

STATE-OF-THE-ART PAPERS

## Development and Pathology of Pulmonary Hypertension

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The Development and Pathology working group was charged with reviewing the present knowledge, gaps in understanding, and areas for further studies in a broad range of themes. These themes in pulmonary vascular biology and pathobiology involved: 1) pulmonary vascular development; 2) pulmonary vascular disease accompanying fetal development and perinatal life; 3) properties of pulmonary vascular endothelial cells; 4) role of bone marrow cells in pulmonary vascular disease; 5) insights into pulmonary thromboembolic disease; 6) role of pathology in the assessment of pulmonary vascular disease; and 7) considerations of tissue banking for research in pulmonary hypertension. These important goals provide a blueprint for future research that may significantly impact our present and future understanding of pulmonary hypertension. (J Am Coll Cardiol 2009;54:S3-9)

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Recent work has shown that in the normal lung, the pre-acinar arteries and post-acinar veins form by vasculogenesis from the splanchnopleural mesoderm of the lung bud (1). Serial reconstruction of human embryos indicates that the pulmonary arteries and veins arise by the sustained addition of newly formed coalescing endothelial tubes derived by vasculogenesis from the mesenchyme of the lung around the airway terminal buds. This occurs while pre-acinar airway branching continues. Angiogenesis appears to predominate after 15 to 17 weeks' gestation during intracinar formation. Many of the signaling molecules expressed in the early human embryo, such as endothelial cell nitric oxide synthase (eNOS), vascular endothelial growth factor and its receptors, angiopoietins, the endothelial-specific receptor TIE-2, transforming growth factor (TGF)- $\beta$ , hypoxia inducible factors 1 $\alpha$  and 1 $\beta$ , and the bone morphogenetic proteins (BMPs) and their receptors, have been studied in vitro and in transgenic models, but how these and other molecules orchestrate developmental processes in life is still unclear. Several important questions remain to be addressed. These include: 1) the elucidation of the signaling processes responsible for the

induction of the pulmonary capillary networks forming around each epithelial bud, the mechanisms regulating the coalescence of the endothelial tubes in the mesenchyme, their fusion with the growing pulmonary artery, and the organization of the randomly formed endothelial cells (e.g., lining up alongside the developing airways, arteries on one side and veins on the other); 2) the precise role of embryonic and fetal lung signaling molecules and how they relate to one another; 3) the role of oxygen-sensing mechanisms, which have been described in the postnatal lung, in utero, and at birth; 4) understanding the crosstalk between epithelial and vascular cells in this complex orchestrated developmental process; 5) clarifying the interplay between central pulmonary arteries and the proximal intrapulmonary arteries during development (e.g., whether they develop simultaneously or independently); 6) determining the extent to which extrapulmonary cells are incorporated into the developing pulmonary vasculature; 7) establishing the basis of the heterogeneity of all the structural components of the vessel wall; 8) determining the potential for vascular cell maintenance of genetic memory that can influence cell phenotype and the response to injury in postnatal life; and 9) ascertaining whether the primordial human lung has a functional circulation throughout its development, as has been shown in the chick embryo.

The developing pulmonary arteries and veins become invested by smooth muscle cells from different anatomic sources. The smooth muscle cells show orderly acquisition of specific cytoskeletal components, but we need to understand the expression patterns of other features, such as K<sup>+</sup> channels, which would allow the identification of

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**Abbreviations  
and Acronyms**

