




# Pulmonary hypertensive vasculopathy in parenchymal lung diseases and/or hypoxia

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**ABSTRACT** Pulmonary hypertension (PH) with complicating chronic lung diseases and/or hypoxia falls into group 3 of the updated classification of PH. Patients with chronic obstructive lung disease (COPD), diffuse lung disease (such as idiopathic pulmonary fibrosis (IPF)) and with sleep disordered breathing are particularly exposed to the risk of developing PH. Although PH in such a context is usually mild, a minority of patients exhibit severe haemodynamic impairment, defined by a mean pulmonary arterial pressure (mPAP) of  $\geq 35$  mmHg or mPAP values ranging between 25 mmHg and 35 mmHg with a low cardiac index ( $< 2 \text{ L}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$ ). The overlap between lung parenchymal disease and PH heavily affects life expectancy in such a patient population and complicates their therapeutic management. In this review we illustrate the pathological features and the underlying pathophysiological mechanisms of pulmonary circulation in chronic lung diseases, with an emphasis on COPD, IPF and obstructive sleep apnoea syndrome.

## Introduction

A wide variety of pulmonary diseases with obstructive and/or restrictive lung function impairment may be associated with pulmonary hypertension (PH). In the updated clinical classification of PH [1], these are placed in group 3, “PH owing to lung diseases and/or hypoxia” (table 1). In this group of diseases, the presence and severity of PH impact unfavourably on prognosis and thus have a bearing on treatment planning. Also, elucidation of the responsible pathogenetic mechanisms may lead to the development of rational and targeted therapies, which are not yet currently available.

PH is defined as a mean pulmonary arterial pressure (mPAP) of at least 25 mmHg at rest, as measured by right heart catheterisation [2]. According to the current classification, the consensus definition for PH in pulmonary parenchymal diseases is determined by an mPAP value of  $\geq 25$  mmHg, while severe PH is qualified by mPAP values  $\geq 35$  mmHg, or mPAP  $\geq 25$  mmHg and  $\leq 35$  mmHg but with a low cardiac

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TABLE 1 Classification of pulmonary hypertension, group 3

- 1 Pulmonary arterial hypertension
- 1' Pulmonary veno-occlusive disease and/or pulmonary capillary haemangiomas
- 1'' Persistent pulmonary hypertension of the newborn
- 2 Pulmonary hypertension owing to left heart disease
- 3 Pulmonary hypertension owing to lung diseases and/or hypoxia
  - 3.1 Chronic obstructive pulmonary disease
  - 3.2 Interstitial lung disease
  - 3.3 Other pulmonary diseases with mixed restrictive and obstructive pattern
  - 3.4 Sleep disordered breathing
  - 3.5 Alveolar hypoventilation disorders
  - 3.6 Chronic exposure to high altitude
  - 3.7 Developmental abnormalities
- 4 Chronic thromboembolic pulmonary hypertension
- 5 Pulmonary hypertension with unclear multifactorial mechanisms

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Data from [1].

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index ( $<2 \text{ L}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$ ) [3]. In idiopathic pulmonary fibrosis (IPF), such severe PH carries a very poor prognosis.

Biopsy pathology has no significant role to play in the demonstration of PH in this group of diseases, or in the assessment of its severity, but it does provide some angle on pathogenesis. Various distinct histological patterns of hypertensive pulmonary vascular disease have been identified in past decades, and these can be linked, to some extent, to different clinical contexts [4]. It is plausible that these various patterns and the differences between them reflect differences in pathogenesis and are, therefore, of potential therapeutic interest. In the rather heterogeneous subgroup of “PH owing to lung diseases and/or hypoxia”, the vascular pathology is heterogeneous as well.

However, our knowledge of histopathology is limited, because it is largely restricted to lung explant and autopsy specimens, except for “pure” hypoxic PH in the absence of pulmonary parenchymal disease, which is easily produced in experimental animals and has its human counterpart in people living at high altitudes, as well as a limited number of conditions of hypoventilation that are unassociated with pulmonary parenchymal disease (see below).

The situation is more complex in lung diseases that, apart from resulting in lung hypoxia, also have associated airway or parenchymal inflammatory or fibrosing disease. The blood vessels of any tissue in the body affected by inflammation and fibrosis display remodelling and the lung is no exception. Such vascular changes are usually a combination of intimal fibrosis with medial and adventitial thickening. Occluding thrombi are not uncommon.

