis the inflammation was confined to fibrosis. Pneumonitis in IPAH cases was milder and consisted only of sparse interstitial infiltrates.

Discussion

In this study, we demonstrate that pulmonary vasculopathic morphology in SScPAH displays heterogeneous features, but is dominated by the presence of intimal fibrosis of both arteries/arterioles and veins/venules, which in some cases is associated with the presence of a PVOD-like pattern. This pattern of vasculopathy, together with the absence of plexiform lesions, sets apart the vasculopathy in SScPAH as an entity different from IPAH.

Previous workers have reported intimal fibrosis and medial hypertrophy in SSc with and without PAH(16;18), and sometimes plexiform lesions(14), focusing on pulmonary pre-capillary lesions. There are few cases reported with, either or not biopsy proven, PVOD associated with SScPAH. (29-31). A recent study by Dorfmueller *et al.*, involving 8 connective tissue disease (CTD)-cases with clinically well defined PAH, including 4 LcSSc patients, demonstrated a PVOD-like pattern in the CTD-population, together with post-capillary fibrous involvement and signs of alveolar hemorrhagic edema(15). Our study, describing the largest series of patients so far of clinically well defined SSc patients with thoroughly documented PAH confirms these findings and extends them by demonstrating small vessel intimal fibrosis, coinciding with a pattern of PVOD in some of the cases, and indicating arteriolar as well as venular intimal involvement.

Interestingly, in SSc without PAH, the arterial and venous vessels of small calibre as well as the microcirculation in SSc-affected hands, demonstrated microvascular involvement (32). In 17 patients with the CREST syndrome (a formerly used term recognised as a variant of SSc characterised by calcinosis, Raynaud's phenomenon, oesophageal dysmotility, sclerodactyly and telangiectasis) which according to present standards defined PAH inadequately, described fibrotic alterations in pulmonary venules in cases both with and without PAH(18). We have observed a similar spectrum of pulmonary vasculopathy in 2 SSc patients without pulmonary hypertension (data not shown).

Convincing examples of plexiform lesions were not detected in the SScPAH group. Some authors have reported the absence of plexiform lesions(15), whereas others have reported the occurrence of such lesions(14). As such, some controversy concerning this topic exists. It is unlikely that in our study interpretations of morphology of plexiform lesions *versus* thrombo-embolic lesions explain this discrepancy, as both types of lesions were rare in our SScPAH group. Rather, in our opinion, the absence of plexiform lesions is another diacritical feature of SScPAH *versus* IPAH.

In our series concentric laminar fibrosis was strictly defined according to previously described criteria(20), whereas an apparently broader definition allowed inclusion of concentric intimal fibrosis consisting of loose fibrous tissue(15). Concentric laminar intimal fibrosis was observed in 3 of the 8 SScPAH cases whereas in the study of Dorfmueller *et al.* in 3 of 4 SScPAH cases(15). Indeed, we observed loose intimal fibrosis in the majority of the SScPAH cases. We did not find concentric laminar fibrosis in our IPAH cases, whereas loose intimal fibrosis was found in two cases. The significance of distinguishing concentric laminar intimal fibrosis from concentric intimal fibrosis may be a matter of debate: whether this lesion represents a distinct entity or whether it represents a stage, or the end result, of intimal fibrosis remains to be determined.

The present data were largely based on autopsy material. The inherent limitations of such a study design are shared by previous studies on this topic. As practically all patients died of PAH-related causes, the pathology in this series represents end-stage disease. Consequently, its features may differ from earlier phases of the same disease. Whatever the way histopathology evolves over time, it is demonstrated in this study, at similar disease duration of PAH that the groups end up with different histopathologic patterns. This suggests that different pathogenetic pathways are involved. The SScPAH population consisted of patients with the limited cutaneous form of SSc (23) with no or mild interstitial disease. As data on

pulmonary vascular changes in SSc with fibrosis are scarce, it is currently not clear as to whether our results can be extrapolated to the SSc patients, with either LcSSc or DcSSc, with major interstitial lung disease. In contrast to previous reports, in the presented study, survival was not significantly different, whereas SScPAH patients are known to have a worse prognosis than IPAH patients, even despite treatment(5;6). It should be noted that the study was not designed to address this issue, and the sample size is rather small to draw firm conclusions in this matter. However, one could postulate that selection may have been biased by recruitment from tertiary referral centers with high awareness for PAH in SSc patients.