<b>BMP</b>	= bone morphogenetic protein
<b>BMPR</b>	= bone morphogenetic protein receptor
<b>BPD</b>	= bronchopulmonary dysplasia
<b>CDH</b>	= congenital diaphragmatic hernia
<b>CTEPH</b>	= chronic thromboembolic pulmonary hypertension
<b>eNOS</b>	= endothelial nitric oxide synthase
<b>EPC</b>	= endothelial progenitor cell
<b>IPAH</b>	= idiopathic pulmonary arterial hypertension
<b>PAH</b>	= pulmonary arterial hypertension
<b>PCH</b>	= pulmonary capillary hemangiomatosis
<b>PH</b>	= pulmonary hypertension
<b>PPHN</b>	= persistent pulmonary hypertension of the newborn
<b>PVOD</b>	= pulmonary veno-occlusive disease
<b>TGF</b>	= transforming growth factor
<b>WHO</b>	= World Health Organization

specific smooth muscle cell phenotypes. Furthermore, it remains unclear whether such phenotypic features would pertain to the specific origin of the smooth muscle cells, either from the lung bud mesenchyme or from a specific extrapulmonary source. We strongly believe that pulmonary vascular development should be considered a critical determinant of the propensity to develop pulmonary vascular disease in later life.

### Mechanisms Regulating Crosstalk Between Airway and Vasculature During Development and the Pathogenesis of Neonatal Lung Diseases

In addition to being among the primary causes of pulmonary hypertension (PH), pulmonary vascular diseases are strongly associated with abnormalities of lung development. Normal lung vascular development is critical for successful adaptation and survival at birth and in postnatal life. Studies of mechanisms that regulate development of the pulmonary circulation have been rela-

tively limited and largely descriptive in nature. However, recent observations have challenged older notions that the development of the blood vessels in the lung passively follows that of the airways. The mechanisms by which the lung successfully achieves normal gas exchange require the growth and maintenance of an intricate system of airways and vessels, including the establishment of a thin yet vast blood-gas interface, which is continuously modulated under both physiologic and pathologic conditions (2,3).

Increasing evidence suggests that lung blood vessels actively promote normal alveolar growth during development and contribute to the maintenance of alveolar structures throughout postnatal life (4-7). Disruption of angiogenesis during lung development can impair alveolarization, and preservation of vascular growth and endothelial survival may promote lung growth and structure of the distal airspace. Understanding how alveoli and the underlying capillary network develop and how these mechanisms are disrupted in disease states is critical for developing efficient therapies for lung diseases characterized by impaired alveolar structure.

Recent advances in the field of vascular biology have provided novel experimental tools to probe how pulmonary blood vessels are assembled during early embryonic and fetal development. These tools may provide important new information regarding pediatric lung diseases associated with PH. Developmental abnormalities of the pulmonary circulation contribute to the pathophysiology of such diseases as persistent PH of the newborn (PPHN), lung hypoplasia, congenital diaphragmatic hernia (CDH), congenital heart disease, and others. This may be especially important in understanding the pathogenesis of bronchopulmonary dysplasia (BPD), which is the chronic lung disease that follows premature birth. BPD is characterized by arrested lung growth, which may play a central role in the ensuing decreased alveolarization and a dysmorphic vasculature (8-10).

Experimental data further suggest a potential therapeutic role for modulation of angiogenesis for lung diseases that are characterized by arrested alveolar growth, such as BPD. The epidemiology and risk factors for PPHN, BPD, and CDH remain to be defined, along with the gene-environment interactions underlying their pathobiology and biomarkers (maternal, neonatal) to identify at-risk infants. Clinical trials are needed to improve therapeutic interventions to treat PH and enhance distal lung growth and function. Noninvasive methods to assess pulmonary hemodynamics and lung vascular growth and structure are also needed. Furthermore, advances in stem cell biology suggest at least potential roles for endothelial progenitor cells (EPCs) and mesenchymal stem cells in the pathogenesis or treatment of lung vascular disease, especially in experimental models of BPD (11,12).

Future work aimed at better defining the basic mechanisms of lung vascular growth and development will likely lead to novel therapeutic approaches to diseases associated with impaired vascular growth or PH. In addition, there is a clear need to better understand the developmental physiology of the lung circulation, especially regarding perinatal (maternal, placental, fetal, and neonatal) mechanisms that: 1) regulate vascular tone and reactivity in utero and maturational changes during normal development; 2) alter vascular tone, reactivity, and function in models of PPHN, including disruption of growth factors, cytokines, and related signaling pathways; and 3) disrupt lung vascular growth and structure. In addition, further studies are needed to clarify the normal physiology and pathobiology of EPCs. Naive or transfected EPC strategies may have a critical role in the restoration of vascular growth and function with impact on maintenance of alveolar structure. EPCs may serve as potential biomarkers used to diagnose neonatal pulmonary vascular disease over the lifetime of an individual.