In this review, we discuss the pathology of vascular disease in this heterogeneous group of lung diseases, with special emphasis on possible pathogenetic mechanisms.

#### “Pure” hypoxic PH: clinical data

In contrast to the systemic circulation, where tissue hypoxia induces arteriolar vasodilatation, hypoxia of lung tissue results in arterial vasoconstriction [5] and, if the hypoxia persists over long periods of time, induces increased muscle in arterial walls, especially of very small branches. This response is presumably beneficial in the case of unequal ventilation of lung tissue, but it results in PH when hypoxia globally affects the lungs.

Hypoxic PH is readily produced in animals exposed to decreased air pressure or decreased oxygen concentration in ambient air. In humans, a very similar situation exists in chronic mountain sickness (Monge’s disease), a potentially lethal form of hypoxic pulmonary hypertensive disease unassociated with any pulmonary parenchymal disease. Not surprisingly, hypoventilation, such as may result from muscle weakness, thoracic deformities and morbid obesity, results in a similar remodelling of pulmonary arteries and PH. In addition, an increased haematocrit may be associated with increased likelihood of thrombosis, so that thromboembolic events, either clinically manifest or occult, may contribute to the PH. Such post-thrombotic vascular disease is hallmarked histologically by focal lesions, which constitute the remnants of organised and recanalised thrombi; (often eccentric) patches of marked arterial intimal fibrosis, and colander lesions; and organised and recanalised thrombi containing multiple lumina.

Hypoxic pulmonary vasoconstriction is the result of different mechanisms, ranging from a direct effect of hypoxia on smooth muscle vascular cells [6, 7], to a decreased release of endothelium-derived vasodilators [8]

and increased secretion of vasoconstrictors [9, 10]. Rodents exposed to chronic hypoxia develop medial thickening of small pulmonary arteries and muscularisation of arterioles, which normally lack a muscle coat, resulting in reversible PH [11, 12]. Similar vascular structural changes have been found in the lungs of healthy high-altitude dwellers [13]. These changes, which are the consequence of hypoxic pulmonary vasoconstriction combined with an increased fluid shear stress, are largely reversible when the hypoxic stimulus is removed [14]. Chronic hypercapnia is also a common finding in patients carrying hypoxic lung disease [15]. Nevertheless, the effect of carbon dioxide on pulmonary vascular tone is controversial, with experimental data providing an effect of both vasoconstriction and vasodilatation [16, 17].

Obstructive sleep apnoea syndrome (OSAS) is characterised by recurrent episodes of upper airway obstruction during sleep, resulting in periodic hypoxia, sleep fragmentation and day-time sleepiness. This condition is associated with increased cardiovascular mortality [18, 19]. There is a strong relationship between OSAS and systemic arterial hypertension [20, 21] with ischaemic heart disease, atrial fibrillation and stroke [22–27]. Data about the occurrence of PH in pure, severe OSAS are extremely scant and indicate that severe PH rarely develops [28]. In addition, some results seem to indicate the reversibility of PH after treatment of the sleep disorder [29, 30].

The mechanisms underlying these associations have only been elucidated incompletely. Excessive sympathetic activity, increased oxidative stress, metabolic alterations and inflammation triggered by intermittent hypoxia are all thought to play a role. Indeed, intermittent hypoxia is known to induce the expression of a variety of inflammatory mediators, including nuclear factor- $\kappa$ B (NF- $\kappa$ B) cells, tumour necrosis factor (TNF)- $\alpha$ , interleukin (IL)-8 and IL-6 [31–33].

Intermittent hypoxia resulting from sleep apnoea can stimulate the carotid body, the principal chemoreceptor detecting the variations of blood oxygen concentration, resulting in increased sympathetic activity and raised blood pressure during apnoeic episodes [34–36]. Rodents (rats and mice) that have been exposed to intermittent hypoxia manifest an enhanced carotid body chemoreflex response when re-exposed to acute hypoxia [37, 38]. As is the case with high-altitude PH, hypoxia triggers vasoconstriction of the lung vessels and may induce vascular remodelling characterised by the thickening of the muscular layer of small pulmonary arteries. In addition, left heart dysfunction, which may arise in OSAS, may lead to increased pulmonary venous pressure, which induces muscularisation of pulmonary veins.

