The 4th World Symposium on Pulmonary Hypertension (PH) took place in Dana Point, California, in February 2008. This 4-day summit of international experts in PH, which highlighted the findings of 11 scientific working groups, was an occasion for looking backward and for looking ahead. Thirty years ago, adults diagnosed with pulmonary arterial hypertension (PAH) could expect to live less than 3 years, and the therapeutic armamentarium was limited to nonselective vasodilators. By 2003, however, when the 3rd World Symposium was held in Venice, Italy, much more was known about the pathologic changes seen in PAH and PH, and several important therapies had been shown to be effective. Today, our selection of therapeutic modalities is broader still, and more than 15 large randomized clinical trials have provided reliable evidence for their benefit. The purpose of the 4th World Symposium was to review the progress we have made in diagnosing and treating PH and PAH; redefine and, when appropriate, reclassify the disease itself; better understand the rationale for ongoing research; and formulate proposals for new investigative paths that may translate into a brighter future for our patients.

The Dana Point meeting opened with an update on the natural history of PH. We learned that the vascular remodeling characteristic of PH may have its origins in disruptions or alterations that take place in lung circulation as early as embryonic and fetal development and certainly plays a role in pediatric lung disease. These early alterations in developmental physiology may determine the likelihood of developing PH in adult life. We now understand that inflammatory processes also contribute to PH, particularly in connective tissue diseases and chronic obstructive pulmonary disease, and inflammation is involved in all of the mechanisms of vascular remodeling.

Pulmonary arterial hypertension is characterized by cellular changes in the walls of pulmonary arteries. Building on the discussion of endothelial dysfunction at the Venice meeting, at Dana Point, we further explored the key role of the endothelium in PAH. In addition, we examined the strong association between mutations in the bone morphogenetic protein receptor type 2 (BMPR2) gene and PH: such mutations have been found in approximately 80% of families with PAH and 25% or less of families with idiopathic PAH. The BMPR2 mutation was discovered in 2000, not long before the Venice meeting; other genetic markers have been discovered in the intervening years.

With the development of new therapeutic options, early and accurate diagnosis of PH becomes increasingly important. One working group at Dana Point examined optimal modalities for diagnosing the disease and predicting outcomes. These include hemodynamic and echocardiographic measures, as well as biomarkers such as brain natriuretic protein.

An increasing number of agents, including prostanoids, endothelin receptor antagonists, and phosphodiesterase-5 inhibitors, continue to provide effective treatment options. One working group at Dana Point developed an algorithm that reflects the best evidence currently available for the appropriate treatment of patients who can benefit from these agents. At the same time, surgical treatment for PH was reviewed, including pulmonary thromboendarterectomy, currently the only cure for chronic thromboembolic PH; atrial septostomy, which decompresses the failing right ventricle; and finally, for those for whom other options are not viable, heart and lung transplantation.

An increase in the number of therapeutic strategies brings new opportunities but also new challenges. Thus, there is a need for well-designed clinical trials with appropriate end points that will enable reliable interpretation of the benefit-to-risk profile of all current and emerging therapies. Consequently, 1 working group dedicated itself to an in-depth examination of the benefits and drawbacks of a variety of clinical trial end points and designs to determine the most interpretable and clinically relevant.

Most often the cause of death from PH is right ventricular failure, and the effect of treatment on right ventricular function is currently under study. Likewise, therapeutic
antiremodeling strategies are under investigation. Dysregulated proliferation of endothelial and smooth muscle cells in PH, along with increased expression of growth factors, have led to the use of antineoplastic drugs, which have shown promise in patients with PH.

Thus, despite the fact that a cure for PAH remains elusive, we concluded the Dana Point meeting with a feeling of optimism. Today, we can improve life expectancy, functional class, and quality of life for many patients with PAH. Tomorrow, as we increase our understanding of specific disease pathways, we will be able to develop targeted therapies that will further improve outcomes.

One of the takeaway lessons from Dana Point is the need for collaborative efforts that will ultimately lead to improved treatment for all patients with PH. Large international registries can help us understand and assess patterns of treatment. Despite complex regulatory complications, we need to establish multi-institutional networks for tissue banking. Multicenter collaboration will also be needed to conduct statistically meaningful genome studies. Such collaborative efforts will be invaluable in providing the necessary resources to help us successfully navigate the road ahead.

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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) © 2009 by the American College of Cardiology Foundation

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 intra-acinar 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)-β, hypoxia inducible factors 1α and 1β, 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+ channels, which would allow the identification of

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Development and Pathology

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 relatively 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.

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.

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 vessel 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 neovascularization 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-β1 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 pathogenic 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 A₂, 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 fibromyocyte, 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 international 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-β 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 microvascularopathy (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 multiplicity and proliferation with variable dilatation. Angioma–like lesions and primary capillary angiomatosis 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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Inflammation, Growth Factors, and Pulmonary Vascular Remodeling

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Inflammatory processes are prominent in various types of human and experimental pulmonary hypertension (PH) and are increasingly recognized as major pathogenic components of pulmonary vascular remodeling. Macrophages, T and B lymphocytes, and dendritic cells are present in the vascular lesions of PH, whether in idiopathic pulmonary arterial hypertension (PAH) or PAH related to more classical forms of inflammatory syndromes such as connective tissue diseases, human immunodeficiency virus (HIV), or other viral etiologies. Similarly, the presence of circulating chemokines and cytokines, viral protein components (e.g., HIV-1 Nef), and increased expression of growth (such as vascular endothelial growth factor and platelet-derived growth factor) and transcriptional (e.g., nuclear factor of activated T cells or NFAT) factors in these patients are thought to contribute directly to further recruitment of inflammatory cells and proliferation of smooth muscle and endothelial cells. Other processes, such as mitochondrial and ion channel dysregulation, seem to convey a state of cellular resistance to apoptosis; this has recently emerged as a necessary event in the pathogenesis of pulmonary vascular remodeling. Thus, the recognition of complex inflammatory disturbances in the vascular remodeling process offers potential specific targets for therapy and has recently led to clinical trials investigating, for example, the use of tyrosine kinase inhibitors. This paper provides an overview of specific inflammatory pathways involving cells, chemokines and cytokines, cellular dysfunctions, growth factors, and viral proteins, highlighting their potential role in pulmonary vascular remodeling and the possibility of future targeted therapy. (J Am Coll Cardiol 2009;54:S10–9) © 2009 by the American College of Cardiology Foundation

Pulmonary arterial hypertension (PAH) constitutes a heterogeneous group of clinical entities sharing similar pathologies that have been subcategorized as idiopathic pulmonary arterial hypertension (IPAH), familial PAH, pulmonary hypertension (PH) associated with other diseases such as connective tissue diseases, (e.g., systemic sclerosis [SSc]), portopulmonary hypertension, and PH related to human immunodeficiency virus (HIV) infection, drugs, and toxins (1). Although modifications to this classification are reviewed elsewhere in this series, this review focuses on inflammatory processes in PAH and other forms of PH, highlighting specific components of inflammation in the development of PH, as well as potential targets for therapy.

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Inflammation in PAH

Inflammation plays a significant role in various types of human PH, such as IPAH and PAH associated with connective tissue diseases and HIV infection and in experimental animal models (e.g., monocrotaline [MCT]-induced PH). A subset of PAH patients have circulating autoantibodies, including antinuclear antibodies (2), and elevated circulating levels of the proinflammatory cytokines interleukin (IL)-1 and IL-6 (3). Although there are serologic and pathologic features suggestive of inflammation in both IPAH and PAH related to SSc (PAH-SSc) or other connective tissue diseases, it is likely that inflammatory pathways and autoimmunity are more pronounced in PAH-SSc. This might explain survival discrepancies and differential response to therapy between the 2 syndromes (4). As such, PAH-SSc might be considered the prototypic syndrome in which to study inflammatory processes potentially operative in the pathogenesis of PAH.

A role for inflammation in PAH is based on the finding of inflammatory cells, including macrophages and T and B lymphocytes, and dendritic cells around the plexiform lesions of PAH (5). Levels of macrophage inflammatory protein-1α, IL-1β and -6 (3,6), and P-selectin (7) are increased in severe IPAH. Involvement of leukocytes, macrophages, and lymphocytes in the complex vascular lesions of IPAH was initially described by Tuder et al. (8) and confirmed in more recent studies by Dorfmüller et al. (9). Cytokine- and chemokine-dependent mechanisms leading to inflammatory cell recruitment in human PAH are also prominent in PAH.

Cytokines and chemokines in PAH. Balabanian et al. (10) demonstrated that fractalkine (CX3CL1), a unique chemokine that promotes the chemokine (C-X3-C motif) receptor 1 (CX3CR1)-expressing leukocyte recruitment, is upregulated in circulating CD4+ and CD8+ T lymphocytes from PAH patients as compared with control subjects. These patients also have elevated soluble CX3CL1 plasma concentrations; their lung tissue samples demonstrate increased CX3CL1 messenger ribonucleic acid (mRNA) expression as compared with control subjects, and pulmonary artery (PA) endothelial cells (ECs) from these lungs express CX3CL1 protein.

Regulated upon Activation, Normal T cell expressed and secreted (RANTES, also known as CCL5) is an important chemoattractant for monocytes and T-cells. CCL5 plays a key role in several vascular inflammatory processes such as glomerulonephritis, Kawasaki disease, and Takayasu’s arteritis. CCL5 might also play an indirect role in PAH through the induction of endothelin (ET)-converting enzyme-1 and ET-1, a potent endothelium-derived factor with strong vasoconstrictive and mitogenic action. Indeed, CCL5 mRNA expression is increased in lung samples from PAH patients as compared with control subjects and probably originates from ECs, as demonstrated by in situ hybridization and immunohistochemistry (11). The exact relevance of these findings to the pathophysiology of PAH requires further investigation.

Two recent studies further suggest that chemokines produced from small PAs of PAH patients might contribute to inflammatory cell recruitment and PA smooth muscle cell (SMC) proliferation. Perros et al. (12) demonstrated that CX3CL1 is expressed by inflammatory cells surrounding PA lesions and that SMCs from these vessels have increased CX3CR1 expression. In addition, cultured rat PA-SMCs express CX3CR1, and CX3CL1 induces proliferation but not migration of these cells. Therefore, fractalkine might act as a growth factor for PA-SMCs. The hypothesis that chemokines might play a role in PA remodeling was further studied by Sanchez et al. (13). Compared with control subjects, IPAH patients have elevated levels of CCL2, also known as monocyte chemotactic protein (MCP)-1, in plasma and lung tissue. In addition, elevated CCL2 release from pulmonary ECs or PA-SMCs was demonstrated. Monocyte migration was markedly increased in the presence of pulmonary ECs (particularly from patients with IPAH) and significantly reduced by CCL2-blocking antibodies. Finally, compared with control subjects, PA-SMCs from patients exhibited stronger migratory and proliferative responses to CCL2, in keeping with the finding that CCR2 was markedly increased in PA-SMCs in these patients (13).

Growth factors and inflammation in PAH. Several growth factors, including platelet-derived growth factor (PDGF) (14,15), epidermal growth factor (EGF) (16), and vascular endothelial growth factor (VEGF) (17), have been implicated in the abnormal proliferation and migration of PA vascular cells. They act as potent mitogens...
and chemoattractants for SMCs, fibroblasts, and ECs and cause resistance to apoptosis.

**VEGF.** Cool et al. (18) demonstrated intense expression of the VEGF receptor KDR, coupled with a reduced expression of p27kip1, a cell cycle inhibitory protein, in the ECs of plexiform lesions. Other markers of angiogenesis, such as VEGF and hypoxia inducible factor-1 subunits α and β, are highly expressed in ECs of plexiform lesions in severe PAH (19). In addition, expression of C-Src kinase (19), a protein that mediates VEGF-induced production of prostacyclin and nitric oxide in ECs, is decreased in PAH. Taken together, these findings suggest a central role in PAH for VEGF, a mediator of angiogenesis but also a factor involved in permeability and inflammatory processes in the vascular endothelium.

**PDGF.** Platelet-derived growth factor is synthesized by many different cell types including SMCs, ECs, and macrophages. PDGF induces the proliferation and migration of SMCs and fibroblasts and has been proposed as a key mediator in the progression of several fibroproliferative disorders such as atherosclerosis, lung fibrosis, and PH (14). As a result, novel therapeutic agents, such as tyrosine kinase inhibitors, have been tested in experimental models of PH (15) and more recently in clinical trials. The rationale for use of these agents is discussed in more detail in later sections. The pathogenic role of PDGF was demonstrated by increased expression of PDGF and platelet-derived growth factor receptors (PDGFRs) by reverse transcription-polymerase chain reaction (PCR) performed on laser-captured micro-dissected PAs from native lungs of patients with severe IPAH who underwent lung transplantation (20). The PDGF-A, PDGF-B, PDGF-Rα, and PDGF-Rβ mRNA expression is increased in small PAs from patients with severe IPAH as compared with control subjects. In small PAs, PDGF-B is mainly expressed in ECs, SMCs, and in some perivascular inflammatory cells, and PDGFR-β is mainly expressed in SMCs. The PDGF-BB–induced proliferation and migration of PA-SMCs is inhibited by imatinib (20). Taken together, these data support the concept that PDGF is overproduced and promotes PA remodeling in PAH.

**EGF.** The EGF-dependent proliferation and migration of SMCs is dependent on the extracellular matrix component tenasin C (TN-C). In addition, EGF colocalizes with TN-C in PAH lesions (21), suggesting a direct role in disease progression. It is noteworthy that the EGF receptor inhibitor PKI166 reverses established MCT-induced PH in rats (16).

**SURVIVIN.** Survivin (16.5 kDa) is the smallest member of the mammalian inhibitor of the apoptosis family. Several malignant processes have been linked to dysregulation of survivin expression. The normal absence of survivin from healthy tissues suggests it is a potential target for therapy. Survivin is overexpressed in PAs from PAH patients and in rats with MCT-induced PAH, compared with control subjects (30). Wild-type survivin delivered via an inhaled adenovirus to normal rats causes PH. Conversely, gene therapy with an adenovirus carrying a phosphorylation-deficient survivin mutant with dominant-negative properties (T34A survivin) reverses established MCT-PAH and prolongs survival (30). Administration of the survivin mutant reduces pulmonary vascular resistance, right ventricular...
(RV) hypertrophy, and PA medial hypertrophy. Both in vitro and in vivo, inhibition of endogenous survivin induces PA-SMC apoptosis, depolarizes mitochondria, causes efflux of cytochrome c in the cytoplasm, translocates apoptosis-inducing factor into the nucleus, and increases voltage-dependent potassium channel (Kv) current, whereas the opposite effects are observed with gene transfer of wild-type survivin. Survivin also induces the production of the PDGF receptor in human vascular SMCs (31). Therefore, the proposed causative role of survivin in PAH and the lack of its expression in normal PA wall and systemic vasculature make this gene attractive for future targeted therapy in PAH.

Transcriptional factors: the nuclear factor of activated T cells in inflammation and vascular remodeling. The nuclear factor of activated T cells (NFAT), originally described in T cells, is a master activator of T cells, increasing the transcription of multiple inflammatory mediators, including many interleukins and tumor necrosis factor (TNF-α), and activating T and B cells (32). Increased [Ca^{2+}]_i activates calcineurin, which dephosphorylates cytoplasmic NFAT, allowing its entry to the nucleus, where it forms complexes with other important transcription factors (e.g., GATA or activator protein-1) and regulates gene transcription (32).

Several recent observations suggest that NFAT might be involved in PAH. The NFAT activation causes downregulation of Kv1.5 (33), which plays a preponderent role in pulmonary vasconstriction. Second, ET (upregulated in PAH) activates NFAT, which in turn increases B-cell lymphoma (bcl)-2 expression, contributing to the prosurvival and antiapoptotic effects of ET in the heart (34). Third, NFAT directly or indirectly regulates the transcription of several genes that regulate mitochondrial function (e.g., pyruvate dehydrogenase and the electron transport chain enzyme cytochrome C oxidase) (35).

The NFAT is upregulated and activated (i.e., translocated in the nucleus) in circulating inflammatory cells in patients with PAH, including IPAH and PAH-SSc. The CD3-positive cells with activated NFAT are also seen in remodeled PAs. Intriguingly, NFAT is also activated in the PA-SMCs of remodeled arteries. The PA-SMCs isolated from PAH patients maintain in culture a unique phenotype (downregulated Kv1.5, upregulated bcl-2, hyperpolarized mitochondria), which is associated with activated NFAT and resistance to apoptosis. The NFAT is not activated in normal lungs and PA-SMCs. The unique phenotype of PAH PA-SMCs is normalized by selective inhibition of NFAT.