For the realization of these goals, there is a pressing need for multicenter interventional studies aimed at the prevention of BPD and the treatment of severe PH in BPD and CDH. These approaches should be spearheaded by inter-

national working groups of pathologists with special expertise in lung development and PH for the diagnostic review of cases. Consortia are needed to collect, process, and study lung tissues in order to enhance clinical research.

### **Pulmonary Endothelial Cell Biology in Health and Disease**

Endothelial cells display remarkable heterogeneity in structure and function, all along the pulmonary arterial-capillary-venous axis. This segment-specific heterogeneity can be illustrated by examining lectin-binding patterns within the pulmonary circulation (13,14). Extra-alveolar endothelial cells (artery and vein) interact with the lectins *Helix pomatia*, but not with *Griffonia simplicifolia*, whereas microvascular endothelial cells (capillary) interact with *Griffonia simplicifolia*, but not with *Helix pomatia*. Using lectin binding as one approach to guide the study of pulmonary endothelial cell function, it has become apparent that microvascular endothelial cells possess greater adhesion strength, unique mechanosensing properties, different organization of signaling networks (e.g., cAMP, calcium, and oxidants), high glycolytic flux, and a discrete distribution of organelles, compared with pulmonary artery endothelial cells (13,14). It is interesting that the site-specific functions of pulmonary artery and microvascular endothelial cells are retained when populations of these cells are studied in culture, providing the opportunity to dissect mechanisms underlying phenotypic heterogeneity. Recent *in vitro* studies have revealed that endothelial cell populations are enriched with progenitor cells, which account for the cells' growth and angiogenic/vasculogenic potential (15). Whereas EPCs comprise only 5% to 10% of the pulmonary artery endothelial cell colony, nearly 50% of microvascular endothelial cells are made up of progenitor cells. EPCs may play a key role in vascular development, maintenance in the post-natal period, and repair following injury. However, progenitor cells that reside within the vascular wall may also play unappreciated roles in vascular disease.

Idiopathic pulmonary arterial hypertension (IPAH) is a prominently pre-capillary disease that is characterized by large and intermediate-sized pulmonary arteries/arterioles in which there is intimal hyperplasia with medial and adventitial hypertrophy and hyperplasia. In advanced stages of PH, cells originating within the vessel wall (smooth muscle cells, endothelial cells, and fibroblasts), and potentially cells from the circulation, assemble in a "plexiform" lesion (16–18). Disordered endothelial cell growth has been documented in patients with IPAH, even in pulmonary artery endothelial cells that are isolated from the patients and grown in culture (16). These findings support the idea that endothelial cells acquire a pro-proliferative, apoptotic-resistant phenotype that, in the case of the plexiform lesion, contributes to the loss of the endothelial cell monolayer (17).

We have only a rudimentary understanding of how endothelia contribute to the vascular pathology in IPAH. At present, the origin of endothelial cells within the plexiform lesion is not resolved; it may involve the participation of large or small pulmonary artery endothelial cells, with variable contribution of bone marrow precursors. However, cells within the lesion interact with *Griffonia simplicifolia*, consistent with a microvascular phenotype. It is not clear whether PH selects for progenitor cells within the vessel wall and whether cells contributing to either the intimal or plexiform lesion represent an overgrowth of EPCs. It is also not clear whether epigenetic modifications or somatic mutations contribute to the uncontrolled growth of endothelial cells in IPAH patients. Indeed, considerable work is needed to address these and related concerns regarding fundamental endothelial cell biology in IPAH.