How can these findings be interpreted? Explanations of the difference in arteriolar and venular intimal fibrosis between the groups are speculative, but might be related to the systemic nature of SSc-disease. For example, mediators of endothelial dysfunction that influence vascular remodeling and fibrogenesis such as endothelin-1 play an important role in SSc pathogenetics(33;34). The perivascular and transmural infiltration of lymphocytes both in SScPAH as well as in IPAH and the surrounding of plexiform lesions by lymphocytes is in line with previous observations by others, supporting the notion that inflammatory mechanisms may be relevant in the pathology of PAH(35-37). Various studies describe specific inflammatory mechanisms concerning SScPAH, such as a different T-cell/endothelium interplay from SSc patients with and without PAH, and the occurrence of anti-endothelial cell and anti-fibroblast antibodies(38-42). In the presented study, in various cases, both SScPAH and IPAH, we observed perivascular and transmural infiltration of lymphocytes, as well as surrounding of plexiform lesions by such infiltrates. Taken together, a-selective intimal fibrosis might be a reflection of the inflammatory nature of SSc.

In a recent report of Montani et al.(43), precipitous pulmonary edema with initiation of PAH therapy occurred in 44% of patients with PVOD. Therefore, SScPAH patients who demonstrate PVOD-like pattern might be susceptible of developing this complication.

Interestingly, cases have been reported, anecdotically, of SScPAH patients developing pulmonary edema upon epoprostenol administration(44;45). In our population, however, no pulmonary edema at initiation of prostacyclines, nor at testing of vasodilation upon NO, occurred. Whether the presence of PVOD-like pattern raises the susceptibility of these patients to pulmonary edema, needs to be confirmed in larger series.

Small vessel intimal fibrosis and the PVOD-pattern occurring at this background may account for the different response to therapy in SScPAH as compared with IPAH(46). Moreover, it could be speculated that this phenomenon explains the lower TLCO values in SScPAH than in IPAH, as post-capillary abnormalities may lead to disturbed gas diffusion at the capillary level(47). Indeed, low TLCO is also a feature of clinical PVOD(46).

It is a matter of speculation to explain the absence of plexiform lesions in SScPAH. Altered angiogenesis as seen in SSc (48) might play a role as, for example, in SSc upregulation of vascular endothelial growth factor (VEGF) and its receptor has been demonstrated(49). Interestingly, in plexiform lesions upregulation of the VEGF/VEGFR axis has been shown (50-52) and as such they are thought to arise from uncontrolled angiogenesis. In this context, it is intriguing that plexiform lesions were not observed in SScPAH in this study, challenging in depth study.

Taken together, this study demonstrates that pulmonary vasculopathic features in SScPAH differ from those seen in IPAH with respect to the occurrence of small vessel intimal fibrosis, a pattern of PVOD and absence of plexiform lesions. These findings may account for differences in terms of TLCO values and response to PAH-therapy between the groups. Moreover, this study supports the notion, as evoked by differences in clinical behaviour, that different pathogenetic mechanisms may underly the development of PAH. This report

underpins the importance of further research into targets for tailored treatment for SSc-associated PAH.

Reference List

- (1) Jimenez SA, Derk CT. Following the molecular pathways toward an understanding of the pathogenesis of systemic sclerosis. *Ann Intern Med* 2004; 140(1):37-50.
- (2) Hachulla E, Gressin V, Guillevin L, Carpentier P, Diot E, Sibilia J et al. Early detection of pulmonary arterial hypertension in systemic sclerosis: a French nationwide prospective multicenter study. *Arthritis Rheum* 2005; 52(12):3792-3800.
- (3) Mukerjee D, St George D, Coleiro B, Knight C, Denton CP, Davar J et al. Prevalence and outcome in systemic sclerosis associated pulmonary arterial hypertension: application of a registry approach. *Ann Rheum Dis* 2003; 62(11):1088-1093.
- (4) Simonneau G, Galie N, Rubin LJ, Langleben D, Seeger W, Domenighetti G et al. Clinical classification of pulmonary hypertension. *J Am Coll Cardiol* 2004; 43(12 Suppl S):5S-12S.
- (5) Fisher MR, Mathai SC, Champion HC, Girgis RE, Houston-Harris T, Hummers L et al. Clinical differences between idiopathic and scleroderma-related pulmonary hypertension. *Arthritis Rheum* 2006; 54(9):3043-3050.
- (6) Kawut SM, Taichman DB, Archer-Chicko CL, Palevsky HI, Kimmel SE. Hemodynamics and survival in patients with pulmonary arterial hypertension related to systemic sclerosis. *Chest* 2003; 123(2):344-350.
- (7) Steen V, Medsger TA, Jr. Predictors of isolated pulmonary hypertension in patients with systemic sclerosis and limited cutaneous involvement. *Arthritis Rheum* 2003; 48(2):516-522.
- (8) Sun XG, Hansen JE, Oudiz RJ, Wasserman K. Pulmonary function in primary pulmonary hypertension. *J Am Coll Cardiol* 2003; 41(6):1028-1035.
- (9) Badesch DB, Tapon VF, McGoon MD, Brundage BH, Rubin LJ, Wigley FM et al. Continuous intravenous epoprostenol for pulmonary hypertension due to the scleroderma spectrum of disease. A randomized, controlled trial. *Ann Intern Med* 2000; 132(6):425-434.
- (10) Badesch DB, Hill NS, Burgess G, Rubin LJ, Barst RJ, Galie N et al. Sildenafil for Pulmonary Arterial Hypertension Associated with Connective Tissue Disease. *J Rheumatol* 2007; 34(12):2417-2422.
- (11) Girgis RE, Mathai SC, Krishnan JA, Wigley FM, Hassoun PM. Long-term outcome of bosentan treatment in idiopathic pulmonary arterial hypertension and pulmonary arterial hypertension associated with the scleroderma spectrum of diseases. *J Heart Lung Transplant* 2005; 24(10):1626-1631.