Inhibition of NFATc2 (predominant NFAT isoform in PAH) by VIVIT (a competitive peptide that inhibits the docking of NFAT to calcineurin) or cyclosporine (inhibitor of calcineurin), restores Kv1.5 expression and current and decreases [Ca^{2+}]_i, [K^+]_i, bcl-2, and mitochondrial membrane potential (ΔΨm), leading to increased apoptosis in vitro (36). In vivo, cyclosporine treatment decreases established MCT-induced PAH in the rat (36). Intriguingly, PA-SMCs exposed to chronic hypoxia display NFAT activation, hyperpolarized mitochondria, and downregulated Kv1.5, similar to the SMC phenotype of PAH. Inhibition with VIVIT or cyclosporine reverses this phenotype, normalizing the mitochondrial membrane potential and level/function of Kv1.5 in these cells. There has been recent interest in developing specific NFAT inhibitors for the treatment of cardiac hypertrophy and failure (37).

Therefore, in PAH, NFAT inhibitors might contribute to reversing RV hypertrophy and pulmonary vascular remodeling through their effects on cardiomyocytes, PA-SMCs, and inflammatory cells.

Viral and Other Infectious Etiologies in PAH

Hypothetically, PH is caused by latent viral infections, because associations between Epstein Barr virus infection and Hodgkin’s disease and parvovirus and cytomegalovirus infection and SSc have been described (38); both diseases have also been associated with PH. Infectious organisms can affect the lung circulation directly, by obliterating lung vessels, or indirectly, by causing and maintaining inflammation.

However, there is little evidence for a “direct” role for infectious agents in the pathogenesis of severe PH. Even in schistosomiasis-associated PH, it is unclear to what extent liver disease and therefore postpulmonary hypertension dominate the pathobiology of PH. Schistosoma eggs modulate regulatory T-cell activity and express a novel member of the transforming-growth factor (TGF)-β superfamily, Schistosoma mansoni inhibin/activin (SmInAct) (39). Recently a mouse model of pneumocystis-induced PH associated with muscularized PAs has been reported (40), and Daly et al. (41) reported a mouse model of highly muscularized PAs after a regimen of aspergillus antigen (ag) immunization.

Role of human herpes virus-8, HIV, and SHIV-Nef in pulmonary vascular remodeling. Pulmonary arterial hypertension has a prevalence of 0.0002% in the general population, but in HIV-infected individuals the prevalence is 0.46% in France (42). The HIV-related PAH (HRPAH) is independent of CD4+ T cell counts (43) and antiviral drug treatment. The clinical features of HRPAH are similar to PAH of other etiologies. Although highly active antiretroviral therapy might have decreased the incidence of HRPAH and might partially reverse PAH in a small number of HIV-1–infected individuals only when combined with PH-specific treatment such as bosentan (44), this disease remains a significant clinical complication in the HIV-1–infected population. Other studies showed no correlation between viral load and right heart changes (45).

Most of the pathways involved in virus pathogenesis converge on either prosurvival or proangiogenic signals, the same signals associated with PH. In the lung, HIV-1 infects primarily macrophages, providing a potential reservoir for the transmission of the virus to circulating T-cells, and is a source
for localized viral proteins such as Nef, Tat, and gp120, which might have direct or indirect effects. Chronic exposure to these viral products as well as deficiency in regulatory T cells and altered production of chemokines/cytokines might contribute to pulmonary vascular dysfunction.

Macaques infected with chimeric SHIV-nef virions (simian immunodeficiency virus [SIV]mac239 Δnef virus containing a cloned HIV-1 nef gene) demonstrate lung vascular changes characteristic of PAH, whereas macaques infected with parental SIV strains containing the native SIV nef allele show no vascular remodeling (46). The Nef was also demonstrated by immunohistochemistry in lungs of HIV-infected patients with PH (47). Thus, HIV-1 Nef protein, perhaps in conjunction with host genetic factors and/or persistent immune dysregulation, contributes to the development of pulmonary vascular remodeling. Foci of mononuclear cells and ectopic lymphoid tissues adjacent to the lesions might be sources of this viral protein.

The HIV-1 Nef is 1 of the accessory proteins made early in HIV infection and whose major effects are downregulating CD4 (48) and blocking major histocompatibility antigen-1 trafficking to the membrane (49), allowing the infected cells to evade immune surveillance (50). In human monocyte-derived macrophages, Nef activates the STAT1 pathway and the secretion of MIP-1, IL-1-α, IL-6, and TNFα (51).

**Human gamma herpes virus 8.** Human gamma herpes virus 8 (HHV8), also known as Kaposi’s sarcoma-associated herpes virus, has been associated with angioproliferation (52). The HHV8 is unquestionably associated with proliferative disorders, including multicentric Castleman’s disease and Kaposi’s sarcoma. Evidence of HHV8 was found in a large percentage of plexiform lesions of one cohort of PH patients, suggesting for the first time that this virus was a contributing factor (53). However, a number of other investigators have attempted without success to find evidence of latent HHV8 infection in lung tissue sections from patients with idiopathic PAH, with immunohistochemistry and PCR methodology (54–57).

**Hepatitis C virus.** Finally, PH represents one of the extrahepatic complications of hepatitis C virus (HCV) infection, with a prevalence of 1% to 5% (58). In the majority of patients, portal hypertension precedes PH (58,59). The pathogenesis is poorly understood, but the histologic hallmarks are similar to IPAH. Whether these lesions are secondary to increased inflammatory cytokine production, direct viral replication, or presence of viral products in the lung remains to be determined. In contrast, an observational study of 823 HIV-infected patients with and without HCV concluded that although age, baseline CD4+ cell count, and duration of highly active antiretroviral therapy were significantly associated with survival, HCV infection was not (60). An associated immune dysregulation might trigger uncontrolled intrapulmonary angiogenesis, as in HIV-mediated PH.

In summary, very little is known about the natural history of any form of virus-related PH or the molecular mechanisms that account for the pathogenesis. Cell biological studies with recombinant viral proteins or with cloned virions might shed some light as to potential molecular mechanisms whereby viral proteins induce angioproliferation.

**PAH-SSc as a Prototypic Inflammatory Disease**

Vascular changes in SSc and evidence for autoimmunity as a central component of remodeling. Vascular changes occur at an early state in SSc and include apoptosis (61), EC activation with expression of cell adhesion molecules, inflammatory cell recruitment, procoagulant state (62), and intimal proliferation and adventitial fibrosis leading to vessel obliteration. Endothelial cell injury is reflected by increased levels of soluble vascular cell adhesion molecule-1 (63), disturbances in angiogenesis as reflected by increased levels of circulating VEGF (64), and presence of angiotrophic factors (64). Dysregulated angiogenesis in PAH-SSc, whether driven by the inflammatory process or other mechanisms, seems to be a predominant feature of the disease and should be a focus of future studies.

**Autoantibodies in scleroderma-related PAH.** A role for an autoimmune process has been proposed in the pathogenesis of PAH-SSc. Antifibrillarin antibodies (anti-U3-RNP) are frequently found in PAH-SSc patients (65), and the poorly characterized anti-endothelial cell antibodies (AECAs) correlate with digital infarcts (66). Antibodies to fibrin-bound tissue plasminogen activator in patients with limited cutaneous SSc (67) and in IPAH patients with HLA-DQ7 antigen (68) and antitopoisomerase II-α antibodies, particularly in association with HLA-B35 antigen (69), are found in PAH-SSc. Nicolls et al. (5) suggested that AECAs—which can activate ECs, induce the expression of adhesion molecules, and trigger apoptosis—play a role in PAH pathogenesis. In vitro experiments using autoantibodies from patients with connective tissue diseases (anti-U1-RNP and –dsDNA) can upregulate adhesion molecules (e.g., endothelial leukocyte adhesion molecule-1) and histocompatibility complex class II molecules on human PA ECs (70), suggesting that an inflammatory process could lead to proliferative and inflammatory pulmonary vasculopathy.

Fibroblasts are essential components of remodeling of the pulmonary vascular wall in PAH and can be found in the remodeled neointimal layer in both PAH-SSc and IPAH. The detection of antifibroblast antibodies in the serum of PAH-SSc and IPAH patients (71,72) has significant pathogenic importance, because these antibodies can activate fibroblasts and induce collagen synthesis, thus potentially contributing directly to the remodeling process. Antibodies from sera of patients with SSc induce a proadhesive and proinflammatory response in normal fibroblasts (72). Immunoglobulin G antifibroblast antibodies are present in sera of patients with IPAH and PAH-SSc and have distinct reactivity profiles in these 2 conditions (71). With
2-dimensional immunoblotting technique, several antigens recognized by serum immunoglobulin G from IPAH and PAH-SSc patients were identified, including proteins involved in regulation of cytoskeletal function, cell contraction, cell and oxidative stress, cell energy metabolism, and different key cellular pathways (73). Although the specific membrane antigens targeted by these autoantibodies remain to be determined, it is likely that they react to membrane components, because they typically bind to unpermeabilized fibroblasts, and might mediate the release of cytokines and growth factors which in turn might contribute to the pathogenesis of vascular remodeling in PAH (71).

Taken together, particularly in light of the positive response to immunosuppressive therapy for one-third of patients with PAH associated with systemic lupus erythematosus and mixed connective tissue disease (74), these studies suggest that inflammation and autoimmunity could play a major role in the pathogenesis of PAH. Thus, a search for specific biomarkers of inflammation could be a focus of future studies in IPAH, PAH-SSc, and other autoimmune conditions associated with PAH.

**Inflammatory genes in SSc and scleroderma-related PAH.** An increasing number of candidate genes have been reported to be associated with SSc in different populations: a variant in the promoter of MCP-1 (75); 2 variants in CD19 (−499G>T, and a GT repeat polymorphism in the 3’-UTR region) (76); a promoter and coding polymorphism in TNF-α (TNF-α 238A>G, TNF-α 489A>G) (77); a variant in the promoter of the IL-1α gene (IL1-α −889T) (78); and a 3-single nucleotide polymorphism haplotype in IL-10 (79). Thus, compelling data support a genetic basis for SSc. Despite these recent advances in genetics, little is known about genetic involvement in PAH-SSc. **BMPR2 mutations** have not been identified in 2 small cohorts of PAH-SSc patients (80,81).

Recently, an association between an endoglin gene (**ENG**; polymorphism and PAH-SSc was identified (82). Endoglin, a homodimeric membrane glycoprotein primarily present on human vascular endothelium, is part of the TGF-β receptor complex. The functional significance of the **ENG** polymorphism in SSc patients remains to be determined.

Aside from the few examples cited in the preceding text, the genes relevant to the pathogenesis and generally poor outcome associated with PAH-SSc have not been identified. Their definition will require robust, well-characterized patient populations to provide adequate power for analysis.

**Inflammation in PH Associated With Chronic Obstructive Pulmonary Disease**

Pulmonary vascular remodeling is a common finding in chronic obstructive pulmonary disease (COPD) and in heavy smokers with normal lung function (83). Inflammatory cells might contribute to the alterations of pulmonary vessels. Indeed, the extent of pulmonary vascular remodel-ling correlates with the severity of the inflammatory cell infiltrate in small airways (84). Furthermore, patients with COPD have an increased number of inflammatory cells infiltrating the adventitia of muscular PAs, as compared with nonsmokers (85). This inflammatory infiltrate is largely constituted by activated T lymphocytes with a predominance of the CD8+ T cell subset (85) without change in neutrophils, macrophages, and B-lymphocytes. **VEGF.** Patients with mild-to-moderate COPD show increased expression of VEGF in PAs compared with control nonsmokers (86). The VEGF expression correlates with arterial wall thickness, suggesting a potential role of VEGF in the pathogenesis of pulmonary vascular remodeling in COPD. In patients with advanced COPD and severe emphysema, the expression of VEGF in PAs is lower than in patients with mild-to-moderate disease and does not differ from control nonsmokers (86), suggesting downregulation of VEGF in patients with emphysema that might lead to EC apoptosis.

**TGF-β.** In COPD, TGF-β has been implicated in connective tissue deposition (87) and airway macrophage recruitment (88). In patients with very severe COPD, the expression of type II receptor (TGF-β RI) but not TGF-β is increased in the tunica media and intima of PAs (89), along with a normal cell proliferation rate in both layers of the vessel wall, suggesting that TGF-β might exert a protective role (restraining cell proliferation) and that growth factors other than TGF-β might be involved in pulmonary vascular remodeling (89).

**Targeting Signaling Pathways: The Role of Antineoplastic Drugs in the Control of Vascular Remodeling in PAH**

The concept of “targeted” therapy holds popular appeal for advancing cancer treatment. Imatinib, an inhibitor of Bcr-Abl kinase, has dramatically changed prognosis for patients with chronic myeloid leukemia (90). Although imatinib is the archetype for targeted cancer therapeutics, it does not exclusively inhibit Bcr-Abl but also inhibits PDGFR (91). Schermuly et al. (15) tested the effects of imatinib in rodent models, on the basis of evidence that PDGF signaling is an important process in the pathophysiology of PAH (92). The effects of MCT on RV systolic pressure, cardiac index, RV hypertrophy, and overall survival were reversed in dose-dependent fashion with administration of imatinib, along with downregulation of phosphorylated PDGFRβ and extracellular signal-related kinase in lung tissue homogenates. Clinical validation of imatinib as PAH therapy was first suggested in case reports (93–95). These led to a Phase II trial to evaluate the safety, tolerability, and efficacy of imatinib in patients with PAH that, at the time of the PH World Congress, was open to accrual at multiple centers in the U.S. and Europe.

**Disrupting PDGF and VEGF signaling.** Although the role for specific disruption of PDGFR signaling in cancer
therapeutic regimens is still under investigation, the efficacy of 2 U.S. Food and Drug Administration-approved agents, sunitinib and sorafenib, is attributed in part to their dual inhibition of VEGF and PDGF signaling pathways. Whereas PDGF is a validated specific target in PH, the rationale for testing antiproliferative drugs in advanced human PAH is also based on the presence of dysregulated proliferation of microvascular ECs and SMCs, monoclonal EC expansion (96), increased expression of secreted growth factors such as VEGF and basic fibroblast growth factor (97), and the fact that this condition—with its poor prognosis—is reminiscent of advanced solid tumors (98). Also at the time of the PH World Congress, a Phase I clinical trial to determine the safety and tolerability of

![Figure 1](image.png)

**Figure 1: Mechanisms of Inflammation-Mediated Remodeling**

This schematic features inflammatory mediators, cells, and mechanisms involved in pulmonary vascular remodeling as well as potential therapeutic targets. Release of cytokines and chemokines in remodeled vessels (e.g., plexiform lesions) or in the circulation, from activated endothelial cells (ECs) and smooth muscle cells (SMCs), mediate the influx of inflammatory cells (e.g., monocytes, T and B lymphocytes). Cellular dysfunction (particularly involving EC and SMC) contributes to release of vaso-motor and growth mediators, activation of transcriptional factors (e.g., nuclear factor of activated T lymphocytes [NFAT]), influx of calcium, and mitochondrial dysfunction. The net effect is a shift of balance in favor of cell proliferation and decreased apoptosis, leading to remodeling and narrowing of the pulmonary vascular lumen. Potential therapeutic target sites include inhibition of growth factors with tyrosine kinase inhibitors, calcineurin with cyclosporine, and prevention of NFAT activation with VIVIT polypeptide (a competitive peptide that inhibits the docking of NFAT to calcineurin). Specific mechanisms are further detailed in the text. bcl2 = B-cell lymphoma 2; CCL2 = chemokine (C-C motif) ligand 2; CCL5 = chemokine (C-C motif) ligand 5 or RANTES (Regulated upon Activation, Normal T cell expressed and secreted); CX3CL1 = chemokine (C-X3-C motif) ligand 1 (fractalkine); CX3CR1 = chemokine (C-X3-C motif) receptor 1; DC = dendritic cells; ET1 = endothelin 1; FB = fibroblasts; FGF = fibroblast growth factor; 5-HT = serotonin; HIV-1 = human immunodeficiency virus 1; IgG = immunoglobulin G; MO = monocyte; NO = nitric oxide; PAH = pulmonary arterial hypertension; PDGF = platelet-derived growth factor; PG2 = prostacyclin; ROK = Rho kinase; VEGF = vascular endothelial growth factor.
sorafenib in PAH patients with stable clinical and hemodynamic status on prostacyclin-based therapy was open and now has since been completed at the University of Chicago. The results of these trials should help advance development of this therapeutic strategy in PAH.