The pathogenetic mechanisms of chronic hypoxia-induced vascular changes are still incompletely understood. In pulmonary arterial smooth muscle cells, hypoxia can lead to the shutdown of  $K^+$  channels, resulting in membrane depolarisation and increased  $Ca^{2+}$  intake. The raised cellular  $Ca^{2+}$  concentration results in sensitisation of the cytoplasmic contractile machinery, leading to arterial vasoconstriction [7]. Hypoxia also generates a stress response in endothelial cells, resulting in the expression of hypoxia inducible factor (HIF)-1 $\alpha$ , HIF-2 $\alpha$  and peroxisome proliferator-activated receptor gamma coactivator (PGC)-1 $\alpha$ , which are pivotal transcriptional regulators [39, 40]. The transcription factor HIF-1 is a major regulator of cell oxygen homeostasis that, under hypoxic conditions, shifts cell metabolism towards anaerobiosis [41]. In addition, HIF-1 induces transcription of *EPO* and *VEGF*, thus increasing blood oxygen carriage capacity and promoting angiogenesis in hypoxic areas [42, 43]. Known HIF target genes include growth factors such as vascular endothelial growth factor (VEGF) and vascular endothelial growth factor receptor (VEGFR)2, pro-inflammatory cytokines such as stromal cell-derived factor (SDF)1 and chemokine receptor type (CXCR)4, glucose metabolic enzymes such as glucose transporter (Glut)-1 and hexokinase (HK)2, and the activation of pro-survival cellular pathways such as NF- $\kappa$ B signalling. In hypoxic lung tissue, macrophages release hypoxia-induced mitogenic factor (HIMF), which promotes inflammation and angiogenesis *via* VEGF and its receptor VEGFR2 [44]. The cytokines released by vascular cells and macrophages may also recruit fibrocytes exhibiting a “inflammatory” phenotype, characterised by the expression of IL-1, IL-6, regulated on activation, normal T-cell expressed and secreted (RANTES), SDF-1 and its receptor CXCR4, osteopontin and the integrin receptor  $\alpha$ v $\beta$ 3 [45]. The role of IL-6 as a mediator of PH in rodents exposed to chronic hypoxia has been well documented [46]. The overexpression of IL-6 in the lungs of mice exposed to chronic hypoxia results in severe PH, characterised by vascular obliterative lesions [47]. Moreover, the increased secretion of IL-6 in hypoxic lungs results in decreased expression of bone morphogenetic protein receptor (BMPR)2, which is mutated in up to 70% of patients with heritable PH [48, 49]. Hypoxic stress also generates endothelial cell production of reactive oxygen species (ROS) and nitrogen species [50], which influence the pulmonary vasomotor state [51]. Thus, chronic hypoxia has an effect on the expression levels of a plethora of vasoconstrictive and inflammatory mediators.

### PH in COPD and interstitial lung disease: clinical data

In chronic obstructive pulmonary disease (COPD), the prevalence of PH closely parallels the severity of the condition, with up to 90% of patients having a mPAP of over 20 mmHg at rest. Although PH is

usually mild, about 1% of patients have severe PH, with mPAP values between 35 and 40 mmHg [52, 53]. The presence and severity of PH in COPD have a substantial impact on survival [53, 54].

In idiopathic pulmonary fibrosis (IPF), mPAP values >25 mmHg are detected in a proportion of patients ranging from 8.1% to 14.9% upon initial examination [55, 56]. In advanced and end-stage disease, these figures have risen to about 50–60% [57, 58]. Clinical, functional and biological indicators of PH in IPF are worsening dyspnoea, impaired gas exchange at rest and low diffusing capacity of the lung for carbon monoxide.

PH is a significant prognostic factor in IPF [56], and the prognostic impact of PH in IPF is related to the severity of PH and pulmonary vascular resistance (PVR) [59] and to a cardiac index below  $2.4 \text{ L}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$  [60]. Patients with combined emphysema and pulmonary fibrosis are also at high risk to develop PH, which is found in 30–50% of these patients [61] and may be disproportionately severe. PH has also been documented as a rare complication occurring in cystic fibrosis [62] and bronchopulmonary dysplasia [63].

The workup for PH assessment in chronic lung disease first includes non-invasive mPAP assessment by echocardiography. The direct measurement of PH in chronic lung diseases by right heart catheterisation is required when lung transplantation is considered, or when a disproportional clinical deterioration compared to ventilatory impairment is noted, or in order to validate the diagnosis of PH in view of patient enrolment in a clinical trial or to confirm left heart dysfunction and to adjust therapy.