- (12) Mathai SC, Girgis RE, Fisher MR, Champion HC, Houston-Harris T, Zaiman A et al. Addition of sildenafil to bosentan monotherapy in pulmonary arterial hypertension. *Eur Respir J* 2007; 29(3):469-475.
- (13) Rubin LJ, Badesch DB, Barst RJ, Galie N, Black CM, Keogh A et al. Bosentan therapy for pulmonary arterial hypertension
1. *N Engl J Med* 2002; 346(12):896-903.
- (14) Cool CD, Kennedy D, Voelkel NF, Tuder RM. Pathogenesis and evolution of plexiform lesions in pulmonary hypertension associated with scleroderma and human immunodeficiency virus infection. *Hum Pathol* 1997; 28(4):434-442.
- (15) Dorfmueller P, Humbert M, Perros F, Sanchez O, Simonneau G, Muller KM et al. Fibrous remodeling of the pulmonary venous system in pulmonary arterial hypertension associated with connective tissue diseases. *Hum Pathol* 2007; 38(6):893-902.
- (16) al Sabbagh MR, Steen VD, Zee BC, Nalesnik M, Trostle DC, Bedetti CD et al. Pulmonary arterial histology and morphometry in systemic sclerosis: a case-control autopsy study. *J Rheumatol* 1989; 16(8):1038-1042.
- (17) Peacock AJ, Murphy NF, McMurray JJ, Caballero L, Stewart S. An epidemiological study of pulmonary arterial hypertension. *Eur Respir J* 2007; 30(1):104-109.
- (18) Yousem SA. The pulmonary pathologic manifestations of the CREST syndrome. *Hum Pathol* 1990; 21(5):467-474.
- (19) Pietra GG, Edwards WD, Kay JM, Rich S, Kernis J, Schloo B et al. Histopathology of primary pulmonary hypertension. A qualitative and quantitative study of pulmonary blood vessels from 58 patients in the National Heart, Lung, and Blood Institute, Primary Pulmonary Hypertension Registry. *Circulation* 1989; 80(5):1198-1206.
- (20) Wagenvoort CA, Mooi WJ. Biopsy pathology of the pulmonary vasculature. Biopsy and Pathology serie 13. 1 ed. London: Chapman and Hall Medical, 1989.
- (21) Wagenvoort CT, Wagenvoort N. Primary Pulmonary Hypertension; A Pathologic Study of the Lung Vessels in 156 Clinically Diagnosed Cases. *Circulation* 1970; XLII:1163-1184.
- (22) Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. *Arthritis Rheum* 1980; 23(5):581-590.
- (23) LeRoy EC, Black C, Fleischmajer R, Jablonska S, Krieg T, Medsger TA, Jr. et al. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. *J Rheumatol* 1988; 15(2):202-205.
- (24) Pietra GG, Capron F, Stewart S, Leone O, Humbert M, Robbins IM et al. Pathologic assessment of vasculopathies in pulmonary hypertension. *J Am Coll Cardiol* 2004; 43(12 Suppl S):25S-32S.