In summary, questions that remain to be answered are: 1) What are the molecular determinants of an EPC? 2) What is the unique cell biology of an EPC? 3) Is there an increased propensity for progenitor cells to cause an endothelial lesion? 4) Do hyperproliferative endothelial cells arise by somatic mutations, epigenetic modifications, or selection of progenitor cells (e.g., apoptosis resistance)? 5) Where are the EPCs located *in vivo*, and which signals activate their growth?

### **Role of Bone Marrow Cells in Pulmonary Vascular Structure and Remodeling**

The term "bone marrow-derived cells" characterizes a wide variety of cell populations that differ with respect to their biological characteristics, expression of marker molecules, and biological functions. These cells range from macrophages to inflammatory cells, EPCs, and fibrocytes. Whereas some of these cell populations may represent targets for potential treatments (e.g., macrophages, inflammatory cells, and fibrocytes), others, such as EPCs, might be utilized as therapeutic agents. However, the rationale for the therapeutic use of such cells is unclear, and the evidence for beneficial effects is still limited. In general, it is accepted that circulating EPCs and fibrocytes play important roles in angiogenesis and vascular remodeling (19,20). It should be pointed out, however, that the literature is replete with conflicting data reporting significant differences in the contribution of EPCs to neoangiogenesis. Since EPCs were first described (21), their identity and relative contribution to neovasculature formation have remained controversial. Conflicting reports of the extent of the contribution of these cells to new blood vessel formation can be ascribed to a limited analysis of the EPC phenotype in each study and a lack of more definitive methods for distinguishing vessel-incorporated bone marrow-derived endothelial cells and intimately associated perivascular cells. Yet another source of variability may result from the specific disease processes being investigated (tumor neovascularization, hypoxia-

induced neovascularization, PAH, cardiac ischemia, limb ischemia, and others).

The incorporation of endothelial or smooth muscle cells from bone marrow into the growing, adult, or aging lungs appears to be negligible (22). Circulating fibrocytes, hybrid cells expressing myeloid and fibroblast markers, may be recruited from the bloodstream to promote tissue remodeling during organ and vascular fibrosis (19,23). These fibrocytes may represent a new target to prevent tissue remodeling, but their precise role in the pathogenesis of PAH remains unclear. It will be interesting to determine whether EPCs contribute to this process via integration into the endothelium and whether the same process also occurs in the lung. The finding that TGF- $\beta$ 1 induces endothelial cells to undergo endothelial-to-mesenchymal transformation, whereas BMP-7 has been found to preserve the endothelial phenotype, may offer an interesting opportunity to intervene in this process.

There is still uncertainty about the therapeutic and pathogenetic impact of bone marrow cells in PH. Therapeutic infusion of in vitro cultured and eNOS-transfected EPCs increased survival and reduced right ventricular pressure and hypertrophy in rats with monocrotaline-induced PH (24). In mice, bone marrow injection attenuated monocrotaline-induced PH but aggravated chronic hypoxic PH (25). An open, placebo-controlled pilot study on the effect of autologous EPC infusion in IPAH reported an improvement in exercise capacity and hemodynamics (26). However, compelling evidence is still lacking that autologous EPC infusion has decisive effects in the development of PH. More studies are required to come to definitive conclusions and to elucidate pathogenetic mechanisms that might explain the effects of circulating bone marrow-derived cells.

In summary, there is a need to clarify: 1) whether bone marrow and mononuclear cells are of potential therapeutic value with respect to pathogenetic mechanisms and clinical efficacy; 2) whether improvement of cell-based therapies will have an impact on clinical outcome; 3) whether the extent of the contribution of EPCs from different organs plays a role in the pathogenesis of PH; 4) whether the contribution of different types of EPCs affects the pathogenesis of PH; and 5) whether there is indeed a relatively low-level contribution of bone marrow-derived EPCs and potentially of smooth muscle cell precursors in pulmonary hypertensive vasculopathy, which emphasizes the requirement for accurate quantitative assessments. All clinical studies should be performed using precisely defined precursor cell populations that have been isolated according to accepted and reproducible protocols.