### Pathogenesis of PH in COPD

The pathophysiology of COPD is hallmarked by irreversible airflow obstruction. Often, there is some degree of inflammation of the airway walls. The inflammation usually represents a response to protracted exposure to toxic substances, such as those present in tobacco smoke. The inflammatory response is marked by increased numbers of macrophages and T-helper lymphocytes in lung tissue. These are a source of a variety of cytokines and growth factors, such as leukotriene B<sub>4</sub>, CXC chemokines, IL-1 $\beta$  and IL-6 and profibrogenic transforming growth factor (TGF)- $\beta$ . In addition, cigarette smoke and small airway inflammation result in oxidative stress, leading to an increased release of proteases by neutrophils and macrophages and the inactivation of antiproteases, such as  $\alpha_1$ -antitrypsin and secretory leukoprotease inhibitors. The smouldering inflammation leads to airway wall thickening, including an increase in airway wall smooth muscle and hyperplasia of bronchial glands, and is accompanied by increased mucous secretion and ciliary dysfunction of the surface epithelium, all contributing to airflow obstruction. The augmented secretion of bronchial mucus is the result of increased numbers of goblet cells within the surface epithelium and of hyperplasia of submucosal glands. The bronchial inflammation may also induce squamous metaplasia and ciliary dysfunction, impeding the clearance of bronchial secretions. The airflow obstruction following the stagnation of mucous secretions progressively leads to air trapping during expiration, resulting in decreased lung capacity. The main airflow obstruction occurs in airways under 2 mm in diameter, where pathological changes are designated respiratory bronchiolitis–interstitial lung disease (RB-ILD). The RB is characterised by the accumulation of macrophages within the respiratory bronchiole and adjacent alveoli and by inflammation with mild thickening of the bronchiolar wall and peribronchiolar alveoli [64].

PH in patients with COPD is the consequence of vasoconstriction as well as structural changes to the vessel walls. The impediments to gas exchange in COPD generate lung tissue hypoxia and hypoxic pulmonary vasoconstriction and remodelling, as outlined in the discussion on high-altitude PH, above. Destruction of the lung parenchyma and the increased airway resistance due to airway wall thickening and bronchial luminal obstruction with mucoid secretions, all contribute to lung hypoxia in COPD [65]. As would be expected, the chronic pulmonary hypoxia in COPD leads to increased medial smooth muscle, especially in small arterial branches [66] (figure 1). In patients with COPD, the hypoxia-induced haemodynamic changes, in conjunction with exposure to toxic agents, engender irreversible vascular structural alterations, characterised by muscularisation of the normally non-muscularised arterioles, as well as medial thickening and neointimal lesions of small and medium-sized pulmonary arteries. Neointimal lesions are characterised by extracellular matrix deposition and proliferation of cells phenotypically similar to smooth muscle cells (SMCs), designated “smooth muscle-like cells” [67, 68]. Smooth muscle-like cells express  $\alpha$ -smooth muscle actin and vimentin, but not desmin, and probably represent a subset of phenotypically immature SMCs [69, 70] (figure 2). They are commonly oriented longitudinally along the vessel lumen, rather than in a circular orientation. Medial hypertrophy of arteries and muscularisation of arterioles are observed in pre-acinar and intra-acinar small pulmonary arterial branches. In severe PH, COPD larger pulmonary arteries also display some degree of medial hypertrophy as well as intimal laminar fibrosis. The occurrence of vascular remodelling in COPD is thought to be related to the synergic action of tobacco smoke and hypoxia. Vascular changes have been documented in non-hypoxaemic COPD

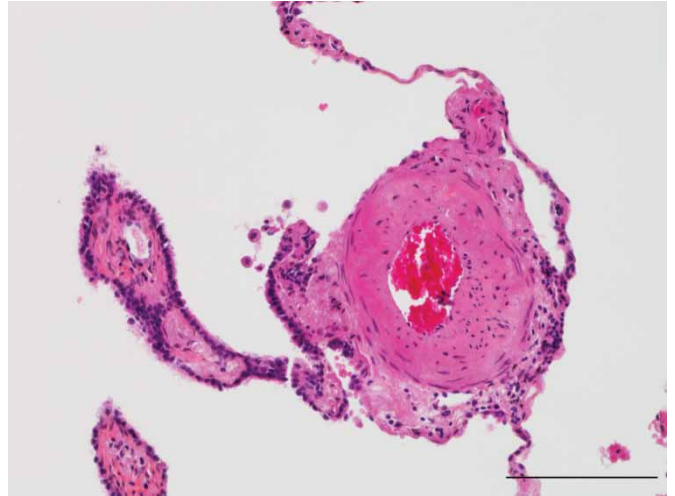


FIGURE 1 Small pulmonary arteries exhibiting irregular intimal and medial thickening within emphysematous lung parenchyma. Haematoxylin-eosin-saffron staining. Scale bar=100  $\mu$ m.

patients, suggesting that the latter can develop independently of hypoxia-induced vasoconstriction. It should be borne in mind, however, that tissue hypoxia may be present focally, in hypoventilated lung areas, so hypoxia-induced haemodynamic changes may occur in the absence of systemic hypoxaemia [71].