Conclusions

It has become clear that inflammatory processes involving cellular effectors, chemokines, cytokines, and growth factors play a preponderant role in the vascular remodeling characteristic of PAH (Fig. 1). Recognition of these specific pathways should allow development of additional targeted therapy in this disease, with the hope of altering a prognosis that has been all too dismal in spite of significant recent progress.

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Cellular and Molecular Basis of Pulmonary Arterial Hypertension

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Pulmonary arterial hypertension (PAH) is caused by functional and structural changes in the pulmonary vasculature, leading to increased pulmonary vascular resistance. The process of pulmonary vascular remodeling is accompanied by endothelial dysfunction, activation of fibroblasts and smooth muscle cells, crosstalk between cells within the vascular wall, and recruitment of circulating progenitor cells. Recent findings have reestablished the role of chronic vasoconstriction in the remodeling process. Although the pathology of PAH in the lung is well known, this article is concerned with the cellular and molecular processes involved. In particular, we focus on the role of the Rho family guanosine triphosphatases in endothelial function and vasoconstriction. The crosstalk between endothelium and vascular smooth muscle is explored in the context of mutations in the bone morphogenetic protein type II receptor, alterations in angiotensin-1/TIE2 signaling, and the serotonin pathway. We also review the role of voltage-gated K⁺ channels and transient receptor potential channels in the regulation of cytosolic [Ca²⁺] and [K⁺], vasoconstriction, proliferation, and cell survival. We highlight the importance of the extracellular matrix as an active regulator of cell behavior and phenotype and evaluate the contribution of the glycoprotein tenascin-c as a key mediator of smooth muscle cell growth and survival. Finally, we discuss the origins of a cell type critical to the process of pulmonary vascular remodeling, the myofibroblast, and review the evidence supporting a contribution for the involvement of endothelial-mesenchymal transition and recruitment of circulating mesenchymal progenitor cells. (J Am Coll Cardiol 2009;54:S20–31) © 2009 by the American College of Cardiology Foundation

Despite the recognized success of existing drug interventions in the relief of symptoms of pulmonary arterial hypertension (PAH), and possibly improvement in survival, most patients eventually become resistant to therapy and succumb to the disease. The past few years have seen a remarkable increase in our knowledge of the cellular and molecular mechanisms responsible for the pathobiology of PAH. This summary aims to present the current state of our understanding of some of the key mechanisms (Fig. 1). We also indicate further areas and directions of research and suggest novel approaches to therapy.

Endothelial Dysfunction in PAH

Endothelial cells (ECs) are recognized as major regulators of vascular function, and endothelial dysfunction has come to mean a multifaceted imbalance in EC production of vasoconstrictors versus vasodilators, activators versus inhibitors of smooth muscle cell (SMC) growth and migration, prothrombotic versus antithrombotic mediators, and proinflammatory versus anti-inflammatory signals.

Rho guanosine triphosphatases (GTPases) in endothelial dysfunction. Rho (Ras homologous) GTP-binding proteins regulate many cellular processes, including gene transcription,
differentiation, proliferation, hypertrophy, apoptosis, phagocytosis, adhesion, migration, and contraction (1). In the prototypical mechanism of RhoA GTPase signaling, environmental cues, acting through G-protein-coupled receptors or receptor-dependent and receptor-independent tyrosine kinases, activate guanine nucleotide exchange factors, which induce exchange of guanosine diphosphate for GTP binding and translocation of GTP-RhoA to the plasma membrane. The membrane translocation requires post-translational prenylation. Upon translocation to the plasma membrane, GTP-RhoA activates its effectors, including the 2 isoforms of Rho kinase (ROCK), ROCK I (ROKβ) and ROCK II (ROKα). Negative regulators of RhoA activation include guanine nucleotide disassociation inhibitors, which oppose the exchange of GTP for guanosine diphosphate; GTPase activating proteins, which catalyze dephosphorylation and inactivation of membrane-bound GTP-RhoA; statins, which inhibit isoprenylation of RhoA and thereby prevent translocation of GTP-RhoA to the cell membrane (2); and protein kinases A and G, which, by phosphorylating RhoA, also prevent membrane translocation of the GTP-bound protein (3).

**Rho GTPases and EC permeability.** An increase in EC permeability may be an important component of the pathogenesis of PAH. The GTPases RhoA and Rac1 play opposing roles in the regulation of EC barrier function. While stimuli such as thrombin activate RhoA/ROCK, which increases formation of F-actin stress fibers, cell contraction, and permeability, barrier-enhancing mediators such as sphingosine-1-phosphate and prostacyclin (PGI2) stimulate Rac1/p21-activated kinase (PAK), which counteracts the effects of RhoA/ROCK and promotes cortical F-actin ring formation and barrier integrity (4). Pulmonary artery ECs cultured from chronically hypoxic piglets demonstrate low Rac1 and high RhoA activities, which correlate with increased stress fiber formation and permeability (5). Activation of Rac1/PAK-1 and inhibition of RhoA reverse these changes.

**Rho GTPases and EC proliferation, migration, and apoptosis.** Rho GTPases participate in EC proliferation and apoptosis. Interestingly, the hyperproliferative, apoptosis-resistant phenotype of PAH ECs may be due to persistent activation of signal transducer and activator of transcription 3 (6), a downstream target of Rho GTPases. Signal transducer and activator of transcription 3 mediates RhoA-induced nuclear factor-κB and cyclin D1 transcription and is involved in nuclear factor-κB nuclear translocation (7).

**Role of rho GTPases in thrombosis.** In situ thrombosis of small peripheral pulmonary arteries contributes to PAH. The ECs are directly involved in the fibrinolytic process through synthesis and release of the profibrinolytic tissue plasminogen activator and the antifibrinolytic/plasminogen activator inhibitor (PAI)-1. The stimulation of systemic artery EC PAI-1 expression by angiotensin II, C-reactive protein, high glucose, and monocyte adhesion is dependent on activation of RhoA/ROCK signaling. Similarly, EC expression of tissue factor, another prothrombotic mediator, increased in the pulmonary arteries (PAs) of PAH lungs, is upregulated by RhoA/ROCK signaling (8). The RhoA/ROCK and Rac/PAK signaling pathways are implicated in thrombin- and thromboxane A2-induced platelet activation and aggregation (9).

**Nitric oxide (NO) and PGI2.** Endothelial dysfunction in PAH is reflected by reduced production of the vasodilators/growth inhibitors NO and PGI2 and increased production of the vasoconstrictor co-mitogens, for example, endothelin-1 and thromboxane A2. Nitric oxide signaling is mediated mainly by the guanylate cyclase/cyclic guanosine monophosphate (cGMP) pathway. Degradation of the second messenger of NO, cGMP, by phosphodiesterases is mainly accomplished by phosphodiesterase-5.

Reduced NO bioavailability in PAH can be due to decreased expression of endothelial NO synthase (eNOS), inhibition of eNOS enzymatic activity, and inactivation of NO by superoxide anion. Activation of endothelial RhoA/ROCK signaling can be involved in at least the first 2 processes. For example, RhoA/ROCK activation mediates hypoxia- and thrombin-induced inhibition of both eNOS expression and its activity in cultured ECs (10). The activity of arginase II, which reduces NO synthesis by competing with eNOS for the substrate L-arginine, is increased in PAH ECs (11), and RhoA/ROCK signaling mediates thrombin- and tumor necrosis factor-α/lipopolysaccharide-induced activation of eNOS (12). Patients with idiopathic PAH (IPAH) have increased plasma levels of the endogenous inhibitor of eNOS, asymmetric dimethylarginine (13), and the levels of asymmetric dimethylarginine and the enzyme that degrades it, dimethylarginine...
dimethylaminohydrolase, are, respectively, increased and decreased in the PA endothelium of IPAH patients (13).

Prostacyclin stimulates the formation of cyclic adenosine monophosphate, which also inhibits the proliferation of SMCs and decreases platelet aggregation. A deficiency of PGI2 and PGI2 synthase and an excess of thromboxane are found in PAH (14). Moreover, PGI2-receptor knock-out mice develop more severe hypoxia-induced pulmonary hypertension (PH) (15). Conversely, PGI2 overexpressing mice are protected against hypoxia-induced PH (16).

**Angiopoietin and TIE2.** Angiopoietin (Ang)-1 is an oligomeric-secreted glycoprotein, which, along with angiopoietin-2 and angiopoietin-3/4, comprises a family of growth factors. The angiopoietin ligands exert their effects through the endothelial-specific tyrosine kinase, TIE2 (17). During lung development, both Ang-1 and TIE2 are expressed in growing blood vessels: Ang-1 is made and

**Figure 1 Potential Mechanisms Involved in the Development of PAH**

Schematic diagram depicting potential mechanisms involved in the development of pulmonary arterial hypertension (PAH). Ang = angiopoietin; AVD = apoptotic volume decrease; BMP = bone morphogenetic protein; BMPR = bone morphogenetic protein receptor; CaM = calmodulin; CREB = cAMP-response element binding protein; DAG = diacylglycerol; Em = membrane potential; EGF = epidermal growth factor; EGFR = epidermal growth factor receptor; ET = endothelin; GAP = GTPase activating protein; GPCR = G protein-coupled receptor; HHV = human herpes virus; HT = hydroxytryptamine (serotonin); HTT = hydroxytryptamine (serotonin) transporter; IP3 = inositol 1,4,5-trisphosphate; Kv = voltage-gated K+; MAPK = mitogen-activated protein kinase; MLC = myosin light chain; MLCK = myosin light chain kinase; NA(D)PH = nicotinamide adenine dinucleotide phosphate; NO = nitric oxide; PASMC = pulmonary artery smooth muscle cell; PDGF = platelet-derived growth factor; PGI2 = prostacyclin; PKC = protein kinase C; PLC = phospholipase C; ROC = receptor-operated Ca2+ channels; ROS = reactive oxygen species; RTK = receptor tyrosine kinase; SR = sarcoplasmic reticulum; SRF = serum response factor; TCF = T cell factor; TIE = endothelial-specific tyrosine kinase; VDCC = voltage-dependent calcium channel.
secreted by vascular SMCs and pericytes, whereas TIE2 is a transmembrane receptor expressed on endothelial cells (18). In the adult, Ang-1 expression in the lung is minimal, whereas TIE2 expression remains constitutive (19).

Several lines of evidence suggest that Ang-1 regulates pathologic SMC hyperplasia in PAH. Ang-1 is overexpressed in most forms of nonfamilial PAH (18,20). In PAH, Ang-1 causes activation of the TIE2 receptor by tyrosine autophosphorylation in the pulmonary vascular endothelium (20,21). Enhanced TIE2 levels and a 4-fold increase in TIE2 phosphorylation are found in human PAH lung tissue, compared with control subjects (20,22).

Virally mediated overexpression of Ang-1 in the rat lung results in PH (21,23). Ang-1 transgenic animals show increased pulmonary vascular endothelial TIE2 phosphorylation and SMC hyperplasia in small pulmonary arterioles. Further, overexpression of a soluble TIE2 ectodomain, which sequesters Ang-1, suppresses the PH phenotype in monocrotoline and hypoxia models of disease (24).

There is a reciprocal relationship between bone morphogenetic protein receptor (BMPR) 1A and Ang-1 expression in the lungs of patients with nonfamilial PAH (20). Ang-1 downregulates BMPRIA expression through a TIE2 pathway in human pulmonary artery endothelial cells (PAECs). Stimulation of human PAECs with Ang-1 induces release of 5-hydroxytryptamine (HT [serotonin]), a potent stimulator of SMC proliferation (21,22). There is controversy in this field. In contrast to a causative role, Ang-1 has been reported to protect against the development of PAH in the rat monocrotoline and hypoxia models of disease (25).

**The SMC in PAH**

**Serotonin, serotonin transporter, and receptors.** Patients with IPAH have increased circulating 5-HT levels, even after heart-lung transplantation (26). In contrast to the constricting action of 5-HT on SMCs, which is mainly mediated by 5-HT receptors 1B/D, 2A, and 2B (27), the mitogenic and co-mitogenic effects of 5-HT require internalization through the serotonin transporter, 5-HTT (28).

That may require co-stimulation of the 5-HT1B receptor (29). Drugs that competitively inhibit 5-HTT block the mitogenic effects of 5-HT on SMCs (30). The appetite suppressants fenfluramine, d-fenfluramine, and aminorex differ from selective 5-HTT inhibitors in that they not only inhibit 5-HT reuptake but also trigger indoleamine release and interact with 5-HTT and -HT receptors in a specific manner (30).

**SEROTONIN TRANSPORTER.** 5-HTT is abundantly expressed on pulmonary artery smooth muscle cells (PASMCs) (31). Mice with targeted 5-HTT gene disruption develop less severe hypoxic PH than do wild-type controls (32,33). Conversely, increased 5-HTT expression is associated with increased severity of hypoxic PH (34,35). Indeed, specific overexpression of 5-HTT in PASMCs is sufficient to produce spontaneous PH (33).

**5-HT receptors in PH.** Of the 14 distinct 5-HT receptors, the 5-HT2A, 5-HT2B, and 5-HT1B receptors are particularly relevant to PAH.

**5-HT2A RECEPTOR.** In most nonhuman mammals, the 5-HT2A receptor mediates vasoconstriction in both the systemic and pulmonary circulations (36). However, the 5-HT2A receptor antagonist ketanserin is not specific for the pulmonary circulation, and systemic effects have limited its use in PAH, where it fails to improve pulmonary hemodynamics significantly (37).

**5-HT2B RECEPTOR.** The development of hypoxia-induced PH in mice is ablated in 5-HT2B receptor knockout mice (38), and this receptor may control 5-HT plasma levels in mice. However, the 5-HT2B receptor may also mediate vasodilation of the PA (39), and loss of the 5-HT2B receptor function may predispose to fenfluramine-associated PH in humans (40).

**5-HT1B RECEPTOR.** The 5-HT1B receptor mediates constriction in human PAs (41) and plays a role in the development of PAH (36,42), because inhibition, either by genetic knockout or pharmacologic antagonism, reduces hypoxia-induced pulmonary vascular remodeling (36). There is cooperation between the 5-HT1B receptor and the 5-HTT in mediating pulmonary vascular contraction (43). In addition, 5-HT1B receptor expression is increased in mice overexpressing the human 5-HTT and in the fawn-hooded rat, which also demonstrates increased 5-HTT expression (43). Both these models are predisposed to hypoxia-induced pulmonary vascular remodeling. Remodeled PAs from patients with PAH overexpress the 5-HT1B receptor. 5-HT1B receptor-mediated changes are specific to the pulmonary circulation, making this receptor an attractive therapeutic target for PH.

**5-HT SYNTHESIS IN PH.** The rate-limiting step in 5-HT biosynthesis is catalyzed by the enzyme tryptophan hydroxylase. Although peripheral 5-HT is synthesized chiefly by the enterochromaffin cells in the gut, human PAECs produce 5-HT and express the tryptophan hydroxylase-1 isoform. Both 5-HT synthesis and tryptophan hydroxylase-1 expression are increased in cells from patients with IPAH compared with controls (44). Mice lacking tryptophan hydroxylase-1 are resistant to hypoxia- and dexfenfluramine-induced PH (45,46).

**K+ and Ca+ channels in PAH.** In PASMCs, the free Ca2+ concentration in the cytosol ([Ca2+]cyt) is an important determinant of contraction, migration, and proliferation. The [Ca2+]cyt in PASMCs can be increased by: 1) Ca2+ influx through voltage-dependent Ca2+ channels, receptor-operated Ca2+ channels, and store-operated Ca2+ channels; and 2) Ca2+ release from intracellular stores (e.g., sarcoplasmic reticulum) through Ca2+ release channels (e.g., inositol 1,4,5-trisphosphate receptors and ryanodine receptors). Inward transport of Ca2+ through Ca2+ transporters in the plasma membrane, such as the reverse mode
of Na⁺/Ca²⁺ exchanger, is also an important pathway for increasing [Ca²⁺]ₜ. In contrast, [Ca²⁺]ₜ in PASMCs can be decreased by: 1) Ca²⁺ extrusion by the Ca²⁺-Mg²⁺ adenosine triphosphatase (Ca²⁺ pump) and by the forward mode of Na⁺/Ca²⁺ exchanger in the plasma membrane; and 2) Ca²⁺ sequestration by the Ca²⁺-Mg²⁺ adenosine triphosphatase in the sarcoplasmic reticulum.