- (25) Mooi WJ, Grünberg K. Histopathology of pulmonary hypertensive diseases. *Current diagnostic pathology* 2006; 12:429-440.
- (26) Lantuejoul S, Sheppard MN, Corrin B, Burke MM, Nicholson AG. Pulmonary veno-occlusive disease and pulmonary capillary hemangiomatosis: a clinicopathologic study of 35 cases. *Am J Surg Pathol* 2006; 30(7):850-857.
- (27) Badesch DB, Abman SH, Simonneau G, Rubin LJ, McLaughlin VV. Medical therapy for pulmonary arterial hypertension: updated ACCP evidence-based clinical practice guidelines. *Chest* 2007; 131(6):1917-1928.
- (28) Rabiller A, Jais X, Hamid A, Resten A, Parent F, Haque R et al. Occult alveolar haemorrhage in pulmonary veno-occlusive disease. *Eur Respir J* 2006; 27(1):108-113.
- (29) Johnson SR, Patsios D, Hwang DM, Granton JT. Pulmonary veno-occlusive disease and scleroderma associated pulmonary hypertension. *J Rheumatol* 2006; 33(11):2347-2350.
- (30) Morassut PA, Walley VM, Smith CD. Pulmonary veno-occlusive disease and the CREST variant of scleroderma. *Can J Cardiol* 1992; 8(10):1055-1058.
- (31) Saito A, Takizawa H, Ito K, Yamamoto K, Oka T. A case of pulmonary veno-occlusive disease associated with systemic sclerosis. *Respirology* 2003; 8(3):383-385.
- (32) Allanore Y, Seror R, Chevrot A, Kahan A, Drape JL. Hand vascular involvement assessed by magnetic resonance angiography in systemic sclerosis. *Arthritis Rheum* 2007; 56(8):2747-2754.
- (33) Abraham D, Distler O. How does endothelial cell injury start? The role of endothelin in systemic sclerosis. *Arthritis Res Ther* 2007; 9 Suppl 2:S2.
- (34) Ahmed SS, Tan FK, Arnett FC, Jin L, Geng YJ. Induction of apoptosis and fibrillin 1 expression in human dermal endothelial cells by scleroderma sera containing anti-endothelial cell antibodies. *Arthritis Rheum* 2006; 54(7):2250-2262.
- (35) Dorfmueller P, Perros F, Balabanian K, Humbert M. Inflammation in pulmonary arterial hypertension. *Eur Respir J* 2003; 22(2):358-363.
- (36) Nicolls MR, Taraseviciene-Stewart L, Rai PR, Badesch DB, Voelkel NF. Autoimmunity and pulmonary hypertension: a perspective. *Eur Respir J* 2005; 26(6):1110-1118.
- (37) Perros F, Dorfmueller P, Souza R, Durand-Gasselin I, Mussot S, Mazmanian M et al. Dendritic cell recruitment in lesions of human and experimental pulmonary hypertension. *Eur Respir J* 2007; 29(3):462-468.
- (38) Baroni SS, Santillo M, Bevilacqua F, Luchetti M, Spadoni T, Mancini M et al. Stimulatory autoantibodies to the PDGF receptor in systemic sclerosis. *N Engl J Med* 2006; 354(25):2667-2676.

- (39) Carulli MT, Handler C, Coghlan JG, Black CM, Denton CP. Can CCL2 serum levels be used in risk stratification or to monitor treatment response in systemic sclerosis? *Ann Rheum Dis* 2008; 67(1):105-109.
- (40) Chizzolini C, Raschi E, Rezzonico R, Testoni C, Mallone R, Gabrielli A et al. Autoantibodies to fibroblasts induce a proadhesive and proinflammatory fibroblast phenotype in patients with systemic sclerosis. *Arthritis Rheum* 2002; 46(6):1602-1613.
- (41) Sanchez O, Marcos E, Perros F, Fadel E, Tu L, Humbert M et al. Role of endothelium-derived CC chemokine ligand 2 in idiopathic pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2007; 176(10):1041-1047.
- (42) Tamby MC, Humbert M, Guilpain P, Servettaz A, Dupin N, Christner JJ et al. Antibodies to fibroblasts in idiopathic and scleroderma-associated pulmonary hypertension. *Eur Respir J* 2006; 28(4):799-807.
- (43) Montani D, Achouh L, Dorfmueller P, Le Pavec J, Sztrymf B, Tcherakian C et al. Pulmonary veno-occlusive disease: clinical, functional, radiologic, and hemodynamic characteristics and outcome of 24 cases confirmed by histology. *Medicine (Baltimore)* 2008; 87(4):220-233.
- (44) Humbert M, Sanchez O, Fartoukh M, Jagot JL, Le Gall C, Sitbon O et al. Short-term and long-term epoprostenol (prostacyclin) therapy in pulmonary hypertension secondary to connective tissue diseases: results of a pilot study. *Eur Respir J* 1999; 13(6):1351-1356.
- (45) Preston IR, Klinger JR, Houtchens J, Nelson D, Mehta S, Hill NS. Pulmonary edema caused by inhaled nitric oxide therapy in two patients with pulmonary hypertension associated with the CREST syndrome. *Chest* 2002; 121(2):656-659.
- (46) Mandel J, Mark EJ, Hales CA. Pulmonary veno-occlusive disease. *Am J Respir Crit Care Med* 2000; 162(5):1964-1973.
- (47) Hughes JM. The single breath transfer factor (Tl,co) and the transfer coefficient (Kco): a window onto the pulmonary microcirculation. *Clin Physiol Funct Imaging* 2003; 23(2):63-71.
- (48) Koch AE, Distler O. Vasculopathy and disordered angiogenesis in selected rheumatic diseases: rheumatoid arthritis and systemic sclerosis. *Arthritis Res Ther* 2007; 9 Suppl 2:S3.
- (49) Distler O, Distler JH, Scheid A, Acker T, Hirth A, Rethage J et al. Uncontrolled expression of vascular endothelial growth factor and its receptors leads to insufficient skin angiogenesis in patients with systemic sclerosis. *Circ Res* 2004; 95(1):109-116.
- (50) Cool CD, Stewart JS, Werahera P, Miller GJ, Williams RL, Voelkel NF et al. Three-dimensional reconstruction of pulmonary arteries in plexiform pulmonary hypertension using cell-specific markers. Evidence for a dynamic and heterogeneous process of pulmonary endothelial cell growth. *Am J Pathol* 1999; 155(2):411-419.