In advanced COPD, destruction of the pulmonary vessels is usually mentioned as a contributor to the elevation of mPAP [52]. Nevertheless, studies correlating computed tomography lung tissue density with haemodynamics did not provide any significant association [72, 73]. Nonetheless, it is generally assumed that the capacity of pulmonary vessels for haemodynamic adaptation may be further impaired in the presence of parenchymal destruction, exposing patients to exercise-induced PH [74].

Hypoxia is not a unique contributor to the development of vascular changes in COPD. Indeed, as indicated above, pulmonary vascular remodelling may also be observed in non-hypoxaemic mild COPD and oxygen therapy is usually insufficient to reverse PH in COPD patients. The sum of these two observations indicates that other mediators are involved in vascular alterations [75, 76]. Cigarette smoking may result in endothelial dysfunction, with enhanced production and release of growth factors and affluence of

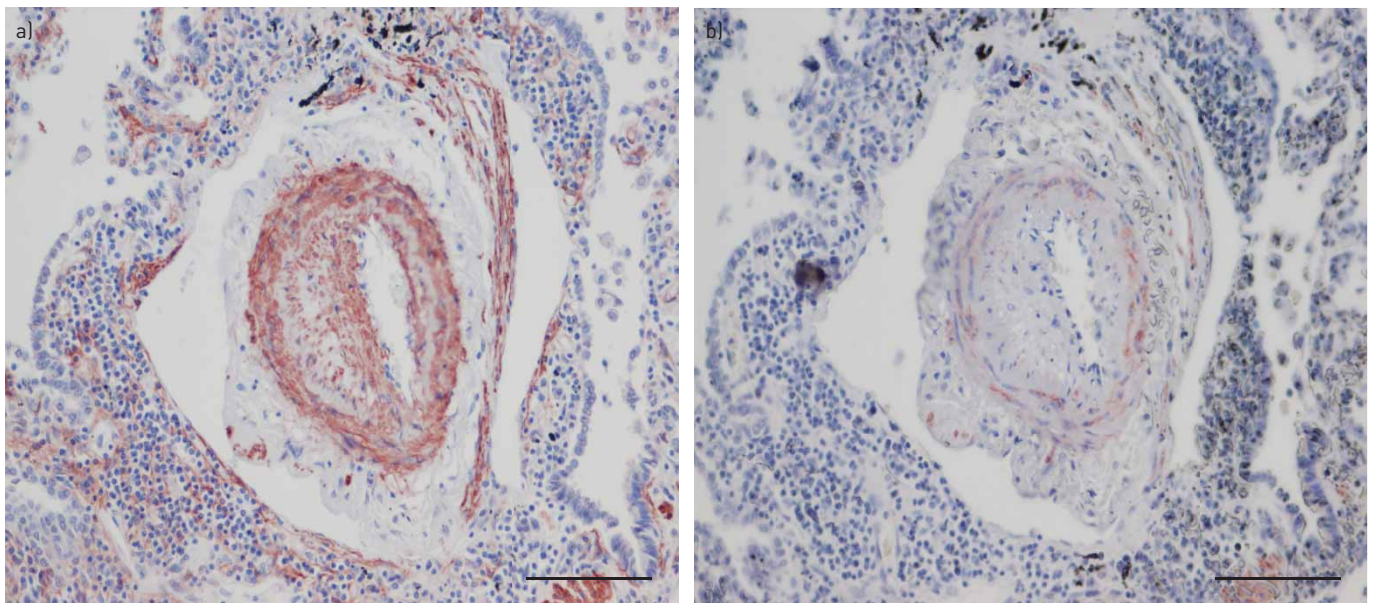


FIGURE 2 a) Actin immunostaining of a remodelled small pulmonary artery. The neointima is thickened by the affluence of cells phenotypically similar to smooth muscle cells (SMC-like cells), expressing actin. b) Desmin immunostaining of the same remodelled pulmonary artery. The neointimal SMC-like cells do not express desmin, unlike medial smooth muscle cells. Both images courtesy of P. Dorfmüller, Hôpital Marie Lannelongue, Le Plessis Robinson, France. Scale bars=100  $\mu$ m.