### **Cellular and Molecular Underpinnings of Large Pulmonary Arteries and Microcirculation in Thromboembolic Disease**

Chronic thromboembolic pulmonary hypertension (CTEPH) is a rare disease that is estimated to result in approximately

3.8% of all cases of acute pulmonary embolism (27). Several mechanisms have been postulated to cause CTEPH after an acute embolic event, including recurrence of embolism in 2.5% to 7% of adequately treated pulmonary embolic events, in situ thrombus propagation into branch pulmonary vessels, and failure to resolve the initial embolus, leading to large- and small-vessel vasculopathy (28). In the proximal pulmonary arterial tree, unresolved pulmonary emboli cause vascular obstruction of the vessel lumen by 2 mechanisms: 1) direct occlusion of the vessel lumen; and 2) induction of secondary endothelial changes of cellular hyperplasia, webbing, and incomplete clot remodeling. In a subset of patients with CTEPH, small pulmonary arterioles also manifest a pathologic process similar to that seen in PAH, whereby these vessels become excessively thickened by muscular hypertrophy and fibrointimal hyperplasia, leading to eventual occlusion (29,30). The importance of pulmonary arteriolar/capillary remodeling in the development of CTEPH is supported by the following facts: 1) there is lack of correlation between elevated pulmonary arterial pressure and the degree of angiographic pulmonary vascular bed obstruction in humans; 2) PH can progress in the absence of recurrent venous thromboembolism; and 3) total pulmonary vascular resistance is still significantly higher in CTEPH patients than in acute pulmonary embolism patients with a similar percentage of vascular bed obstruction (31,32).

In addition to physically occluding the vessel lumen, thrombi act as a physical trap for circulating mitogenic, inflammatory, and vasoreactive factors, many of which can interfere with or alter the function of the adjacent endothelium. Endothelial barrier dysfunction, leading to increased endothelial permeability, is evident in response to barotrauma, inflammation, acute lung injury, and clot retention (33). Changes in shear stress due to vascular occlusion may also influence endothelial cell function and production of vasoreactive and mitogenic factors. Because platelets are a source of numerous inflammatory, vasoactive, mitogenic, and chemotactic factors (e.g., thromboxane A<sub>2</sub>, serotonin, platelet-activating factor, angiopoietin-1), their aggregation in the thrombus should influence endothelial function. In summary, a retained clot in the pulmonary vascular tree is thought to be an instigator of endothelial permeability, resulting in access of growth factors, cytokines, mitogens, and vasoreactive factors to both pulmonary artery endothelial cells and pulmonary artery smooth muscle cells. There is also preliminary evidence that thromboemboli in the proximal pulmonary arterial tree contains EPCs that migrate into the vessel wall and contribute to the remodeling process (34).

Although the clinical characteristics of CTEPH have been well defined, understanding of its cellular and molecular mechanisms is lacking. The prevailing opinion is that PH resulting from chronic thromboembolism is a consequence of unresolved pulmonary emboli with adjacent vascular remodeling as well as a secondary vasculopathy in small pulmonary arterioles and capillaries. Mechanistic parallels with other forms of PH (e.g., IPAH) have been

drawn, particularly in light of similar histopathology in some distal arteriolar beds (35). Many questions remain, the most fundamental of which deal with elucidation of risk factors and pathogenesis of CTEPH. Future research should answer several questions, including: 1) why most patients with pulmonary embolism do not develop CTEPH; 2) why some individuals fail to resolve acute thromboembolic obstruction of the pulmonary vascular tree; 3) why some CTEPH patients have postoperative residual PH; and 4) whether patients with unsatisfactory postoperative outcomes have cellular, molecular, and genetic abnormalities in the pulmonary vasculature similar to those in patients with IPAH. Furthermore, investigation is needed to define the role of: 1) inherited defects in coagulation, incomplete/defective fibrinolytic pathways; 2) pulmonary artery endothelium contributing to the intimal changes that occur adjacent to a thromboembolism in main, lobar, and segmental pulmonary arteries; 3) infiltrating cell types from the thromboemboli; cellular proliferation of fibrocyte, myocyte, and endothelial subtypes within the vessel wall; 4) bone marrow progenitors as either bystanders or active remodeling agents; and 5) bone morphogenetic protein receptor (BMPR)-1A and angiopoietin-1/TIE-2 signaling in CTEPH. From a scientific and diagnostic point of view, there is need: 1) to develop an animal model for CTEPH; 2) to design better diagnostic modalities to distinguish CTEPH from IPAH; and 3) to study signaling pathways and patterns of gene expression and understand their potential clinical significance in the treatment of patients before and after pulmonary endarterectomy.