- (51) Lee SD, Shroyer KR, Markham NE, Cool CD, Voelkel NF, Tudor RM. Monoclonal endothelial cell proliferation is present in primary but not secondary pulmonary hypertension. *J Clin Invest* 1998; 101(5):927-934.
- (52) Tudor RM, Chacon M, Alger L, Wang J, Taraseviciene-Stewart L, Kasahara Y et al. Expression of angiogenesis-related molecules in plexiform lesions in severe pulmonary hypertension: evidence for a process of disordered angiogenesis. *J Pathol* 2001; 195(3):367-374.

Table 1. Patient Characteristics of systemic sclerosis

N=8	
LcSSc, no. (%)	8 (100)
Disease duration*, yr	10 ± 4
Antibody profile (Antinuclear antibody/Anti-centromere/Anti-ribonucleoprotein) no. (%)	8/6/1(100/75/12.5)
Anti-phospholipid auto-antibodies	0
Pericardial effusion, n	3
CT abnormalities associated with PVOD (n=7)	
Lymphadenopathy, n	3
Septal lines, n	3
Ground glass, n	6

Values expressed as mean ± SE or otherwise as stated. Definition of abbreviations: LcSSc: limited cutaneous systemic sclerosis. * Since first non-Raynaud symptom, at time of diagnosis of pulmonary arterial hypertension;[†]

Table 2. Haemodynamic, lung function, exercise data at PAH diagnosis

	SScPAH N=8	IPAH N=11	p
mRpa, mmHg	8 (3-15) (n=6)	10 (4-19) (n=9)	0.30
sPpa, mmHg	73 (35-101) (n=8)	100 (62-128) (n=11)	0.15
mPpa, mmHg	45 (25-71) (n=8)	60 (43-76) (n=9)	0.08
PCWP, mmHg	7 (4-12) (n=8)	7 (5-12) (n=9)	0.57
PVR, dynes·s·cm ⁻⁵	654 (227-3278) (n=6)	920 (346-1587) (n=9)	0.41
CI, l/min·m ²	2.4 (1.1-3.6) (n=6)	2.5 (1.3-3.2) (n=9)	0.69
FEV1, %	88 (77-97)	80 (66-86)	0.15
TLC, %	88 (81-115) (n=6)	93 (78-115) (n=9)	0.78
VC, %	97 (84-115) (n=7)	89 (58-110) (n=8)	0.34
TLCO, %	40 (26-58) (n=7)	68 (39-89) (n=10)	0.01
TLCO/VA, %	39 (26-69) (n=7)	58.5 (52-87) 4 (n=10)	0.01
PaO ₂ , mmHg	73 (58-88)	65.5 (52-80)	0.46
6 MWD, m	278 (0-382)	400 (100-570)	0.15
Smoking history (never/current/former)	0/0/3	0/0/3	
Therapy at time of death			
Prostacycline (n)	7	10	
ERA (n)	1	1	
PDE-5 inhibitor (n)	1	-	
ABS	-	2	

Values expressed as median (range) or otherwise as stated. Definition of abbreviations: SScPAH: systemic sclerosis-associated pulmonary arterial hypertension; IPAH: idiopathic PAH; mRpa: mean right atrial pressure; sPpa, mPpa: systolic, mean pulmonary artery pressures; PCWP: pulmonary capillary wedge pressure; PVR: pulmonary vascular resistance; CI: cardiac index; FEV1, %: percentage predicted of forced expiratory volume; TLC, %: percentage of predicted total lung capacity; VC: percentage predicted of vital capacity; TLCO %: percentage predicted of transfer factor of the lung for carbon monoxide; VA: alveolar volume; 6 MWD: 6-minute walk distance; ERA: endothelin receptor antagonist; PDE-5: phosphodiesterase 5; ABS: atrial balloon septostomy

Table 3 Parameters of pulmonary vascular morphology
Systemic-sclerosis associated pulmonary arterial hypertension:

Case no.	Lung sample	Plexiform lesions	Post-thrombotic eccentric intimal fibrosis	Concentric laminar intimal fibrosis	Small vessel intimal fibrosis	PVOD/CH	Hypoxic vasculopathy	Congestion*	Intimal fibrosis venules/ interlobular veins	Pulmonary vascular inflammation/ vasculitis [†]	Pneumonitis [‡]	Fibrosis [§]
1	LTX	N	Y	Y	Y	N	N	1	N	2	3	1
2	A	N	Y	N	Y	Y	N	1	Y	1	1	N
3	A	N	N	N	Y	N	N	N	Y	1+3	2	N
4	A	N	Y	N	Y	Y	N	1	Y	N	1	N
5	B	N	Y	Y	Y	N	N	N	Y	N	2	N
6	A	N	Y	Y	Y	Y	N	2	Y	1	2	N
7	LTX	N	Y	N	Y	N	N	N	Y	N	4	2+3
8	A	N	Y	N	Y	Y	N	1	Y	1	N	N

Idiopathic pulmonary arterial hypertension:

9	A	N	Y	N	Y	N	N	N	N	2	1	N
10	A	Y	Y	N	N	N	N	3	N	2	1	N
11	LTX	Y	Y	N	N	N	N	N	N	2	1	N
12	A	Y	Y	N	N	N	N	1	Y	2	N	N
13	A	Y	Y	N	N	N	N	1+3	N	N	1	N
14	A	Y	Y	N	Y	N	N	1+3	Y	1	N	N
15	A	Y	Y	N	N	N	N	N	N	N	1	N
16	A	Y	Y	N	N	N	N	1+3	N	N	N	N
17	A	Y	Y	N	N	N	N	N	Y	1	N	N
18	A	Y	Y	N	Y	N	N	N	N	N	1	N
19	A	Y	Y	N	N	N	N	N	N	N	N	N

Definition of abbreviations: LTX: lung explantation. A: autopsy. B: Biopsy. N = No, Y = yes. PVOD/CH: pulmonary veno-occlusive disease/ capillary hemangiomatosis;

* Congestion described as: 1 = diffuse; 2 = patchy; 3 = vasodilation-related. [†] Vasculitis described as: 1 = lymphocytic; 2 = perivascular infiltrate, insufficient for vasculitis; 3 = granulocytic. [‡] Pneumonitis described as: 1 = sparse interstitial aggregates; 2 = diffuse interstitial infiltrate, mild, no fibrosis; 3 = diffuse interstitial infiltrate, moderate, no fibrosis; 4 = diffuse interstitial infiltrate and fibrosis; 5 = infiltrate confined to fibrotic areas. [§] Fibrosis described as: 1 = mild; some interstitial fibrosis; 2 = moderate; architectural changes; 3 = severe; honeycombing. ^{||} = Predominant pattern

Figure 1. Intimal fibrosis of alveolar interstitial vessels. No distinction could be made between arteriole and venule on the basis of anatomic localisation. Concentric intimal fibrosis in **A** (Case 2, Table 3), complete obliteration in **B** and **C** (Case 6, Table 3) (elastica von Gieson stain; A and B: original magnification x 200, C: 100x).