**INHIBITION OF K⁺ CHANNEL ACTIVITY.** Decreased expression and/or function of K⁺ channels leads to membrane depolarization and contributes to sustained elevation of [Ca²⁺]ₜ by: 1) activating voltage-dependent calcium channel (VDCC); 2) facilitating the production of inositol 1,4,5-trisphosphate, which stimulates the release of sarcoplasmic reticulum Ca²⁺ into the cytoplasm; and 3) promoting Ca²⁺ entry through the reverse mode of Na⁺/Ca²⁺ exchange.

**ROLE OF RECEPTOR-OPERATED AND STORE-OPERATED CA²⁺ CHANNELS IN REGULATING [Ca²⁺]ₜ.** The influx of Ca²⁺ through store-operated calcium channels, referred to as capacitative Ca²⁺ entry, is critical for refilling the empty sarcoplasmic reticulum with Ca²⁺. Store-operated calcium channels in vascular SMC include the transient receptor potential channels. Some canonical transient receptor potential (TRPC) channel genes are expressed in human PASMCs and PAECs.

Proliferation of PASMC is associated with a significant increase in messenger ribonucleic acid and protein expression of TRPC channels such as TRPC1, TRPC3, and TRPC6 (47,48). Inhibition of TRPC expression with antisense oligonucleotides markedly decreases the amplitude of capacitative calcium entry and significantly inhibits PASMC proliferation. Thus, upregulation of TRPC channels may be a significant mechanism in the induction of PASMC proliferation.

**PATHOGENIC ROLE OF DOWNREGULATED KV CHANNELS AND UPREGULATED TRP CHANNELS.** In PASMCs from IPAH patients, the amplitude of whole-cell Iₚ(V) and mRNA/protein expression levels of Kv channel subunits (e.g., Kv1.2 and Kv1.5) are both significantly decreased in comparison with cells from controls or patients with secondary PH (49). The downregulated Kv channels and decreased Iₚ(V) are associated with a more depolarized Eₚ in IPAH PASMCs, and the resting [Ca²⁺]ₜ is much higher than in PASMCs from controls. The magnitude of capacitative calcium entry, evoked by passive store depletion with cyclopiazonic acid, is significantly greater in PASMCs from IPAH patients than in cells from secondary PH patients. Enhanced capacitative calcium entry, possibly by upregulation of TRPC channels, may represent a critical mechanism involved in the development of severe PAH.

**KV CHANNELS, MITOCHONDRIAL METABOLISM, AND PAH.** Warburg (50) proposed that a metabolic shift from oxidative phosphorylation to glycolysis, occurring despite adequate oxygen availability, was a characteristic of cancers. Recent data suggest that PAH and cancer share this "Warburg phenotype" (51,52). Both are characterized by mitochondrial hyperpolarization, depressed pyruvate dehydrogenase complex activity, and depressed H₂O₂ production (53). In both, there is also an O₂-independent perpetuation of the rapid, reversible metabolic/redox shifts that normally occur in response to hypoxia and initiate hypoxic pulmonary vasoconstriction (54,55). This metabolic shift creates a "pseudohypoxic environment" with glycolytic predominance and normoxic hypoxia-inducible factor-1α activation. The metabolic shift suppresses Kv1.5 expression, leading to membrane depolarization and elevation of cytosolic K⁺ and Ca²⁺. In both PAH PASMCs and cancer cell lines, this creates a proliferative, apoptosis-resistant phenotype.

As in familial PAH, PAH in the fawn-hooded rat is heritable. The fawn-hooded rat’s PASMC mitochondrial reticulum is fragmented even before PAH develops. The observed hyperpolarization of ΔѰₘ and reduction in production of reactive oxygen species also occurs in PASMCs from IPAH patients (51). In PAH, mitochondrial abnormalities that shift metabolism away from oxidative phosphorylation toward glycolysis lead to a decreased electron flux and reduced reactive oxygen species production, which falsely signifies hypoxia, resulting in normoxic hypoxia-inducible factor-1α activation. Both the hypoxia-inducible factor-1α activation and the related decrease in Kv1.5 expression are reversed by low doses of exogenous H₂O₂, consistent with the redox theory for their etiology. A hypoxia-inducible factor-1α dominant-negative construct also restores Kv1.5 expression in fawn-hooded rat PASMC (51). Decreased Kv expression is an emerging hallmark of the PAH PASMC, occurring in human PAH (49,51) and all known experimental models (56–58). Interestingly, both Kv channels involved in hypoxic pulmonary vasoconstriction (Kv1.5 and Kv2.1) are inhibited by the anorexigens (59) and by 5-HT (60). In addition, endothelin-1 reversibly reduces the Kv1.5 currents (61). Restoring Kv1.5 expression reduces chronic hypoxic PH and restores hypoxic pulmonary vasoconstriction (62).

Mitochondrial therapy, for example, inhibition of pyruvate dehydrogenase kinase by dichloroacetate or Kv1.5 gene therapy partially regresses both PAH and cancer (51,52,62), consistent with the concept that PAH and cancer share a mitochondrial basis. Dichloroacetate restores oxidative metabolism in fawn-hooded rat PASMCs, shifting them away from the proliferative/apoptosis resistant glycolytic state. Dichloroacetate also causes regression of PAH induced by chronic hypoxia or monocrotaline (51,56,57).

**RhoA/ROCK-mediated vasoconstriction.** It is now clear that activation of RhoA/ROCK signaling is a major regulator of vascular tone (63). Smooth muscle cell tension is determined primarily by phosphorylation (contraction) and dephosphorylation (relaxation) of the regulatory myosin light chain (MLC), as described in the preceding text. At a given level of cytosolic Ca²⁺, second messenger-mediated pathways can modulate the activity of myosin light chain kinase (MLCK) and myosin light chain phosphatases.
(MLCPs) (e.g., MYPT1) to modify MLC phosphorylation and force, namely, to modify the Ca\(^{2+}\) sensitivity of contraction. Two major pathways in vascular smooth muscle (SM) are inhibition of MLCP action by ROCK-mediated phosphorylation of MYPT1, and protein kinase C-mediated phosphorylation and activation of the MLCP-inhibitor protein CPI-17.

Deactivation of Ca\(^{2+}\) is also a mechanism of vasodilation. Besides inducing SMC relaxation by desensitizing receptors and decreasing cytosolic [Ca\(^{2+}\)] and MLCK activity, the NO/soluble guanylate cyclase/cGMP/PKG pathway also decreases Ca\(^{2+}\) sensitivity by phosphorylating and inactivating RhoA protein, or by directly phosphorylating MLCP, which increases MLCP activity (3). Similarly, vasodilation by stimuli that activate the adenylate cyclase and cAMP/PKA pathway is also attributable partly to inhibition of RhoA/ROCK signaling (3).

**RhoA/ROCK in acute pulmonary vasoconstriction.** ROCK-mediated Ca\(^{2+}\) sensitization is necessary for the sustained phase of acute hypoxic pulmonary vasoconstriction (64). Similarly, hypoxia directly activates RhoA in cultured PASMCs (65). Many studies have demonstrated the participation of ROCK in acute pulmonary vasoconstriction due to a variety of stimuli.

**RhoA/ROCK in human PAH.** Studies of RhoA/ROCK signaling in human PAH are limited. Low intravenous doses of fasudil acutely cause modest decreases in pulmonary vascular resistance in patients with PAH (66). Clinical trials examining the inhibition of RhoA/ROCK are under way.

### Crosstalk Between Vascular Cells

Whether SM hyperplasia results from inherent characteristics of PASMCs or from dysregulation of molecular events that govern PASMC growth, such as signals originating from PAECs, remains an open question (67). In addition, there is evidence of crosstalk between adventitial cells and medial SMCs.

Endothelial dysfunction in PAH may follow excessive release of paracrine factors that act either as growth factors to induce PASMC proliferation or as chemokines to recruit circulating inflammatory cells (44,68). Thus, exposure of PASMCs to culture medium from PAECs induces PASMC proliferation, and this effect is exaggerated when PAECs from patients with PAH are used (44).

The role of ECs in angiogenesis and remodeling is now better understood (69,70). In maturation, ECs no longer proliferate or migrate but promote vessel stabilization by recruiting periendothelial support cells, which differentiate into SM-like cells (71). Failure of interactions between the 2 cell types, as seen in numerous genetic mouse models, results in severe and often lethal cardiovascular defects. Deficiencies in this process may lead to abnormal dilation of resistance pulmonary vessels, such as that seen in hereditary hemorrhagic telangiectasia. Several studies suggest that the crosstalk between PAECs and PASMCs may be under the control of diverse pathways including the angiopoietin-1/TIE2, transforming growth factor (TGF)-\(\beta\)/activin receptorlike kinase (ALK)-1, and bone morphogenetic protein (BMP)/BMPR-II pathways (21,22,72). PAECs constitutively produce and release excessive amounts of soluble factors that act on PASMCs and inflammatory circulating cells to initiate or enhance pulmonary vascular remodeling and inflammation.

### Cellular and Molecular Consequences of BMPR-II Mutation

Mutations in the BMPR2 gene have been found in \(\approx70\%\) of families with PAH (73,74). In addition, up to 25% of patients with apparently sporadic IPAH harbor mutations (75).

**Normal BMP/TGF-\(\beta\) signaling.** BMPs are the largest group of cytokines within the TGF-\(\beta\) superfamily (76). BMPs are now known to regulate growth, differentiation, and apoptosis in a diverse number of cell lines (77). The TGF-\(\beta\) superfamily type II receptors are constitutively active serine/threonine kinases. BMPR-II initiates intracellular signaling in response to specific ligands (78). Ligand specificity for different components of the receptor complex may have functional significance to the tissue-specific nature of BMP signaling (79,80). Recently, BMP9 was identified as a ligand that signals through a complex comprising BMPR-II and ALK-1 (81). This important finding might provide a mechanism for the rare occurrence of severe PAH in some families with hereditary hemorrhagic telangiectasia due to ALK-1 mutations (82). After ligand binding, the type II receptor phosphorylates a glycine-serine–rich domain on the proximal intracellular portion of an associated type I receptor (usually BMPR-IA [ALK-3] or BMPR-IB [ALK-6]). Activated type I receptors in turn phosphorylate cytoplasmic signaling proteins known as Smads, which are responsible for TGF-\(\beta\) superfamily signal transduction (83). BMPs signal through a restricted set of receptor-mediated Smads (R-Smads), Smads-1, -5, and -8, which must complex with the common partner Smad (Co-Smad), Smad-4, to translocate to the nucleus. Switching off Smad signaling in the cell is achieved by Smad ubiquitination and regulatory factors (Smurfs) (84) and by recently identified Smad phosphatases (85).

**The consequences of BMPR2 mutation for BMP/TGF-\(\beta\) signaling.** The mechanism by which BMPR-II mutants disrupt BMP/Smad signaling is heterogeneous and mutation specific (86). Of the missense mutations, substitution of cysteine residues within the ligand binding or kinase domain of BMPR-II leads to reduced trafficking of the mutant protein to the cell surface. At least for the ligand binding domain mutants, the mistrafficking can be rescued with chemical chaperones, resulting in improvements in Smad signaling (87). In contrast, noncysteine mutations within the kinase domain reach the cell surface but fail to
activate Smad-responsive luciferase reporter genes. Many mutations lead to nonsense-mediated mRNA decay of the mutant transcript, leading to a state of haploinsufficiency. PASMCs from mice heterozygous for a null mutation in the BMPR2 gene are also deficient in Smad signaling (88,89). Thus, haploinsufficiency or missense mutation leads to a loss of signaling by the Smad1/5 pathway in response to BMP2 and BMP4. However, marked siRNA knockdown of BMPR-II leads to increased Smad signaling in response to some ligands, for example, BMP7 (80,89). This effect is mediated by increased signaling through the ActR-II receptor. In PASMCs, BMPR-II appears to mediate growth inhibition and differentiation, whereas ActR-II mediates osteoblastic differentiation (90).

Studies of BMP signaling cells and tissues from PAH patients. In the lung, BMPR-II is highly expressed on the vascular endothelium of the PAs (91) and at a lower level in PASMCs and fibroblasts. The expression of BMPR-II is markedly reduced in the pulmonary vasculature of patients with mutations in the BMPR-II gene (91). BMPR-II expression is also reduced in the pulmonary vasculature of patients with IPAH in whom no mutation in the BMPR2 gene was identified. A reduction in the expression of BMPR-II may be important to the pathogenesis of PAH, whether or not there is a mutation in the gene. In addition, since the level of BMPR-II expression in familial cases was considerably lower than predicted from the state of haploinsufficiency, this suggests that some additional environmental or genetic factor may be necessary to further reduce BMPR-II expression below a threshold that triggers vascular remodeling.

Phosphorylation of Smad1/5 is also reduced in the pulmonary arterial wall of patients with underlying BMPR-II mutations and in patients with IPAH with no identifiable mutation (92). The response of PASMCs to BMP ligands depends to some extent on the anatomical origin of cells. The serum-stimulated proliferation of cells harvested from the main or lobar PAs tends to be inhibited by TGF-β1 and BMPs 2, 4, and 7 (92). Indeed, BMPs may induce apoptosis in these cells (93). The growth inhibitory effects of BMPs have been shown to be Smad1 dependent (92). In contrast, in PASMCs isolated from PAs of 1 to 2 mm diameter, BMPs 2 and 4 stimulate proliferation (92). This pro-proliferative effect of BMPs is dependent on the activation of ERK1/2 and p38MAPK. Both Smad and MAPK pathways are activated to a similar extent in cells from both locations, but the integration of these signals by the cell differs. This integration may be at the level of an important family of transcription factors, the inhibitors of DNA binding (Id genes) (94).

The response of vascular ECs to BMPs is dependent on the specific BMP ligand. Endothelial cells proliferate, migrate, and form tubular structures in response to BMP4 and BMP6 (95). In addition, BMPs in general protect endothelial cells from apoptosis (96). Interestingly, BMP9, which acts through BMPR-II and ALK-1, seems to inhibit PAEC proliferation. Knockdown of BMPR-II with siRNA increases the susceptibility of PAECs to apoptosis (96).

The contrasting effects of BMPs in pulmonary vascular ECs and the underlying PASMCs provide a hypothesis for pulmonary vascular damage and remodeling in familial PAH. A critical reduction in BMPR-II function in the endothelium may promote increased endothelial apoptosis, which compromises the endothelial barrier. This would allow ingress of serum factors and stimulate activation of vascular elastases. High rates of apoptosis in the endothelium could favor the development of apoptosis-resistant clones of ECs and lead to pleuroparenchymal lesion formation. In the underlying media, PASMCs already compromised in their ability to respond to the growth-suppressive effects of BMPs are exposed to growth factors stimulating proliferation.

BMP signaling in rodent models of PAH. Reduced mRNA and protein expression of BMPR-II have been reported in the lungs of animals with experimental PH (97,98). In the monocrotaline rat model, adenoiral delivery of BMPR-II through the airways failed to prevent PH (99). However, targeted gene delivery of BMPR-II to the pulmonary endothelium did significantly reduce PH in chronically hypoxic rats (100).

Studies in knockout mice reveal the critical role of the BMP pathway in early embryogenesis and vascular development (101). However, heterozygous BMPR-II +/− mice survive to adulthood with no discernable phenotype (88). When heterozygotes are exposed to lung overexpression of interleukin-1β (102) or chronically infused with 5-HT (88), they develop more PH compared with wild-type littermates. Thus, BMPR-II dysfunction increases the susceptibility to PH when exposed to other environmental stimuli. The relatively low penetrance of PAH within families supports a “two-hit” hypothesis, in which the vascular abnormalities are triggered by accumulation of genetic and/or environmental insults in a susceptible person.

Transgenic mice overexpressing siRNA targeting BMPR-II exhibit ~10% of the normal levels of BMPR-II during development. These mice survive but do not develop spontaneous PAH. Intriguingly, they display a phenotype suggestive of hereditary hemorrhagic telangiectasia, with vascular ectasia and anemia (103). Conditional overexpression of a dominant negative kinase domain mutant BMPR-II in vascular SMCs of adult mice causes increased pulmonary vascular remodeling and PH (104). Conditional knockout of endothelial BMPR-II in adult mice has also been shown to predispose to PH (105).