### Pathology of PH: Evian, Venice, and Beyond

The pathologic interpretation of pulmonary vascular remodeling in PH has been central in studies related to the disease. As outlined by Zaiman *et al.* (36), the importance of pathology in the clinical management or even the diagnosis of PH has fallen behind the direct assessment of pulmonary artery pressures using pulmonary arterial catheterization or estimation of right ventricular pressures using echocardiography. Infrequently, lung biopsies have served as a basis for the diagnosis of PH. Furthermore, the importance of proper identification of the different forms of pulmonary vascular remodeling, or so-called pulmonary vascular lesions, in studies of the pathogenesis of PH and the effects of potential treatments cannot be underestimated. Key questions concern the role of pathology of PH in the near future. Which recommendations regarding pathology of PH will best serve clinicians and, ultimately, patients? Is there a classification that should be added to or substituted for prior classifications?

To answer these questions, it is useful to review the different classifications used for the last 50 years. These originated from international conferences, that is, the 1973 World Health Organization (WHO) symposium, the 1998 Evian, France, international meeting, and the 2003 inter-

national meeting held in Venice, Italy. The 1973 WHO symposium stressed the contribution of pulmonary vascular pathology in the classification of the disease, an emphasis that was eventually superseded by developments in hemodynamic assessment and novel clinical algorithms. The Evian meeting recommended a descriptive approach, based on acknowledged limitations in interpreting and correlating pathology with specific causes, severity grades, and outcomes (37). This descriptive approach emphasized a combination of the classic microanatomic and lesional nomenclature with the underlying cellular components that characterize the lesion. It therefore followed the recommendation that lesional descriptors, such as eccentric or concentric, plexiform or dilation lesions, be identified based on their location in the intimal, medial, and/or adventitial regions. An attempt to complement this approach with one based on the recognition of particular cells involved in the structure of a lesion implied that investigation of the baseline abnormalities of pulmonary vascular cells might offer an insight into the underlying diagnosis and potential therapeutic responses of different forms of PH. This approach attempted to incorporate the use of immunohistochemical markers and opened the possibility that functional markers related to disease pathogenesis might serve as potential tools for enhancing the importance of pathology in the diagnosis and prognosis of PH. As these dysfunctions are segregated by vascular cell types, most prominently endothelial (38) or smooth muscle cells, the application of these tools is expected to provide a much-needed insertion of pathology into the clinical workup of patients with PH. The identification of mutations of receptors in the TGF- $\beta$  family, particularly of BMPR-2, further highlights the overall need for such an approach.

The Venice symposium (39) made several recommendations, the intent of which was to “provide a more descriptive approach to . . . the main vascular changes and the associated pathological alterations.” The report discussed the descriptions of intimal, medial, and adventitial thickening. The intimal lesions were segregated into concentric cellular, concentric acellular, and eccentric lesions. Complex vascular lesions were categorized as an individual category of lesions. These included plexiform and dilation lesions, arteritis, occlusive venous thrombotic lesions, and pulmonary microvasculopathy (also known as pulmonary alveolar capillary hemangiomatosis). Given the common denominator of BMPR-2 mutations in some forms of pulmonary veno-occlusive disease and pulmonary microvasculopathy, the recommendations leave open a potential continuum between pre-capillary PH and venous disease associated with PH.