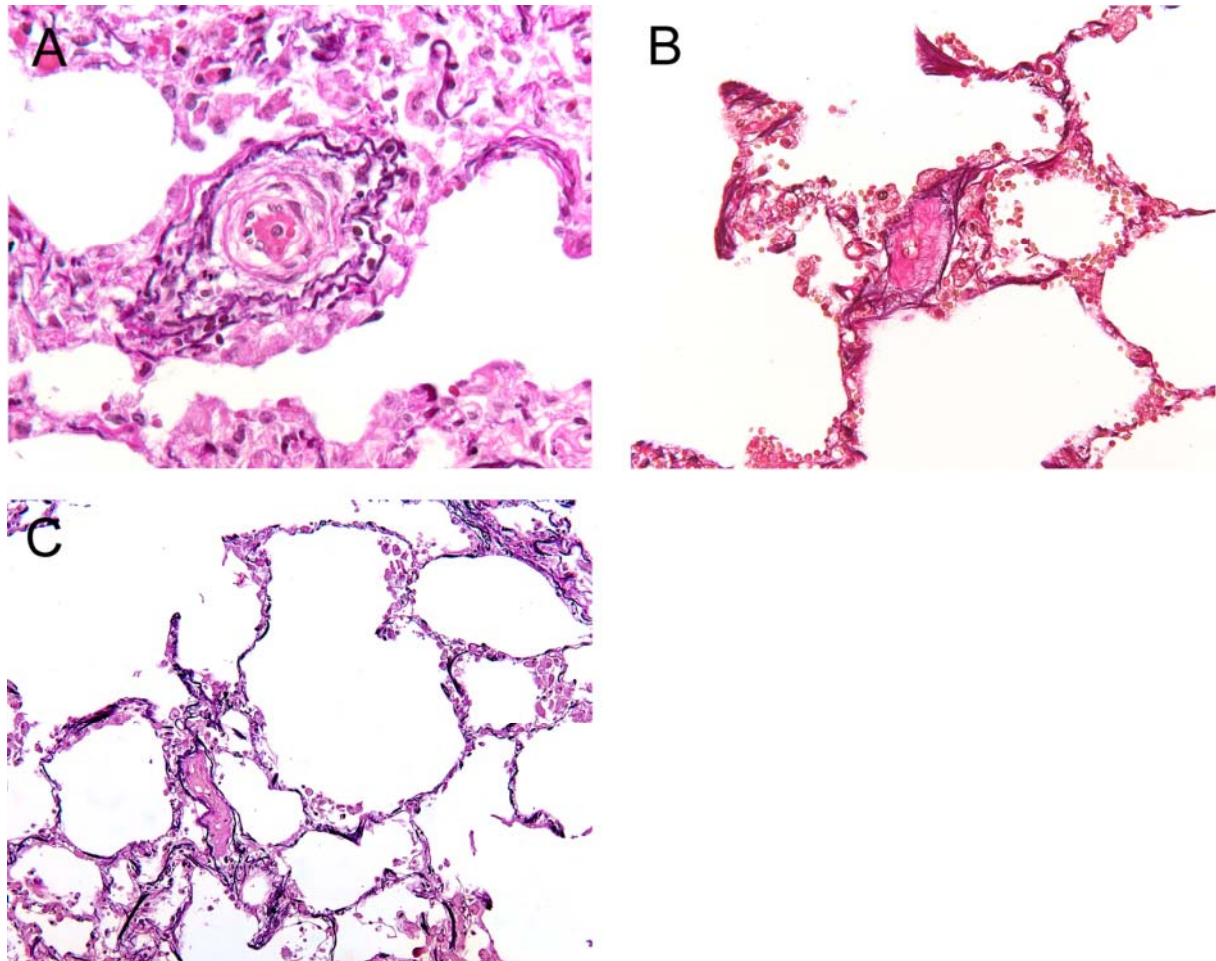


Figure 2. **A** (Case 4, Table 3): PVOD pattern with patchy capillary congestion (arrows). **B** (Case 2, Table 3): PVOD pattern with patchy capillary congestion (arrow), deposition of iron salts on elastine fibers (arrow head) and giant cells (hollow arrows). **C** (Case 2, Table 3): PVOD- pattern with patchy capillary congestion (arrows), iron deposition on elastine fibers and vasculitis with transmural infiltration of lymphocytes (arrow head) (haematoxylin-eosin stain; original magnification x 100).