The Extracellular Matrix

The extracellular matrix (ECM) not only represents a substrate for tissue morphogenesis, but also instructs almost all forms of cell behavior at the biophysical and biochemical levels through interactions with multiple receptors, including heterodimeric integrins composed of α and β subunits (106). Importantly, major qualitative and quantitative
changes in the ECM underscore a number of human pathologies, including cancer and PAH. Functional differentiation of the breast epithelium relies upon contact with an appropriate basement membrane by β1 integrins that promote both proper cell polarity and patterns of gene expression (107). Similarly, the underlying ECM dictates whether human stem cells will differentiate into adipocytes or osteoblasts (108). Many studies highlight the critical importance of understanding the reciprocal relationships between the ECM and signaling pathways, such as Rho GTPases. The connections between integrins, ECM ligands, and actin-based microfilaments inside the cell are indirect and are linked through scaffolding proteins, such as talin, paxillin, and α-actinin (106). These scaffolds activate or recruit numerous signaling molecules, including focal adhesion kinase and Src kinase family members, which then phosphorylate their substrates (109).

Tenascin-C in PAH. Tenascin-C, a large ECM glycoprotein, is expressed within the medial SMC layer of injured and remodeling PAs from hypertensive animals (110) and humans (111,112). It surrounds proliferating PASMCs within arteries from hypertensive individuals (110,111). Furthermore, tenascin-C promotes PASMC proliferation and survival. For example, exogenous tenasin-C protein amplifies the SMC proliferative response to soluble growth factors, including epidermal growth factor and basic fibroblast growth factor (110), by promoting clustering and activation of receptor tyrosine kinases, such as epidermal growth factor receptors (113). Moreover, studies using isolated PASMCs and PAs from monocrotaline-exposed hypertensive rats revealed that suppression of tenasin-C using an antisense approach induces SMC apoptosis and regression of pulmonary vascular lesions (114).

Origins of the Myofibroblast in PAH

Pulmonary hypertension is characterized by cellular changes in the walls of PAs. Virtually all of these changes are characterized by increased numbers of cells expressing α-SM actin (115). It has been thought that the SM-like cells that express α-SM actin and accumulate in vascular lesions were derived from the expansion of resident vascular SMCs or adventitial fibroblasts. However, new data suggest other possible sources of α-SM actin-expressing cells (SM-like cells and/or myofibroblasts) in various vascular diseases. Circulating progenitor cells can assume an SM-like phenotype (116). Resident vascular progenitor cells have also been demonstrated to express SM-like characteristics in several vascular injury states (117). Finally, the possibility that both epithelial and endothelial cells have the capability of transitioning into a mesenchymal or SM-like phenotype has been raised.

Endothelial-mesenchymal transition. The term endothelial-mesenchymal transition (EnMT), rather than transformation or transdifferentiation, relates to epithelial biology, where the process of epithelial-mesenchymal transition has been more thoroughly investigated. Epithelial-mesenchymal transition is a process in which epithelial cells lose cell-to-cell contacts and polarity and undergo dramatic remodeling of the cytoskeleton (118), with repression of epithelial markers. Concurrently, cells begin to express mesenchymal antigens, including FSP-1, α-SM actin, fibronectin, and types I and III collagens, and manifest a proliferative and migratory phenotype. The transition of epithelial cells toward a mesenchymal phenotype occurs during embryonic development, and recent data suggest that epithelial-mesenchymal transition is important in cancer biology. A role for epithelial-mesenchymal transition during tissue injury leading to organ fibrosis is also becoming clear.

Less is known regarding EnMT than epithelial-mesenchymal transition. However, several groups have provided evidence that EnMT is critical in aortic and PA development (119). Endothelial cells labeled at an early stage of development appear later (at the onset of SMC differentiation) in the subendothelial space of the developing aorta and express α-SM actin (120). Morphologic studies in human embryos suggest that endothelial-like cells may give rise to SMC during the maturation of both PAs and veins (121). Findings in experimental wound repair have suggested that EnMT may also take place in the adult. Similarly, microvascular ECs transition into mesenchymal cells in response to chronic inflammatory stimuli (122). A role for EnMT in the neointimal thickening observed in transplant atherosclerosis and restenosis has also been suggested (120).

Endothelial cells from a variety of vascular beds retain the capability of transitioning into mesenchymal or even SM-like cells under several culture conditions (119). Endothelial cells derived from the adult bovine aorta convert to spindle-shaped α-SM actin-expressing cells when treated with TGFβ-1 (123). Human dermal microvascular ECs can be induced to transform into myofibroblasts in vitro, after long-term exposure to inflammatory cytokines (124). Recent studies have demonstrated that hypoxia is also capable of inducing transdifferentiation of PAECs into myofibroblast or SM-like cells in a process regulated by myocardin (125).

Circulating Mesenchymal Progenitor Cells in Pulmonary Vascular Remodeling

Bone marrow–derived circulating cells, known as fibrocytes, may be a source for myofibroblast accumulation during reparative processes in the lung (126). Fibrocytes are mesenchymal progenitors that coexpress hematopoietic stem cell antigens, markers of the monocyte lineage, and fibroblast products. They constitutively produce ECM components as well as ECM-modifying enzymes and can further differentiate into myofibroblasts. These cells can contribute to the new population of fibroblasts and myofibroblasts that emerge at tissue sites during normal or aberrant wound
healing, in ischemic or inflammatory fibrotic processes, and as part of the stromal reaction to tumor development (127). The fibrocyte may differentiate into mature mesenchymal cells in vivo. Differentiation of fibrocytes into myofibroblast-like cells occurs where there is increased production of TGFβ-1 and/or endothelin. In these settings, fibrocytes or fibrocyte precursor cells demonstrate downregulation of leukocytic markers (e.g., CD34 and CD45) with a concomitant upregulation of mesenchymal markers. A causal link between accumulation of fibrocytes at injured sites and ongoing tissue fibrogenesis or vascular remodeling has been provided in animal models of pulmonary disease (116). Inhibition of fibrocyte accumulation results in reduced collagen deposition and reduced accumulation of myofibroblasts. In the chronically hypoxic rat, monocyte/fibrocyte depletion markedly attenuated pulmonary vascular remodeling (116).

The transition of any cell type including ECs, progenitor cells, fibroblasts, or even SMC into a myofibroblast becomes relevant to a better understanding of PH, as myofibroblasts can generate long-lasting constriction regulated at the level of Rho/Rho-kinase–mediated inhibition of MLC phosphorylation (128). Thus, cells that have transitioned into fibroblast-like and myofibroblast-like cells may play a role in the inability of the vessel wall to dilate in response to traditional vasodilating stimuli.

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Key Words: pulmonary arterial hypertension • cellular • molecular basis.
Pulmonary arterial hypertension (PAH) is a rare disorder that may be hereditable (HPAH), idiopathic (IPAH), or associated with either drug-toxin exposures or other medical conditions. Familial cases have long been recognized and are usually due to mutations in the bone morphogenetic protein receptor type 2 gene (BMPR2), or, much less commonly, 2 other members of the transforming growth factor-β superfamily, activin-like kinase-type 1 (ALK1) and endoglin (ENG), which are associated with hereditary hemorrhagic telangiectasia. In addition, approximately 20% of patients with IPAH carry mutations in BMPR2. We provide a summary of BMPR2 mutations associated with HPAH, most of which are unique to each family and are presumed to result in loss of function. We review the finding of missense variants and variants of unknown significance in BMPR2 in IPAH/HPAH, fenfluramine exposure, and PAH associated with congenital heart disease. Clinical testing for BMPR2 mutations is available and may be offered to HPAH and IPAH patients but should be preceded by genetic counseling, since lifetime penetrance is only 10% to 20%, and there are currently no known effective preventative measures. Identification of a familial mutation can be valuable in reproductive planning and identifying family members who are not mutation carriers and thus will not require lifelong surveillance. With advances in genomic technology and with international collaborative efforts, genome-wide association studies will be conducted to identify additional genes for HPAH, genetic modifiers for BMPR2 penetrance and genetic susceptibility to IPAH. In addition, collaborative studies of BMPR2 mutation carriers should enable identification of environmental modifiers, biomarkers for disease development and progression, and surrogate markers for efficacy end points in clinical drug development, thereby providing an invaluable resource for trials of PAH prevention. (J Am Coll Cardiol 2009;54:S32–42) © 2009 by the American College of Cardiology Foundation

Pulmonary arterial hypertension (PAH) is a rare disorder with an estimated incidence of approximately 2 cases per million per year (1,2). It is characterized by a sustained increase in mean pulmonary artery pressure (>25 mm Hg at rest or 30 mm Hg with exercise), normal pulmonary capillary wedge pressure, and increased pulmonary vascular resistance. For adults, mean age at presentation ranges from 36 to 50 years, although individuals of any age can be affected (2,3). Prior to the advent of modern therapies, life expectancy for adults with idiopathic pulmonary arterial hypertension (IPAH) was <3 years from diagnosis; for children, it was <10 months (4).

Pulmonary arterial hypertension may be heritable (HPAH), idiopathic, or associated with drug or toxin exposures (fenfluramine derivatives or toxic oil syndrome), or other medical conditions, including connective tissue diseases, human immunodeficiency virus infection, congenital heart disease, and portal hypertension. Familial cases have long been recognized (5), and in 2000, bone morphogenetic protein receptor type 2 (BMPR2) was identified following linkage analysis (6-8) as the gene responsible for more than 70% of HPAH and approximately 20% of IPAH cases (9-12). Crude indirect estimates of the population carrier frequency for BMPR2...
mutations lie in the frequency range of 0.001% to 0.01% (13). Two further receptor members of the transforming growth factor (TGF)-β cell signaling superfamily are also recognized as uncommon causes of HPAH. Heterozygous mutations in activin-like kinase-type 1 (ALK1) (14) and endoglin (ENG) (15) cause hereditary hemorrhagic telangiectasia (HHT) and may rarely lead directly to the development of PAH.

Heritable PAH is inherited as an autosomal dominant trait with incomplete penetrance and an estimated lifetime risk of 10% to 20% (16). The disease is more frequent in women, with a ratio of at least 1.7:1 women to men (2,17,18). Both incomplete penetrance and the significantly skewed gender ratio suggest interactions between BMPR2 disease mutations and environmental exposures that may include hormones, together with a role for modifying genes. The latest classification scheme now replaces the term familial PAH with HPAH, at least in part to recognize the fact that up to 20% of cases previously thought to be IPAH harbor identifiable mutations in BMPR2 and therefore pose a hereditary risk to other family members. Only 6% of PAH patients reported a family history of PAH in the prospective National Institutes of Health registry (18). A family history of PAH may go unrecognized in IPAH cases with BMPR2 mutations, as a consequence of either incomplete penetrance or de novo (spontaneous) mutations. Heterozygous BMPR2 sequence variants have been identified in a small subset of patients with PAH associated with relatively brief exposure to fenfluramine (13,19) or with congenital heart disease (20), raising the question as to whether such factors represent disease triggers in the face of inherited susceptibility in some patients. In contrast, BMPR2 mutations have not been identified in PAH associated with the scleroderma-spectrum of disease or with human immunodeficiency virus (21,22).

HPAH and IPAH have a similar clinical course. HPAH is associated with a slightly younger age of onset and a slightly more severe hemodynamic impairment at diagnosis, but with similar survival (23). Patients with PAH and disease-causing BMPR2 mutations are, however, less likely to respond to acute vasodilator testing during right heart catheterization and are unlikely to benefit from treatment with calcium channel blockade (23–25).

Genetic Anticipation

Families with BMPR2 mutations have been reported to have genetic anticipation, or earlier age of diagnosis in subsequent generations (17). However, no systematic population-based study has been performed to avoid the ascertainment bias that could result in the recruitment and study of families associated with earlier-onset disease in more recent generations. Furthermore, the usual genetic mechanisms for anticipation, including trinucleotide repeat expansions, are not present in BMPR2. The question of genetic anticipation can be better addressed in future registries in which all patients with HPAH and IPAH can be genetically characterized and unbiased family studies can be performed.

The TGF-β Family and PAH

The TGF-β superfamily comprises a large series of cytokine growth factors that control a host of cellular functions, among them proliferation, migration, differentiation, apoptosis, and extracellular matrix secretion and deposition. Displaying high evolutionary conservation across species, TGF-β members are segregated into several subfamilies, notably the prototypic TGF-β ligands, receptors, and accessory molecules, activins, and the largest of these groups, the bone morphogenetic proteins (26). The implication of BMPR2, ALK-1, and ENG as causal factors in hereditary and associated forms of PAH has emphasized the critical importance of this pathway to the integrity of the pulmonary vasculature (27).

BMPR-II Structure and Signal Transduction

The 4 functional domains of TGF-β type II receptors are typically highly conserved across the family. They consist of an N-terminal ligand binding domain, a single transmembrane region, a serine/threonine kinase, and a cytoplasmic tail domain. Particular to BMPR-II, however, is the presence of an exceptionally long postkinase cytoplasmic domain, primarily encoded by exon 12 of the gene. A second isoform, generated by alternative splicing of exon 12, is expressed ubiquitously at the mRNA level, although the in vivo function of the mature polypeptide remains enigmatic (28).

Signal transduction routed through the TGF-β pathway has been extensively interrogated over the last 2 decades; in contrast, BMP signaling remains less well described. The paradigmatic BMP pathway is a phosphorylation relay of signaling intermediaries initiated at the cell surface and culminating in the nucleus (Fig. 1) (29). A heterotetrameric complex consisting of type I receptors, for example ALK1, BMPR1A or BMPR1B, and BMPR-II, amalgamate to bind extracellular dimeric ligand. These interactions promote close receptor species proximity and activation of the type I receptor by the constitutive kinase BMPR-II. The type I receptors, in turn, bind and phosphorylate members of the receptor R Smad family, namely SMAD1/5 or 8. When activated, the affinity of the R-Smads for a nuclear chaperone, Smad–4, common across the TGF-β system, is
augmented. These complex-bound signaling molecules shuttle to the nucleus, where, in concert with specific nuclear cofactors and repressors, they influence regulation of a limited set of target genes (26). Within the framework of an essentially uncomplicated canonical pathway, the specificity and flexibility of signaling outcomes are tightly maintained by the myriad, cell-specific levels of regulation extending from sequestration of extracellular ligand to inhibitive competition of type I receptors by nonfunctional decoy receptors and extensive cross-talk with other signaling pathways including p38MAPK, the cytoskeleton-associated proteins Tctex-1 and LIMK-1 (26,30–32), and c-src and RACK-1 (33,34).

The burgeoning understanding of TGF-β receptor structure and function has been an invaluable aid in determining the likely impact of BMPR2 mutations in PAH. Conversely, and of equivalent importance, analysis of the spectrum and pattern of these defects, in conjunction with directed functional studies, has confirmed and better resolved those regions of the receptor necessary for normal physiologic activity.

**Major Genetic Risk Factors in PAH**

Through the compilation of previously reported mutation data, combined with an ongoing global collaboration targeted at conducting systematic and comprehensive screening of patient samples, a combined total of 298 BMPR2 mutations have been identified (35). Of these, 88 are novel (Table 1) (36–39). These mutations were identified in independent probands, including those with a known PAH family history, sporadic onset of disease, and PAH associated with other disorders. All mutations studied to date appear to have risen on unrelated genetic backgrounds, suggesting that genetic founder events are uncommon (35). Indeed, approximately 41% (122 of 298) of all recorded BMPR2 mutations are small deletions or insertions at sites of low-complexity sequence or as a consequence of C>T transitions, presumably resulting from the relatively common process of spontaneous cytosine deamination. Thus, despite the fact that PAH typically presents as a late-onset disease, ancestral mutations in this gene are at best rare, possibly because of a deleterious effect on reproductive fitness. In addition, we describe uncommon genetic susceptibilities, including ALK-1 defects in PAH and PAH associated with HHT.

**Mutation in BMPR2 Constitutes the Primary Genetic Risk in PAH**

A wide range of mutation detection methodologies have been employed to screen patients for point mutations and
large gene rearrangements, among them direct sequencing, melting curve analysis, denaturing high-performance liquid chromatography, Southern blotting, and multiplex ligation-dependent probe amplification. To date, mutations have been recorded in over 70% of subjects with 1 or more affected relatives, whereas in idiopathic cases, mutation detection rates between centers range from 10% to 40% (12,19,36,40). Of interest, mutations of TGF-β have been recorded in over 70% of subjects with 1 or more dependent probe amplification. To date, mutations have been predicted to introduce premature truncation of the polypeptide chain are the most prevalent (203 of 298, 68%). Although there is another putative PAH locus at 2q32 that was mapped in part by using stress echocardiography to detect asymptomatic obligate carriers (41), no gene has yet been identified from this interval. Thus, to date, mutations in BMP2 remain the primary genetic susceptibility for PAH.