inflammatory cells, leading to smooth muscle cells proliferation and vessel wall thickening [77–79]. The biological pathways disrupted by tobacco smoke are largely unexplored, but it is thought that they may be related to oxidative damage [80]. ROS are effective in destroying lung parenchyma by direct damage as well as by inducing inflammation [81]. Nitric oxide (NO) may combine with superoxide ( $O_2^-$ ) to form the strong oxidant peroxynitrite ( $ONOO^-$ ) [82].  $ONOO^-$  may induce both apoptosis and low proliferation in alveolar epithelial cells [83]. The impact of  $ONOO^-$  on cell proliferation and survival is achieved *via* c-Jun N-terminal kinases (JNK) and Src (sarcoma-family kinases) phosphorylation and upregulation of Rtp801 [83]. Moreover,  $ONOO^-$  can also react with protein tyrosine residues to form the nitrotyrosine, which is considered a marker of cell damage and is increased in COPD lungs [84, 85]. Tobacco smoke also generates products of lipid peroxidation, such as F2-isoprostanes, 4-hydroxy-2-nonenal, acrolein and malondialdehyde, which trigger cellular redox signalling effects. The oxidative stress can influence primal cell signalling and induce nuclear histone modifications, enhancing the tissue inflammatory response. Investigations of the impact of tobacco smoke on lung circulation in animals have confirmed the hypothesis that tobacco exposure induces muscularisation of pre-capillary vessels and that this happens before the occurrence of emphysema [86, 87]. Nevertheless, there is no doubt that hypoxia plays a decisive role in COPD vascular changes leading to PH. As airflow obstruction increases, the effects of hypoxaemia on pulmonary circulation may add to the detrimental effects of tobacco smoke.

### Pathogenesis of PH in IPF

IPF is characterised by the progressive scarring of lung tissue, which leads functionally to the deterioration of gas exchange capacity through a restrictive lung function abnormality, in combination with shunting of blood through the vessels running by the fibrotic lung. Usual interstitial pneumonia (UIP) is the histopathological equivalent of IPF. UIP is characterised by a heterogeneous distribution of the fibrosis, predominating in subpleural and basal zones, and results from the accumulation and confluence of fibrosis, which characteristically begins as so-called fibroblast foci, small foci of subepithelial fibroblasts and increased matrix. Scarred areas are juxtaposed next to conserved parenchymal fields, without intervening transition zones. Areas of “active” ongoing fibrosis (represented by fibroblastic foci) coexist with areas of hyaline fibrosis within the scarred regions. Fibroblastic foci are characterised by aggregates of actively proliferating myofibroblasts within a myxoid matrix, oriented with the long axis parallel to the long axis of the alveolar septa. Such aggregates are thought to derive from the organisation of small interstitial exudates. Fibroblastic foci can be readily identified because of their light-staining matrix, rich in proteoglycans, contrasting with the “dark-staining” fibrosis or honeycomb areas. “Honeycomb” changes, which consist of large airspaces surrounded by fibrotic tissue, covered by bronchiolar-type epithelium or hyperplastic type 2 pneumocytes, result from lung architectural restructuring following alveolar injury. A mild inflammatory infiltrate, consisting of small lymphocytes, plasma cells and sometimes neutrophils and eosinophils, is often seen within fibrotic or honeycomb areas. Remodelling of pulmonary vessels is observed both within the fibrotic areas and in unscarred lung parenchyma (figure 3). In the fibrotic zones, small pulmonary arteries display substantial intimal fibrosis and medial hypertrophy. The pulmonary veins are also affected by remodelling,