In the present discussions, it was agreed that pathology should have a primary role in documenting the types and extent of vascular lesions and associated morphologic alterations in lung tissue from patients and animals with PH. This systematic approach should serve to correlate pulmonary vascular pathology with clinical presentation/outcome.

A systematic approach based a multilevel analysis should be outlined, primarily at the examination of hematoxylin- and eosin-stained slides, further supported by cell structural immunohistochemical markers, and potentially in the future, complemented by the detection of pathobiologically relevant markers. These recommendation are very much in line with those made in the Evian meeting (37).

### **Pulmonary Vascular Pathology: Recommendation on Reporting and Examination of Pulmonary Venopathy**

The proper interpretation of pulmonary vascular remodeling requires that the pulmonary vessels be correctly identified, with determination of topography of the vascular lesions. Arteries have to be named according to their location and accompanying airway structure, with approximate estimation of diameter. For example, pulmonary arteries can be properly labeled as intralobular arteries if they are seen accompanying terminal bronchioles. A similar approach can be applied to veins, including their location in the lobule (intralobular), or in pre-septal or intraseptal locations. Pulmonary arterial lesions may involve isolated medial hypertrophy, medial hypertrophy and intimal thickening, concentric lamellar, eccentric, concentric nonlamellar, complex lesions with plexiform parts, and arteritis. Plexiform/complex lesions are usually similar in the lungs of individual patients. Plexiform lesions with venous and capillary changes may coexist in similar parts of the lung. Vascular changes can be segmental.

Coexisting venous-venular changes should be noted. These include several potential occlusive lesions, such as intimal thickening/obstruction (fibrosis, cells), luminal septa, recanalization, adventitial thickening, muscularization, iron and calcium incrustation, and foreign body reaction. Associated capillary changes may be present with multiplication and proliferation with variable dilatation. Angioma-like lesions and primary capillary angiomas may be present. Most if not all cases have associated variable arterial changes. Other changes include dilated lymphatics, hemosiderin-laden alveolar macrophages, and type II cell hyperplasia.

In addition to an extensive literature on the arterial findings in PH (40,41), there is increasing awareness of compromise of the venous circulation in PAH. Pulmonary veno-occlusive disease (PVOD) now belongs together with pulmonary capillary hemangiomatosis (PCH) to the PAH group, with predominance of vascular disease at the post-capillary level of the pulmonary vasculature. There is a possible overlap between PVOD and cases of PCH, a disease classically characterized by an aggressive, patchlike, capillary angioproliferation, as outlined in a report of 35 cases of PVOD and PCH sharing similar histologic patterns (42). The observed post-capillary lesions involve septal veins and pre-septal venules and frequently consist of a loose, fibrous remodelling of the intima that may totally occlude the lumen. The involvement of pre-septal venules should be

considered as necessary for the histologic diagnosis of PVOD, as fibrous occlusion of large septal veins may be seen in many forms of pulmonary venous hypertension, including a frequently reported obstruction of large pulmonary veins following catheter ablation for cardiac atrial fibrillation (43).

### **Tissue Banking for Pulmonary Vascular Research**

Currently, there are some initiatives to bank human lung tissue for pulmonary vascular research. In North America, the Cardiovascular Medical Research and Educational Fund has set up a multi-institutional effort to collect diseased lung tissue for research in IPAH, under the Pulmonary Hypertension Breakthrough Initiative (44,45). This network interfaces multiple transplant centers with tissue processing sites, genomics, cell processing, and proteomics centers. The process of organization and operational infrastructure has proved to be complex but, since the network has succeeded in its goals, it provides an example that can be followed in the future. Furthermore, it has highlighted the complexities of setting up a bank of tissue, including delicate regulatory issues that have to be methodically addressed prior to setting up this type of enterprise. At this time, local or regional tissue banks involving various institutions may be more feasible. However, exchange of paraffin blocks of diseased lungs with PH among centers that study the disease is highly desirable. More importantly, this effort may offer a unique opportunity to better define the natural history of IPAH and potentially to identify early vascular lesions indicative of the disease.

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