**Truncating Mutations of BMP2 in Heritable and Idiopathic PAH**

Mutations predicted to introduce premature truncation codons to the BMP2 open reading frame encompass nonsense (85 of 298, 29%), frame-shift (73 of 298, 24%), splice-site (26 of 298, 9%), and gene duplications/deletions (19 of 298, 6%). An exception of note is a double substitution of 2 consecutive bases (GC>AT) identified in a large PAH kindred 944 base pairs upstream of the translation start site (36). Bioinformatic assessment indicated that the mutation, within the context of flanking sequence, would likely generate the formation of an aberrant translational start signal, consequently leading to incorporation of a premature stop codon in the first exon of the gene. Allele-specific polymerase chain reaction assays utilizing variant and cell-based studies to examine activation of the nonsense-mediated decay (NMD) surveillance machinery have confirmed both degradation and loss of the mutant-harbor ing transcript. Indeed, both conventional studies on patient cell lines and novel in vitro technologies demonstrate that the majority of mutations in this class are rapidly removed from the cell via the NMD pathway (36,42). Along with the observation of large proximal and whole deletions of BMP2, these findings now firmly substantiate haplinsufficiency as the predominant molecular mechanism underlying BMP2 predisposition to hereditary and idiopathic forms of disease (11,13,43).

**Distribution and Impact of Missense Mutations Across Conserved Functional Domains of BMP2**

Missense mutations of BMP2 in HPAH/IPAH cluster mainly in regions of the gene-encoding receptor domains indispensable to signaling activity and are confined to exons 2, 3, 6 to 9, 11, and 12. The extracellular ligand-binding domain of BMP2-II adopts a precisely folded conforma-

tion, exquisitely dependent on the formation of 5 disulphide bridges by 10 cysteine residues dispersed across exons 2 and 3, invariant in the majority of type II receptors (44). Amino acid substitutions in this domain are common, particularly at the cysteine residues, with 17 of 22 independent cysteine mutations affecting 8 of the 10 conserved residues. Subcellular analysis of all tested cysteine mutant constructs demonstrates substantial cytosolic retention, likely to be due to a profound loss of conformational integrity (45,46).

The BMP-II catalytic domain shares the fundamental structural and functional characteristics of members of the extensive eukaryotic protein kinase superfamily. The kinase region is compartmentalized into 12 subdomains of variable importance to the processes of adenosine triphosphate binding, substrate recognition, and phosphate group transfer (47). However, dispersed across the subdomains are 12 critical, highly conserved, amino acid residues. Missense mutations are typically restricted to regions crucial to kinase activity, as best exemplified by substitution of the native arginine residue at the invariant position 491, a site of frequent and recurrent mutation in PAH. Disruption of the key structural interaction between this residue and a glutamic acid at position 386 effectively renders the receptor kinase inactive. Whereas noncysteine substitutions traffic normally to the cell surface, all kinase mutations occurring in these catalytically important domains display a near complete abolition of signaling through the Smad pathway (45,46).

**Variants of Unknown Significance in PAH**

Amino acid substitutions are seldom observed in the cytoplasmic domain of BMP2-II (7 of 298, 2%), in contrast to truncating mutations, which account for approximately 15% of the overall mutation load in PAH. Furthermore, missense mutations in this region are functionally distinct from classical PAH-causing defects, as they are associated with normal levels of Smad activation (45,46). Instead, in transient transfection-based assays, disease-specific substitutions of the cytoplasmic tail appear to constitutively activate p38MAPK and impede phosphorylation of the dynein light chain Tctex-1 (32,46). Thus, this domain of the receptor is significant in relaying receptor signal through Smad-independent pathways. The relevance of this finding to disease pathogenesis is currently under investigation.

The mutation spectrum in PAH associated with other conditions or acknowledged risk factors, for example, fenfluramine use, is distinct from the mutation spectrum in hereditary/idiopathic disease (35). PAH patients with fenfluramine exposure exclusively harbor missense mutations, in comparison to only one-third of patients with HPAH. Moreover, the substitutions occur at positions not recognized as having a major impact on receptor function. For example, none of the 5 reported variants in the extracellular domain observed in patients with PAH and congenital heart
Table 1 Pathogenic BMPR2 Mutations in PAH

<table>
<thead>
<tr>
<th>Location</th>
<th>Mutation Category</th>
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<th>Nucleotide Change</th>
<th>Amino Acid Change</th>
<th>Frequency in This Study</th>
<th>Comment</th>
<th>Reference</th>
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<td>p.L97P</td>
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</tr>
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<td></td>
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<td>p.Y42H</td>
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<td>ECD</td>
<td>c.248_2_418del</td>
<td>2 H,H</td>
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</tr>
<tr>
<td>Exon 2–3</td>
<td>Deletion</td>
<td>ECD</td>
<td>c.248_2_418del</td>
<td>5 H,H,H,H,H</td>
<td></td>
<td>This analysis</td>
<td></td>
</tr>
<tr>
<td>Intron 3</td>
<td>Splice-site</td>
<td>ECD</td>
<td>c.248_2_418del</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Splice-site</td>
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<td>c.248_2_418del</td>
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<td></td>
<td>This analysis</td>
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</tr>
<tr>
<td>Exons 4–5</td>
<td>Deletion</td>
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<td></td>
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<tr>
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<td>c.583G&gt;T</td>
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<tr>
<td>Missense</td>
<td>TM</td>
<td>c.604A&gt;T</td>
<td>p.N202Y</td>
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</tr>
<tr>
<td>Exon 6</td>
<td>Frameshift</td>
<td>KD</td>
<td>c.612delA</td>
<td>p.L204fsX5</td>
<td>1 H</td>
<td></td>
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</tr>
<tr>
<td>Non-sense</td>
<td>KD</td>
<td>c.631C&gt;T</td>
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<td>2 H</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Frameshift</td>
<td>KD</td>
<td>c.660insG</td>
<td>p.G220fsX4</td>
<td>1 NK</td>
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<td></td>
</tr>
<tr>
<td>Frameshift</td>
<td>KD</td>
<td>c.690_691delAGinsT</td>
<td>p.K239fsX21</td>
<td>1 H</td>
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<td></td>
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<tr>
<td>Missense</td>
<td>KD</td>
<td>c.794A&gt;G</td>
<td>p.E226G</td>
<td>1 I</td>
<td></td>
<td>This analysis</td>
<td></td>
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<td>Missense</td>
<td>KD</td>
<td>c.797G&gt;C</td>
<td>p.R266T</td>
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<td>This analysis</td>
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<td>Missense</td>
<td>KD</td>
<td>c.806G&gt;T</td>
<td>p.A268V</td>
<td>1 I</td>
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<td>Baloira et al. (37)</td>
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<td>Intron 6</td>
<td>Splice-site</td>
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<td>c.852_1G&gt;C</td>
<td>1 I</td>
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<td></td>
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<td>Splice-site</td>
<td>KD</td>
<td>c.853_1G&gt;C</td>
<td>1 I</td>
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<td>This analysis</td>
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</tr>
<tr>
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<td>Non-sense</td>
<td>KD</td>
<td>c.928A&gt;T</td>
<td>p.R310X</td>
<td>1 I</td>
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<td></td>
</tr>
<tr>
<td>Missense</td>
<td>KD</td>
<td>c.932G&gt;A</td>
<td>p.G311E</td>
<td>1 H</td>
<td></td>
<td>This analysis</td>
<td></td>
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<tr>
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<td>KD</td>
<td>c.1019T&gt;C</td>
<td>p.L340P</td>
<td>1 H</td>
<td></td>
<td>This analysis</td>
</tr>
<tr>
<td>Non-sense</td>
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<td>p.C347X</td>
<td>1 H</td>
<td></td>
<td>This analysis</td>
<td></td>
</tr>
<tr>
<td>Missense</td>
<td>KD</td>
<td>c.1042G&gt;A</td>
<td>p.V348E</td>
<td>1 H</td>
<td></td>
<td>This analysis</td>
<td></td>
</tr>
<tr>
<td>Frameshift</td>
<td>KD</td>
<td>c.1095delC</td>
<td>p.R365fsX8</td>
<td>1 H</td>
<td></td>
<td>This analysis</td>
<td></td>
</tr>
<tr>
<td>Frameshift</td>
<td>KD</td>
<td>c.1099_1103delGGGA</td>
<td>p.E368fsX1</td>
<td>1 H</td>
<td></td>
<td>This analysis</td>
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<tr>
<td>Deletion</td>
<td>KD</td>
<td>c.968_1129del</td>
<td>1 H</td>
<td></td>
<td></td>
<td>This analysis</td>
<td></td>
</tr>
</tbody>
</table>

Continued on next page
disease or associated with appetite suppressant intake alter cysteine residues, and 2 residues altered in exon 5 encode a receptor region of no known functional significance (13, 20, 47). Significantly, a recent in vitro study of this mutation series has indicated that the signaling capacity of receptors harboring atypical variation approaches physiologic levels (42). Akin to the cytoplasmic tail substitutions, the relationship of these variants to the etiology of PAH is difficult to assess. A possible interpretation may be that these alleles produce PAH in the setting of additional genetic or environmental risk factors.

**Rare Disease Alleles Underlying PAH**

HHT is an autosomal dominant vascular disorder characterized by the appearance of cutaneous telangiectasias and arteriovenous malformations. The disease is caused by pathogenic mutations of either the TGF-β type I receptor ALK-I or accessory receptor endoglin (ENG) (Table 2) (15, 27, 48, 49), and, rarely, Smad 4, which is also associated with juvenile polyposis. A small proportion of HHT patients have PAH that is clinically and histopathologically indistinguishable from other heritable forms of PAH, whereas others have PAH due to pulmonary arteriovenous fistulas (27). The underlying causative factor in these patients is, typically, mutations of ALK-I. Up to 20% (16 of 83) of all detected mutations in ALK-I are associated with the development of PAH, and of these, 81% (13 of 16) are consistently observed with PAH (50, 51). The majority of these defects comprise missense mutations and cluster in functional domains of the receptor, namely the kinase domain and NANDOR box. In rare instances, mutations of ALK-I (n = 9) appear to cause IPAH or HPAH without HHT (Table 3) (27, 38, 48). Of interest, 4 mutations are confined to 2 discrete positions (amino acids 479 and 484)
of the kinase domain, suggesting that the native residues, both arginines, may be important for maintenance of the pulmonary architecture (45). However, an important caveat is that these patients usually present with early-onset disease and may go on to develop HHT at a later stage (38, 48).

**Genetic Testing for PAH**

Clinical genetic testing is available for PAH for BMPR2, ALK1, and ENG. In most cases, genetic analysis will commence with analysis of BMPR2 unless there are specific clinical symptoms or family history to suggest HHT, such as mucocutaneous telangiectasias, recurrent epistaxis, gastrointestinal bleeding, or arteriovenous malformations in the pulmonary, hepatic, gastrointestinal, or cerebral circulations. Genetic testing may be offered to any individual with a family history of PAH or IPAH (without other known affected family members), and physicians may have a duty to inform these patients of the possibility that PAH could develop in other family members. It may be necessary to go back to stored blood or DNA if the only affected family member is deceased and if those materials are available for testing.

Evaluation of BMPR2 should begin with full sequencing of all 13 exons, including splice junctions. If no mutation is identified by sequence analysis, further characterization for genomic deletions and rearrangements should be evaluated by an appropriate methodology, such as multiplex ligation-dependent probe amplification. Using these combined approaches, approximately 70% of HPAH patients will have identified mutations in BMPR2 (36). Clinical genetic testing is available in North America and Europe, with the current cost of testing ranging from approximately U.S. $1,000 to $3,000 to analyze the first member of a family. Testing other family members for a family-specific mutation is U.S. $300 to $500. Genetic testing should involve pre-test and post-test genetic counseling, ideally with a genetic counselor experienced in pulmonary hypertension. As a consequence of the incomplete penetrance and variable age of onset, identification of a BMPR2 mutation may have a complex and serious psychosocial impact on the family and is often associated with feelings of guilt in the parent who has passed on mutation to the children. Genetic testing is most helpful when it is able to identify members of the family who are not genetically at risk for PAH, and who can then forgo serial evaluation for detection of PAH.

The most common reasons that persons pursue genetic testing are to inform their children of their hereditary predisposition or to make informed decisions about family planning (52). In the past, many patients opted not to pursue genetic testing because of anxiety regarding genetic discrimination. Recognition of these concerns has led a number of countries to introduce either voluntary or legal codes to protect individuals requesting genetic counseling and formal testing. For example, in the U.S., the Genetic Information Nondiscrimination Act, passed in May 2008, protects members of both individual and group health insurance plans from discrimination in coverage or cost of health insurance coverage and also protects against discrimination in employment based upon a genetic predisposition (53). Genetic testing of children should be performed with caution, because of the potentially significant psychological impact on a child, particularly overt anxiety for the future development of a potentially fatal disease in the absence of currently known effective disease-prevention strategies.

**Clinical Monitoring of Individuals at Risk**

Clinical monitoring of patients with a family history of PAH or carriers of the BMPR2 mutation has not been evaluated rigorously. Consideration has been given to annual clinical examination, echocardiogram, stress echocardiography, Doppler echocardiography during supine bicycle exercise, and right heart catheterization at rest and with exercise. The 1998 World Pulmonary Hypertension Conference suggested that first-degree relatives of known HPAH patients should be screened annually using clinical examination and echocardiography. It is hoped that with regular surveillance, individuals can be diagnosed earlier in their disease and benefit from early treatment. Although there are currently no data to suggest that early diagnosis

---

**Table 3**

<table>
<thead>
<tr>
<th>Location</th>
<th>Domain</th>
<th>PAH Classification</th>
<th>Nucleotide Change</th>
<th>Amino Acid Change</th>
<th>Age at Onset</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Exon 2</td>
<td>ECD</td>
<td>IPAH</td>
<td>c.430C&gt;T</td>
<td>p.R144X</td>
<td>NK</td>
<td>This analysis</td>
</tr>
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<td>Exon 5</td>
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<td>IPAH</td>
<td>c.536A&gt;C</td>
<td>p.D179A</td>
<td>51 yrs</td>
<td>Harrison et al. (27)</td>
</tr>
<tr>
<td>Exon 7</td>
<td>KD</td>
<td>HPAH</td>
<td>c.936C&gt;G</td>
<td>p.H312Q</td>
<td>14 yrs</td>
<td>Fujiwara et al. (38)</td>
</tr>
<tr>
<td>Exon 8</td>
<td>KD</td>
<td>IPAH</td>
<td>c.1142T&gt;C</td>
<td>p.L381P</td>
<td>9 yrs</td>
<td>Fujiwara et al. (38)</td>
</tr>
<tr>
<td>Exon 9</td>
<td>KD</td>
<td>HPAH</td>
<td>c.1270C&gt;A</td>
<td>p.P424T</td>
<td>7 yrs</td>
<td>Fujiwara et al. (38)</td>
</tr>
<tr>
<td>Exon 10</td>
<td>KD</td>
<td>IPAH</td>
<td>c.1436G&gt;A</td>
<td>p.R479Q</td>
<td>7 yrs</td>
<td>Fujiwara et al. (38)</td>
</tr>
<tr>
<td>KD</td>
<td>IPAH</td>
<td>c.1436G&gt;A</td>
<td>p.R479P</td>
<td></td>
<td>NK</td>
<td>This analysis</td>
</tr>
<tr>
<td>KD</td>
<td>HPAH</td>
<td>c.1451G&gt;A</td>
<td>p.R484Q</td>
<td>18 months</td>
<td>Harrison et al. (27)</td>
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<tr>
<td>KD</td>
<td>HPAH</td>
<td>c.1451G&gt;A</td>
<td>p.R484Q</td>
<td>2 yrs</td>
<td>Fujiwara et al. (38)</td>
<td></td>
</tr>
</tbody>
</table>

Numbering is based on +1 of the initiation methionine of ALK1.

IPAH = Idiopathic pulmonary arterial hypertension; other abbreviations as in Tables 1 and 2.
will improve the outcome, such studies are in progress (54,55).