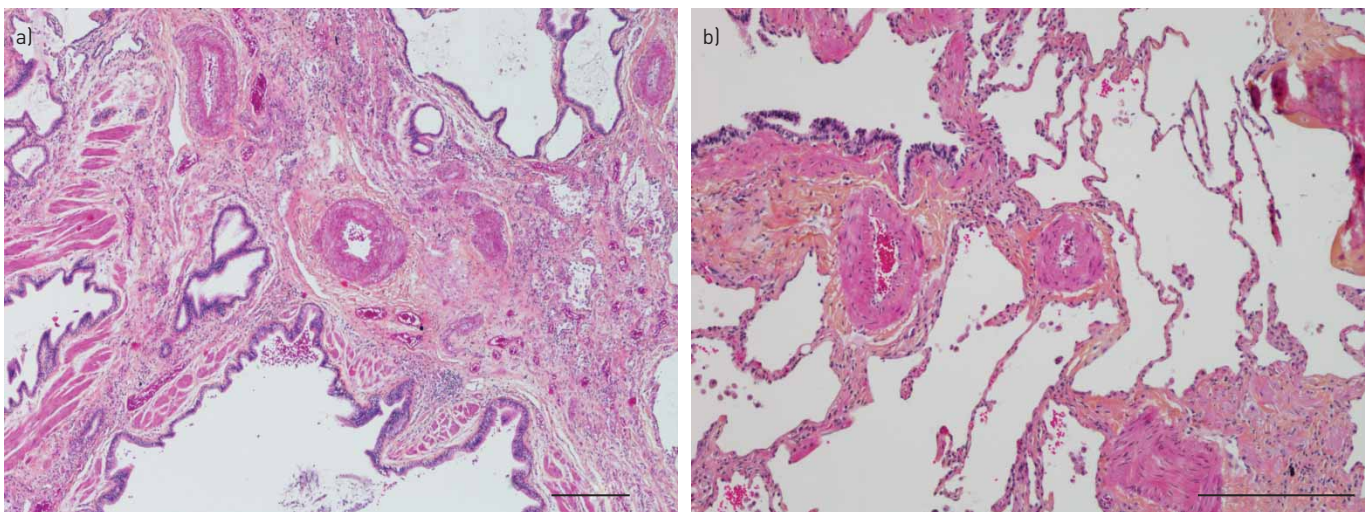


FIGURE 3 a) Small pulmonary arteries within a fibrotic area (usual interstitial pneumonia lung). The narrowing of the vascular lumen is the result of both intimal fibrosis and medial smooth muscle increase. Haematoxylin–eosin–saffron staining. Scale bar=250  $\mu$ m. b) Remodelled small pulmonary arteries outside fibrosis in usual interstitial pneumonia lung. Haematoxylin–eosin–saffron staining. Scale bar=200  $\mu$ m.

exhibiting luminal narrowing by an intimal fibrosis. In architecturally conserved lung zones, some remodelling is seen in pulmonary venules, exhibiting luminal occlusion by acellular fibrous tissue. Such a finding often coexists with alveolar capillary multiplication and muscularisation of pulmonary arterioles [88].

PH in IPF is increasingly prevalent as IPF progresses. In view of the histological features mentioned above, the occurrence of PH in IPF cannot be explained solely by hypoxic vasoconstriction and the loss of capillary bed through the scarring tissue. To improve the therapeutic management of IPF-associated PH, there is a need to understand the pathobiology of the fibrotic process itself, as well as of the associated vascular remodelling. The density of blood vessels is low in scarred lung regions, while the non-fibrotic tissues at the edge of such regions are richly vascularised. In scarred areas, complex biological processes perturbing the vessels' homeostasis are ongoing. Notably, the imbalance between angiogenetic mediators (such as VEGF) and angiostatic factors (such as pigment epithelium-derived factor (PEDF)) dramatically contributes to pulmonary circulation restructuring, with an increased risk of developing PH. In addition, the effects of oxidative substances and of paracrine molecules released in scarring areas may trigger cellular stress responses and pathobiological processes such as an endothelial to mesenchymal transition, participating in the vascular remodelling.

The pathobiology of vascular remodelling in IPF is complex, and diverse molecules are involved. Such molecules are of potential interest as biomarkers of the progression of the disease and as potential targets of novel therapies.