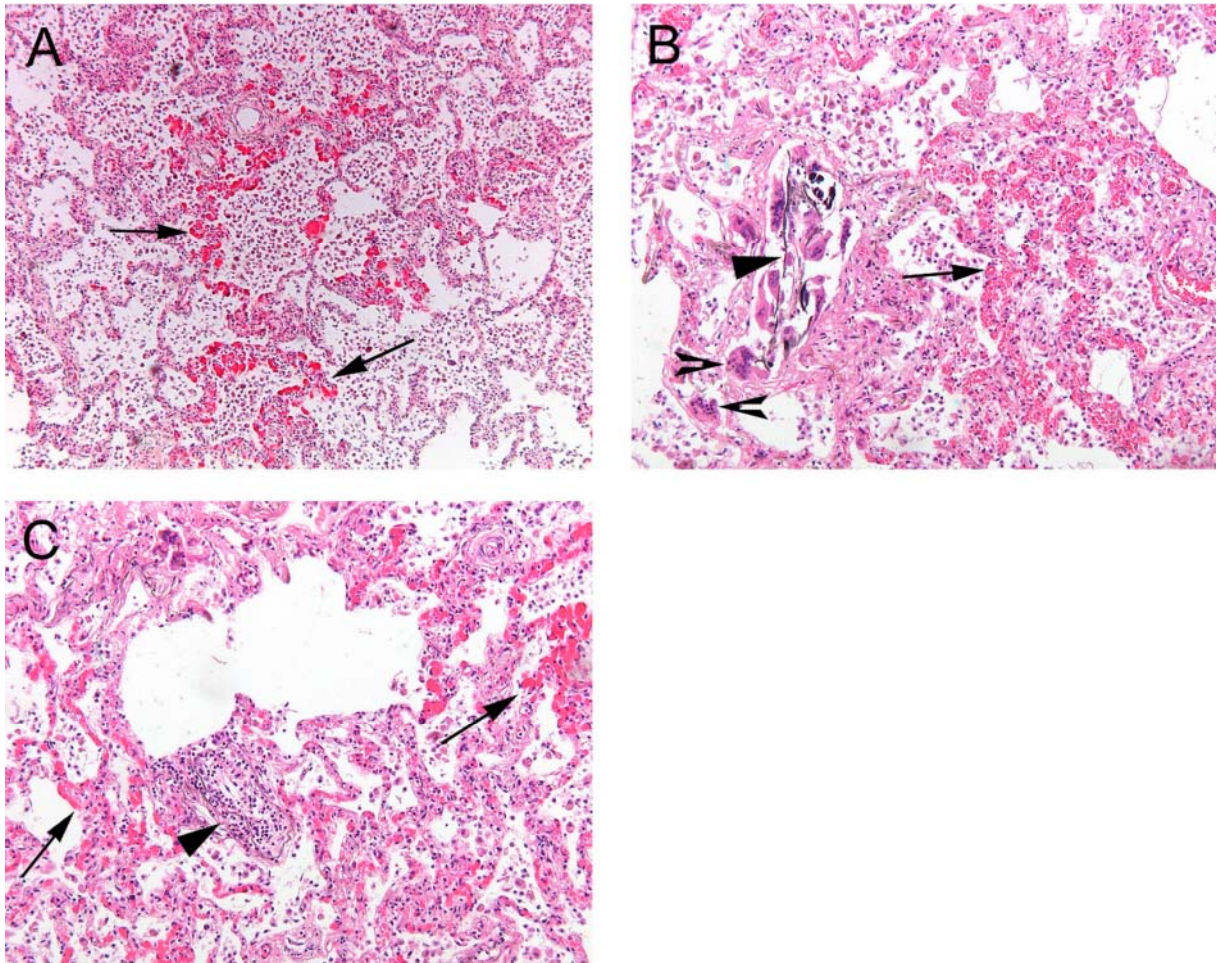


Figure 3. **A** (Case 3, Table 3): Single one lesion in the systemic sclerosis-associated pulmonary arterial hypertension (PAH) group mostly resembling a plexiform lesion: localisation adjacent to a bronchiolus (arrow); intimal fibrosis with recanalization (arrow heads); dilated vessel segments (block arrow) (original magnification x 200). **B** (Case 19, Table 3): Plexiform lesion in a patient with idiopathic PAH adjacent to a feeding artery, with intimal fibrosis and slit-like channels lined by cuboidal endothelial cells (arrow) and dilated vessel segments (block arrow). (A: elastica von Gieson stain; B: haematoxylin-eosin stain; original magnification x 200)

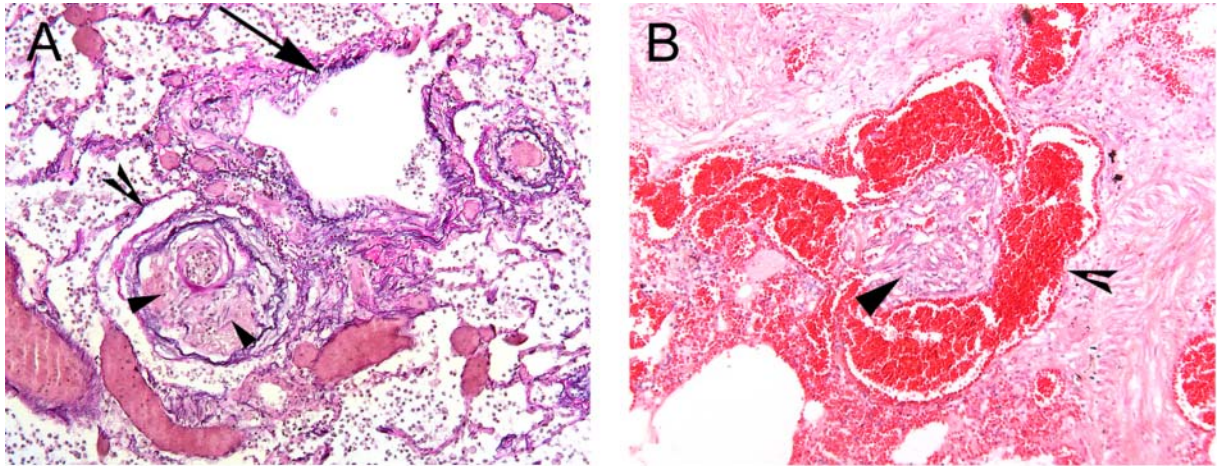


Figure 4. A and B (Case 6, Table 3): Pulmonary artery with concentric laminar intimal fibrosis and some perivascular mononuclear cell infiltration (A: haematoxylin-eosin stain; B: elastica von Gieson stain; original magnification x 200)