**Reproductive Planning**

Prenatal testing is available for BMPR2 mutations if a familial mutation has been identified. For a prospective parent carrying a known BMPR2 mutation, there is a 50% risk of transmitting this mutation to any offspring (56). Prenatal testing can be performed by chorionic villus sampling as early as the 10th week of pregnancy. However, partly in consequence of the reduced penetrance of PAH disease mutations, in our experience, very few families have pursued this option. Additionally, pre-implantation genetic diagnosis is available, whereby families use in vitro fertilization, and genetic testing is performed on the embryo prior to embryo transfer. Only embryos without the familial BMPR2 mutation are transferred to the uterus. This option may prove more appealing, particularly to women who are carriers of the BMPR2 mutation and have been advised not to pursue a pregnancy because of the increased risk of developing PAH symptoms themselves. In such cases, in vitro fertilization is already required for surrogacy. However, pre-implantation genetic diagnosis with in vitro fertilization is costly, may not be covered by insurance in the U.S., and is not available in all European countries. Pre-implantation genetic diagnosis has some associated diagnostic errors (57).

**Identifying Novel, Highly Penetrant Genes for HPAH**

A small percentage of HPAH families have multiple affected individuals but do not have identified mutations in BMPR2, despite full sequencing of exons and splice junctions and testing for genomic alterations (9–12). Although many of these families have limited numbers of living affected family members available for research, HPAH in these families may be due to locus or allelic heterogeneity that could be identified by sequencing of candidate genes, or in rare cases, analyzed by linkage if sufficient numbers of affected family members are available. As our ability to cost-effectively sequence and interpret greater amounts of DNA grows, we should be able to analyze the BMPR2 mutation-negative HPAH cases for mutations in the whole BMPR2 genomic locus and for other physiologic candidate genes.

**Genetic Modifiers of Risk for PAH**

The complex clinical features of PAH, including variable age of disease onset both within and between families and sex-dependent penetrance, imply the existence of additional factors capable of modulating disease susceptibility (58). Moreover, the likely existence of environmental modifiers is highlighted by our observation of at least 7 pairs of monozygotic twins discordant for disease (R. C. Trembath, personal communication, September 2008). To date, a number of studies examining the contribution of variations in candidate genes considered to play a biological role in the etiology of PAH have been conducted (40,59–68). However, all the case/control analyses suffer from critically small sample cohorts that would be unlikely to identify genetic modifiers of moderate effect size. Further, validation through independent replication studies, a crucial second stage of association analyses, has been lacking in most of the studies performed to date (Table 4) (35,59–64,66–68).

**Genome-Wide Association (GWA) Analysis in IPAH**

Driven by the HapMap project (69) and the development of genotyping platforms facilitating analysis of several hundred thousand independent loci, an understanding of the human haplotypic architecture of sequence variation and the identification of loci conferring even modest risks for disease are now feasible. To achieve the power required to conduct a GWA study and to detect signals of modest genotypic relative risk (GRR), large numbers of cases and controls and

### Table 4 Candidate Gene Association Studies Conducted in PAH

<table>
<thead>
<tr>
<th>Gene</th>
<th>PAH Classification</th>
<th>Case Sample (n)</th>
<th>Control Sample (n)</th>
<th>Association (Patient/Control Frequency)</th>
<th>Significance (p Value)</th>
<th>Reference</th>
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<td>ACE</td>
<td>Not specified</td>
<td>60</td>
<td>158</td>
<td>DD genotype (0.45/0.28)</td>
<td>0.01</td>
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<td>Not specified</td>
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<td>200</td>
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<td>N/A</td>
<td>Hooper et al. (63)</td>
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<tr>
<td>Hypox</td>
<td>48</td>
<td>30</td>
<td>I allele (0.67/0.38)</td>
<td>0.003</td>
<td>Alistashev et al. (60)</td>
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</tr>
<tr>
<td>5-HTT</td>
<td>PAH = appetite suppressants</td>
<td>89</td>
<td>84</td>
<td>II genotype (0.65/0.27)</td>
<td>&lt;0.001</td>
<td>Eddahibi et al. (62)</td>
</tr>
<tr>
<td>IPAH/CTEPH</td>
<td>74/35</td>
<td>Unknown</td>
<td>No association</td>
<td>N/A</td>
<td>Koehler et al. (64)</td>
<td></td>
</tr>
<tr>
<td>HPAH/IPAH/APH</td>
<td>133/259/136</td>
<td>253</td>
<td>No association</td>
<td>N/A</td>
<td>Machado et al. (35)</td>
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<td>HPAH</td>
<td>166/83</td>
<td>125</td>
<td>II genotype with early onset in FPAH</td>
<td>&lt;0.02</td>
<td>Willers et al. (67)</td>
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<tr>
<td>Endoglin</td>
<td>PAH = systemic sclerosis</td>
<td>23</td>
<td>140</td>
<td>6bINS (0.11/0.24)</td>
<td>&lt;0.01</td>
<td>Wipff et al. (68)</td>
</tr>
<tr>
<td>PGIS</td>
<td>CTEPH</td>
<td>90</td>
<td>144</td>
<td>No association</td>
<td>N/A</td>
<td>Amano et al. (61)</td>
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<td>Kv1.5</td>
<td>IPAH</td>
<td>NO responders</td>
<td>NO nonresponders</td>
<td>SNP4 a allele; SNP17 a allele (0.07/0.23; 0.016/0.06)</td>
<td>0.01/0.05</td>
<td>Remillard et al. (66)</td>
</tr>
</tbody>
</table>

ACE = angiotensin converting enzyme; APAH = associated pulmonary arterial hypertension; CTEPH = chronic thromboembolic pulmonary hypertension; D = deletion; HPAH = heritable pulmonary arterial hypertension; HTT = hydroxytryptamine (serotonin) transporter; I = insertion; Kv1.5 = potassium channel subunit 1.5; NO = nitric oxide; PGIS = prostacyclin synthase gene; PH = pulmonary hypertension; other abbreviations as in Tables 1 and 3.
GWA Study Design in IPAH

A staged approach will be taken, using a common genotyping platform and concurrent screens. This will provide a dataset of >1,000 cases and ≤3,000 controls. All patients will be screened for mutations in BMPR2 and ALK-1, and mutation carriers will be removed from the analysis. Data from the 2 screens may be combined in a meta-analysis (72). Based on power calculation simulations, these numbers are considered sufficient to detect variants of moderate effect size (GRR 1.3). The second stage of the study will be independent replication by a GWA performed on a combined cohort of North American IPAH cases and controls. Again, the datasets will be combined in a meta-analysis, and loci displaying significant association across the 3 studies will be chosen for further interrogation. In this way, the false-positive report rate is anticipated to be limited and the likelihood of true associations with IPAH increased (73).

Publicly available data from the HapMap consortium on the extent of linkage disequilibrium in regions displaying positive signals will be used to fine-map associated loci and establish a panel of tagging single nucleotide polymorphisms. Where strong association is identified with low-frequency haplotypes, candidate genes will be resequenced to capture rare, potentially causal variants. All potentially functional single nucleotide polymorphisms will subsequently be analyzed in all patient and control cohorts. A range of well-established statistical tools, including conditional logistic regression, will be used to identify those variants most likely to be true susceptibility alleles.

Modifiers of BMPR2

Mutation in Heritable Disease

The major PAH centers worldwide have access to at least 250 kindreds with identified BMPR2 mutations. Risk alleles from the IPAH GWA study will be assessed in affected probands from these families by comparison to age- and sex-matched unaffected mutation carriers to determine whether, in the framework of BMPR2 mutation, they act as modifiers in familial disease as well as IPAH.

Future Research

Identification of a substantial number of genetically at-risk individuals offers the potential to develop an extremely valuable resource for future studies, including assessment of the natural history of PAH, development of biomarkers for disease onset and progression, the identification of environmental and genetic modifiers, and the opportunity to test methods for primary prevention. Biomarker development will benefit from advances in expression profiling, proteomics, and metabolomics, by longitudinally testing individuals at increased genetic risk. Interestingly, the largest number of genetically at-risk individuals is within families of patients currently diagnosed with IPAH, given the relatively higher prevalence of this form of PAH. Through clinical genetic testing programs or by enrollment in research programs, such kindreds will provide an invaluable resource for studying PAH and potentially identifying preventive measures. Clearly these types of ambitious studies will require large numbers of patients as well as collaboration among multiple centers around the world. However, such a cohort of asymptomatic BMPR2 mutation carriers is likely to provide one of the most powerful resources for an in-depth understanding of PAH pathogenesis and prospects for prevention.

Author Disclosures

Dr. Elliott is employed by Intermountain Healthcare. Intermountain Healthcare has filed a patent based on Dr. Elliott’s invention for the use of BMPR2 mutation analysis to assess vasoreactivity in pulmonary arterial hypertension. Intermountain Healthcare, with Dr. Elliott as Principal Investigator, has received grant support from Actelion, Pfizer, Encysive, CoTherix, and United Therapeutics. Dr. Elliott serves as a member of the Registry to Evaluate Early and Long Term PAH Disease Management sponsored by Actelion. Drs. Machado, Eickelberg, Geraci, Hanaoka, Loyd, Newman, Phillips, Soubrier, Trembath, and Chung report no conflicts of interest.

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REFERENCES


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Updated Clinical Classification of Pulmonary Hypertension

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Clamart, France; Nashville, Tennessee; Geneva, Switzerland; La Jolla, California; Leuven, Belgium; London, United Kingdom; Salt Lake City, Utah; Dublin, Ireland; Pittsburgh, Pennsylvania; Shanghai, China; Rochester, Minnesota; Montréal, Québec, Canada; Osaka, Japan; and São Paulo, Brazil

The aim of a clinical classification of pulmonary hypertension (PH) is to group together different manifestations of disease sharing similarities in pathophysiology, clinical presentation, and therapeutic approaches. In 2003, during the 3rd World Symposium on Pulmonary Hypertension, the clinical classification of PH initially adopted in 1998 during the 2nd World Symposium was slightly modified. During the 4th World Symposium held in 2008, it was decided to maintain the general architecture and philosophy of the previous clinical classifications. The modifications adopted during this meeting predominantly concern Group 1, pulmonary arterial hypertension (PAH). This subgroup includes patients with PAH with a family history or patients with idiopathic PAH with germline mutations (e.g., bone morphogenetic protein receptor-2, activin receptor-like kinase type 1, and endoglin). In the new classification, schistosomiasis and chronic hemolytic anemia appear as separate entities in the subgroup of PAH associated with identified diseases. Finally, it was decided to place pulmonary veno-occlusive disease and pulmonary capillary hemangiomatosis in a separate group, distinct from but very close to Group 1 (now called Group 1'). Thus, Group 1 of PAH is now more homogeneous. (J Am Coll Cardiol 2009;54: S43–54) © 2009 by the American College of Cardiology Foundation

The classification of pulmonary hypertension (PH) has gone through a series of changes since the first classification was proposed in 1973 at an international conference on primary PH (PPH) endorsed by the World Health Organization (1, 2). The initial classification designated only 2 categories, PPH or secondary PH, depending on the presence or absence of identifiable causes or risk factors. Twenty-five years later, the 2nd World Symposium on Pulmonary Arterial Hypertension (PAH) was held in Evian, France. The “Evian classification” attempted to create categories of PH that shared pathologic and clinical features as well as similar therapeutic options (3). This was a much broader, more encompassing classification, with 5 major categories; it allowed investigators to conduct clinical trials in a well-defined group of patients with a shared underlying pathogenesis. This has led to multiple clinical trials and the approval of 8 different medications worldwide for the treatment of PAH.

The 3rd World Symposium on PAH was held in Venice, Italy, 5 years after the Evian conference. At this conference, the impact and usefulness of the “Evian classification” was reviewed, and modest changes were made. The most notable change was to abandon the term PPH in favor of idiopathic pulmonary arterial hypertension (IPAH); familial PAH if there is a family history of PAH; or associated PAH if another cause, such as connective tissue disease or human immunodeficiency virus (HIV), is present. Although the term PPH had become well ingrained in the literature after Dresdale first used it in 1951 (4), it had become clear that

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the pathologic changes and response to therapy were similar in several other conditions or diseases. The term "secondary PH" had been abandoned at the Evian meeting because it was confusing and did not help with diagnosis or in directing treatment (5) (Table 1). The other prominent change made at the Venice meeting was to move pulmonary veno-occlusive disease (PVOD) and pulmonary capillary hemangiomatosis (PCH) from separate categories into a single subcategory of PAH. These 2 entities have many similarities with each other, which will be discussed later in this article, as well as some similarities with PAH. The 2008 4th World Symposium on PH held in Dana Point, California, provided the opportunity to slightly modify the previous clinical classifications.

### Dana Point Classification

During the 4th World Symposium on PH held in 2008 in Dana Point, California, the consensus of an international group of experts was to maintain the general philosophy and organization of the Evian-Venice classifications. However, in response to a questionnaire regarding the previous classification, a majority of experts (63%) felt that modification of the Venice classification was required to accurately reflect information published over the past 5 years, as well as to clarify some areas that were unclear. The current Dana Point classification is listed in Table 2, with major changes highlighted.

### Group 1: PAH

Pulmonary arterial hypertension has been the focus of the classification of PH since the first classification in 1973. The nomenclature of the subgroups and associated conditions has evolved since that time, and additional modifications were made in the Dana Point classification.

1.1. Idiopathic and heritable PAH. Pulmonary arterial hypertension may occur in different clinical conditions depending on associated diseases. Idiopathic PAH corresponds to sporadic disease in which there is neither a family history of PAH nor an identified risk factor. When PAH occurs in a familial context, germline mutations in the bone morphogenetic protein receptor type 2 (BMPR2) gene, a member of the transforming growth factor β signaling family, can be detected in approximately 70% of cases (6,7). More rarely, mutations in activin receptor-like kinase type 1, or endoglin, also members of the transforming growth factor β signaling family, have been identified in patients with PAH, predominantly with coexistent hereditary hemorrhagic telangiectasia. Recently, it has been suggested that patients with PAH associated with BMPR2 mutations may represent a subgroup of patients with more severe disease who are less likely to demonstrate vasoreactivity than those with IPAH without BMPR2 mutations (8–10).

Because BMPR2 mutations have also been detected in 11% to 40% of apparently idiopathic cases with no family history (11,12), the distinction between idiopathic and familial BMPR2 mutations is artificial. All patients with BMPR2 mutations have heritable disease, whether the patient is the first identified case, possibly with a de novo mutation, or other family members were previously diagnosed with PAH. In addition, in 30% or fewer families with PAH, no BMPR2 mutation has been identified. Thus, it was decided to abandon the term “familial PAH” in the new classification and to replace it with the term “heritable PAH.” Heritable forms of PAH include IPAH with germline mutations (mainly BMPR2 but also activin receptor-

### Table 1: Venice Clinical Classification of Pulmonary Hypertension (2003)

<table>
<thead>
<tr>
<th>Classification</th>
<th>Notes</th>
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<tbody>
<tr>
<td>1. Pulmonary arterial hypertension (PAH)</td>
<td></td>
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<tr>
<td>1.1. Idiopathic (IPAH)</td>
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</tr>
<tr>
<td>1.2. Familial (FPAH)</td>
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<tr>
<td>1.3. Associated with (APAH)</td>
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<tr>
<td>1.3.1. Collagen vascular disease</td>
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<td>1.3.2. Congenital systemic-to-pulmonary shunts</td>
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<td>1.3.3. Portal hypertension</td>
<td></td>
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<td>1.3.4. HIV infection</td>
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<tr>
<td>1.3.5. Drugs and toxins</td>
<td></td>
</tr>
<tr>
<td>1.3.6. Other (thyroid disorders, glycogen storage disease, Gaucher disease,</td>
<td></td>
</tr>
<tr>
<td>hereditary hemorrhagic telangiectasia, hemoglobinopathies, myeloproliferative</td>
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<tr>
<td>disorders, spleenectomy)</td>
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<tr>
<td>1.4. Associated with significant venous or capillary involvement</td>
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<tr>
<td>1.4.1. Pulmonary veno-occlusive disease (PVOD)</td>
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<td>1.4.2. Pulmonary capillary hemangiomatosis (PCH)</td>
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<tr>
<td>1.5. Persistent pulmonary hypertension of the newborn</td>
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<tr>
<td>2. Pulmonary hypertension with left heart disease</td>
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<tr>
<td>2.1. Left-sided atrial or ventricular heart disease</td>
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<tr>
<td>2.2. Left-sided valvular heart disease</td>
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<tr>
<td>3. Pulmonary hypertension associated with lung diseases and/or hypoxemia</td>
<td></td>
</tr>
<tr>
<td>3.1. Chronic obstructive pulmonary disease</td>
<td></td>
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<td>3.2. Interstitial lung disease</td>
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<td>3.3. Sleep-disordered breathing</td>
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<td>3.4. Alveolar hypoventilation disorders</td>
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<td>3.5. Chronic exposure to high altitude</td>
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<td>3.6. Developmental abnormalities</td>
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<tr>
<td>4. Pulmonary hypertension owing to chronic thrombotic and/or embolic disease</td>
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<tr>
<td>4.1. Thromboembolic obstruction of proximal pulmonary arteries</td>
<td></td>
</tr>
<tr>
<td>4.2. Thromboembolic obstruction of distal pulmonary arteries</td>
<td></td>
</tr>
<tr>
<td>4.3. Nonthrombotic pulmonary embolism (tumor, parasites, foreign material)</td>
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<tr>
<td>5. Miscellaneous</td>
<td></td>
</tr>
<tr>
<td>Sarcoidosis, histiocytosis X, lymphangiomatosis, compression of pulmonary</td>
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</tr>
<tr>
<td>vessels (adenopathy, tumor, fibrosing mediastinitis)</td>
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like kinase 1 or endoglin) and familial cases with or without identified germline mutations (13,14). The new category of “heritable PAH” does not mandate genetic testing in patients with IPAH or in familial cases of PAH. Genetic testing, when called for, should be performed as a part of a comprehensive program that includes genetic counseling and discussion of the risks, benefits, and limitations of such testing (15).