TGF- $\beta$  is a master regulator of extracellular matrix deposition and fibroblasts proliferation, and it is an acknowledged profibrogenic factor. In the lung, TGF- $\beta$  is produced by macrophages as well as epithelial cells and fibroblasts. It is secreted in the form of an inactive precursor that is activated by proteolytic cleavage. Smad2/3, mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K) and Rho GTPase pathways are activated by interaction of the TGF- $\beta$  receptor with its ligand. Notably, activation of the Smad pathway appears to be crucial for fibroblast proliferation and extracellular matrix (ECM) production culminating in fibrosis [89]. TGF- $\beta$  influences the inflammatory response through the inhibition of immune cell proliferation [90], and is also involved in angiogenesis in a dose-dependent manner [91]. It promotes endothelial cell (EC) apoptosis, differentiation and vascular smooth muscle cell (VSMC) recruitment at high doses, while activating EC at low doses [92]. High TGF- $\beta$  levels in fibrotic areas may indeed contribute to local EC apoptosis, leading to vascular depletion and to an increase in muscularisation of the pulmonary arteries. Indeed, apoptotic ECs in pulmonary fibrotic areas may release growth factors driving VSMC proliferation, such as the platelet-derived growth factor (PDGF) [93–95]. Moreover, the depletion of ECs and endothelial dysfunction in those areas may lead to a decreased production and dropping of vasodilators and inhibitors of VSMC proliferation, such as prostacyclin and NO [95]. In parallel with such phenomena, enhanced release of vasoconstrictors such as endothelin-1, angiotensin and thromboxane A<sub>2</sub> is documented, contributing to vascular remodelling and to the increase in mPAP [96, 97].

VEGF is a chief driver of angiogenesis, with a major role, also, in the homeostasis maintenance of the vascular system. ECs need VEGF signalling for survival and proliferation, and VEGF inhibition leads to capillary alveolar damage, resulting in emphysema. VEGF expression is induced by hypoxia and TGF- $\beta$  stimulation. In lungs, VEGF is mainly secreted by macrophages, epithelial cells, ECs and mesenchymal cells [98]. In IPF, VEGF is markedly reduced within fibrotic areas [99, 100], where its depletion is correlated with EC apoptosis. The level of VEGF is also reduced in the bronchoalveolar lavage fluid of IPF patients.

PDGF is suggested to play a role in fibrosis progression and in pulmonary artery remodelling in IPF, by promoting fibroblast and VSMC proliferation and migration, leading to excessive arterial muscularisation and intimal fibrosis [101]. This finding is also sustained by investigations on experimental models [102]. It is postulated that PDGF acts in an early stage of IPF vascular remodelling, because it can be detected in ECs and VSMCs. PDGF is no longer found in lung vessels in late-stage disease [103]. PDGF could be a relevant target for the inhibition of fibrogenesis and vascular remodelling.

PEDF is found in lung mesenchymal cells, ECs and in bronchial cells. This molecule has a strong angiostatic effect, inhibiting VEGF-induced angiogenesis and inducing EC apoptosis. PEDF can be involved in lung fibrosis, by promoting fibroblast growth, because PEDF is found in the extracellular matrix [104]. Moreover, PEDF inhibition may be an interesting therapeutic target.

Other potential molecular targets limiting fibrosis and vascular remodelling include endostatins, endothelin-1 and angiotensin-II (ATII). In particular, endostatins have an angiostatic effect, through the inhibition of VEGFR2 and matrix metalloproteinase 2 (MMP-2), and are potentially involved in pulmonary artery remodelling in IPF [105, 106]. Those molecules are generated by the injured lung epithelium from the proteolytic cleavage of collagen XVIII, and their levels in lung tissue seem to be correlated to the degree of lung parenchymal damage in IPF [107, 108]. Endothelin-1 has vasoconstrictive effects and is also an

inductor of VSMC growth [97]. Although endothelin seems to be involved in IPF PH, the pharmacological inhibition of its receptor did not result in sufficient clinical improvement [109]. The elevation of ATII within fibrotic areas may therefore contribute to vascular remodelling, providing a solid rationale for research focused on AT inhibition. Moreover, the relevance of ATII as a prognostic biomarker can also be kept in consideration [110].

### Conclusion

Diverse mechanisms are involved in the pathogenesis of pulmonary hypertensive vasculopathy in the setting of parenchymal lung diseases and/or hypoxia. Pulmonary vasoconstriction, triggered by hypoxia, represents the main pathogenetic mechanism in PH in high-altitude dwellers and in patients with sleep disordered breathing. The persistence of vasoconstriction leads to structural changes in lung vasculature, affecting mostly the small pulmonary arteries. In COPD, hypoxia, the exposure to tobacco smoke and the loss of pulmonary vessels contribute to pulmonary haemodynamic impairment, through vascular remodelling. Finally, PH in UIP is characterised by structural changes involving pulmonary vessels both within the fibrotic areas and outside fibrosis. The biological mechanisms underlying vascular remodelling in UIP are incompletely understood and encompass an altered balance between angiostatic and angioproliferative mediators and the effects of both oxidative and paracrine molecules perturbing vascular cell homeostasis. The occurrence of PH in parenchymal lung disease complicates the therapeutic attitude and requires the guidance of specialist consultation.

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