1.3. Drug- and toxin-induced PAH. A number of risk factors for the development of PAH have been identified and were included in the previous Evian and Venice classifications (3,5). Risk factors for PAH include “any factor or condition that is suspected to play a predisposing or facilitating role in the development of the disease. Risk factors may include drugs and chemicals, diseases, or phenotype (age, gender).” Risk factors were categorized as definite, very likely, possible, or unlikely, based on the “strength of their association with PH and their probable causal role.” In the current classification, the categorization of risk factors and the likelihood of developing PAH have been modified. Updated risk factors and associated conditions for PAH are presented in Table 3. A “definite” association is defined as an epidemic, such as occurred with appetite suppressants in the 1960s, or large, multicenter epidemiologic studies demonstrating an association between a drug and PAH. A “likely” association is defined as a single-center, case-control study demonstrating an association or a multiple-case series. “Possible” is defined as drugs with similar mechanisms of action as those in the “definite” or “likely” categories but which have not yet been studied (e.g., drugs used to treat attention-deficit disorder). Lastly, an “unlikely” association is defined as one in which a drug has been studied in epidemiologic studies and an association with PAH has not been demonstrated.

Aminorex, fenfluramine derivatives, and toxic rapeseed oil represent the only identified “definite” risk factors for PAH (3,5). A recent retrospective analysis of more than 100 cases of PAH associated with fenfluramine exposure showed that this category shares clinical, functional, hemodynamic, and genetic features with IPAH, suggesting that fenfluramine exposure represents a potential trigger for PAH without influencing its clinical course (16).

The most recent surveillance study of PH, Surveillance of Pulmonary Hypertension in America (SOPHIA), enrolled 1,335 subjects at tertiary PH centers in the U.S. between 1998 and 2001 (17). This study confirmed the association of fenfluramine and dexfenfluramine intake with the development of PAH. The average monthly number of IPAH cases did not change during the study, which was, however, conducted after fenfluramine and its derivates had been withdrawn from the U.S. market. A novel finding was that St. John’s Wort (odds ratio [OR]: 3.6, vs. thromboembolic PH) and over-the-counter antiobesity agents containing phenylpropanolamine (OR: 5.2, vs. thromboembolic PH) also increased the risk of developing IPAH.

The SOPHIA study examined intake of a variety of nonselective monoamine reuptake inhibitors, selective serotonin reuptake inhibitors, antidepressants, and anxiolytics, and found no increased risk for developing PAH (17). However, a recent case-control study of selective serotonin

### Table 3 Updated Risk Factors for and Associated Conditions of PAH

<table>
<thead>
<tr>
<th>Definite</th>
<th>Possible</th>
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<tbody>
<tr>
<td>Aminorex</td>
<td>Cocaine</td>
</tr>
<tr>
<td>Fenfluramine</td>
<td>Phenylpropanolamine</td>
</tr>
<tr>
<td>Dexfenfluramine</td>
<td>St. John’s Wort</td>
</tr>
<tr>
<td>Toxic rapeseed oil</td>
<td>Chemotherapeutic agents</td>
</tr>
<tr>
<td>L-tryptophan</td>
<td>Oral contraceptives</td>
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<tr>
<td>Methamphetamine</td>
<td>Estrogen</td>
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Likely

| Unlikely
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<tbody>
<tr>
<td>Oral contraceptives</td>
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<tr>
<td>Estrogen</td>
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<tr>
<td>Cigarette smoking</td>
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PAH = pulmonary arterial hypertension; SSRI = selective serotonin reuptake inhibitor.
reuptake inhibitor use in pregnant women after 20 weeks of gestation showed an increased risk (OR: 6.1) in the offspring of developing persistent PH of the newborn, a form of PAH (18). Based on this study, selective serotonin reuptake inhibitors may play a role in the development of PH, at least in association with pregnancy, and therefore they have been reclassified in the “possible” category.

Amphetamine use represents a “likely” risk factor for PAH, although they are rarely taken as a single agent and are frequently used in combination with fenfluramine. A recent comprehensive retrospective study suggested a strong relationship with the use of methamphetamine (inhaled, smoked, oral, or intravenous) and the occurrence of IPAH (19). Based primarily on the results of this study, methamphetamine use is now considered a “very likely” risk factor for the development of PAH. Additional changes in drug- and toxin-induced PAH will be discussed later. With the exception of hereditary hemorrhagic telangiectasia associated with PAH, the first 3 subcategories of Group 1, idiopathic, heritable, and drug- and toxin-induced PAH, are all associated with the development of isolated pulmonary arterial diseases.

1.4.3. Portopulmonary hypertension. The development of PAH in association with elevated pressure in the portal circulation is known as portopulmonary hypertension (POPH) (39,40). Portal hypertension, rather than the presence of underlying liver disease, is the main determining risk factor for the development of POPH. Prospective hemodynamic studies have shown that 2% to 6% of patients with portal hypertension have PH (41,42). Right heart catheterization is absolutely mandatory for the definitive diagnosis of POPH because several factors may increase pulmonary arterial pressure (PAP) in the setting of advanced liver disease (e.g., high flow associated with the hyperdynamic circulatory state and increased pulmonary capillary wedge pressure owing to fluid overload and/or diastolic dysfunction). Pulmonary vascular resistance (PVR) is usually normal in these cases. Pathologic changes in the small arteries appear identical to those seen in IPAH. A recent multicenter case-control study identified 2 risk factors for the development of POPH: female sex and autoimmune hepatitis (43). Interestingly, hepatitis C infection was associated with a decreased risk. A recent, large cohort study of POPH showed that long-term prognosis was related to the presence and severity of cirrhosis and to cardiac function (44).

1.4.4. Congenital heart diseases. A significant proportion of patients with congenital heart disease (CHD), in particular those with relevant systemic-to-pulmonary shunts, will develop PAH if left untreated. Persistent exposure of the pulmonary vasculature to increased blood flow, as well as increased pressure, may result in pulmonary obstructive arteriopathy, which leads to increased PVR that will result in shunt reversal. Eisenmenger syndrome is defined as CHD with an initial large systemic-to-pulmonary shunt that induces progressive pulmonary vascular disease and PAH, with resultant reversal of the shunt and central cyanosis (45,46). Eisenmenger syndrome represents the most advanced form of PAH associated with CHD. The histopathologic and pathobiologic changes seen in patients with PAH associated with congenital systemic-to-pulmonary shunts (e.g., endothelial dysfunction of the associated PAH was evaluated more recently and showed a stable prevalence of 0.46% (95% confidence interval: 0.32% to 0.64%) (35). Human immunodeficiency virus-associated PAH has clinical, hemodynamic, and histologic characteristics similar to those seen in IPAH. The mechanism for the development of PH remains unclear. Because neither the virus nor viral DNA has been found in pulmonary endothelial cells, an indirect action of virus through secondary messengers such as cytokines, growth factors, endothelin, or viral proteins is strongly suspected.

Uncontrolled studies suggest that patients with severe HIV-associated PAH could benefit from bosentan or long-term infusion of epoprostenol (36,37). Interestingly, in a substantial number of cases, normalization of pulmonary vascular hemodynamics can be obtained with therapy indicated for PAH; this is very rarely seen in IPAH (38).
pulmonary vasculature) are similar to those observed in idiopathic or other associated forms of PAH.

It has been reported that a large proportion of patients with CHD develop some degree of PAH (47–49). The prevalence of PAH associated with congenital systemic-to-pulmonary shunts in Europe and North America has been estimated to range between 1.6 and 12.5 cases per million adults, with 25% to 50% of this population affected by Eisenmenger syndrome (50). Following the Dana Point meeting, it was decided to update the pathologic and pathophysiologic classification of CHD with systemic-to-pulmonary shunts (Table 4) to provide a more detailed description of each condition. This anatomic and pathophysiologic classification may be too complex to be used in clinical practice; however, 4 quite distinct phenotypes can be recognized (Table 5).

1.4.5. Schistosomiasis. Another important modification of the new classification is the inclusion of PH associated with schistosomiasis in Group 1.

In the previous classification, this form of PH was subcategorized in Group 4 as PH owing to chronic thrombotic and/or embolic disease. Embolic obstruction of pulmonary arteries by schistosoma eggs was thought to be the primary mechanism responsible for the development of PH (51). However, more recent publications indicate that PH associated with schistosomiasis can have a similar clinical presentation to IPAH (52), with similar histologic findings, including the development of plexiform lesions (53). The mechanism of PAH in patients with schistosomiasis is probably multifactorial. It may include POPH, a frequent complication of this disease (54), and local vascular inflammation as a result of impacted schistosoma eggs, whereas mechanical obstruction by schistosoma eggs seems to play a minor role. PAH associated with schistosomiasis represents a frequent form of PAH, especially in countries in which the infection is endemic. It is estimated that more than 200 million people are infected with any of the 3 species of schistosoma and that 4% to 8% of patients will develop hepatosplenic disease. Data from a recent study based on invasive hemodynamics showed that the prevalence of PAH in patients with hepatosplenic disease was 4.6%; also important was the prevalence of post-capillary hypertension in patients with hepatosplenic disease (55). However, more recent publications indicate that PH associated with schistosomiasis represents a frequent form of PAH, especially in countries in which the infection is endemic. It is estimated that more than 200 million people are infected with any of the 3 species of schistosoma and that 4% to 8% of patients will develop hepatosplenic disease. Data from a recent study based on invasive hemodynamics showed that the prevalence of PAH in patients with hepatosplenic disease was 4.6%; also important was the prevalence of post-capillary hypertension in patients with hepatosplenic disease (55). However, more recent publications indicate that PH associated with schistosomiasis represents a frequent form of PAH, especially in countries in which the infection is endemic. It is estimated that more than 200 million people are infected with any of the 3 species of schistosoma and that 4% to 8% of patients will develop hepatosplenic disease. Data from a recent study based on invasive hemodynamics showed that the prevalence of PAH in patients with hepatosplenic disease was 4.6%; also important was the prevalence of post-capillary hypertension (3.0%), reinforcing the need for invasive hemodynamics for the proper diagnosis of PAH in schistosomiasis (55).

1.4.6. Chronic hemolytic anemia. The chronic hemolytic anemias represent a new subcategory of PAH; these were previously categorized under “other” as conditions associated with the development of PAH. Since the Venice classification, there has been increasing evidence that PAH is a complication of chronic hereditary and acquired hemolytic anemias, including sickle cell disease (SCD) (56,57), thalassemia (58), hereditary spherocytosis (59), stomatocytosis (60), and microangiopathic hemolytic anemia (61).

Pulmonary hypertension has been described most frequently in patients with SCD with histologic lesions similar
to those found in IPAH, including plexiform lesions in 1 case series (62). However, the prevalence of PAH in SCD is not clearly established. The largest study of patients with SCD, which defined PH echocardiographically by the presence of tricuspid regurgitation jet velocity (TRV) ≥2.5 m/s, found that 32% of patients had PH (57). However, using a TRV >2.5 m/s on echocardiography to define PH can lead to a substantial number of false positive cases of PH not confirmed by right heart catheterization (35,63). When a TRV >3.0 m/s was used, corresponding to an estimated systolic PAP of >41 mm Hg, only 9% of the cohort met the criteria for PH. Right heart catheterization was carried out in only 18 of 63 patients with TRV >2.5 m/s. In this subpopulation, PH defined by a mean PAP >25 mm Hg was confirmed in 17 patients; however, pulmonary wedge pressure was elevated in some patients. A substantial proportion of patients with SCD have pulmonary venous hypertension: 46% in 1 study of 26 patients with SCD and PH (64). In addition, some patients present with a hyperkinetic state with moderate elevation in mean PAP and normal PVR. Thus, although it appears that some patients with SCD do develop PAH, the prevalence of PAH in SCD is undoubtedly much lower than 32%. Prospective epidemiologic studies using echocardiographic screening and direct hemodynamic confirmation with right heart catheterization in all patients with suspected PH are ongoing and will evaluate the precise prevalence of PAH in SCD. The mechanism of PAH in SCD remains uncertain. A probable hypothesis is that chronic hemolysis results in high rates of nitric oxide consumption and produces a state of resistance to nitric oxide bioactivity (65). Consequently, smooth muscle guanosine monophosphate, a potent vasodilator/antiproliferative mediator, is not activated (66).

**Group 1**: PVOD and/or PCH

The conditions of PVOD and PCH are uncommon, but they are increasingly recognized as causes of PH (67). In the Evian classification, these 2 entities were placed in 2 different groups, both distinct from PAH: PVOD was included in the pulmonary venous hypertension category, and PCH was included in the heterogeneous group of disorders believed to directly affect the pulmonary vasculature. Pathologic studies indicate, however, that PVOD and PCH are often quite similar in terms of changes in the pulmonary parenchyma (i.e., pulmonary hemosiderosis, interstitial edema, and lymphatic dilation) and the development of pulmonary arterial intimal fibrosis and medial hypertrophy. Similarities in pathologic features and clinical presentation suggest that these disorders may overlap. Accordingly, in the Venice classification, PVOD and PCH were placed together as a subgroup of PAH.

PVOD and PCH were included in Group 1 for a number of reasons. First, the histologic changes in the small pulmo-

nary arteries (i.e., intimal fibrosis and medial hypertrophy) are also found in PAH. Second, the clinical presentations of PVOD/PCH and PAH are often indistinguishable and unrecognized antemortem (5). Third, PVOD/PCH and PAH share similar risk factors. These include the scleroderma spectrum of disease (68), HIV infection (69,70), and the use of anorexigen. Fourth, in addition to the well-described familial association with PAH, familial occurrence has been reported with both PVOD and PCH (67). Lastly, mutations in BMPR2, the gene associated with familial PAH and IPAH, have been documented in patients with PVOD (71,72). These findings suggest that PVOD, PCH, and PAH may represent different components of a single spectrum of disease.

The present decision to leave PVOD and PCH together in the same subgroup is supported by a recent clinicopathologic study (73) analyzing 38 specimens (autopsies [n = 15], surgical biopsies [n = 15], explants [n = 7], and pneumonectomy [n = 1]) from 35 patients diagnosed as having either PVOD (n = 30) or PCH (n = 5). PCH was identified in 24 (73%) patients diagnosed as having PVOD, either as perivenular foci or diffuse involvement of the pulmonary parenchyma. In 5 patients diagnosed with PCH, 4 showed the venous and arterial changes characteristic of PVOD. These findings suggest that PCH could be an angioproliferative process frequently associated with PVOD.

Recent evidence supports leaving PVOD and PCH together; however, it is apparent that although they may present similarly to IPAH, there are a number of important differences. These include the presence of crackles and clubbing on examination, ground glass opacities, septal thickening, mediastinal adenopathy on chest computed tomography (74–76), hemosiderin-laden macrophages on bronchoalveolar lavage (77), and a lower carbon monoxide diffusing capacity and PaO2 in patients with PVOD or PCH (71). In addition, the management, response to medical therapy, and prognosis of PVOD/PCH are quite different than that of PAH. A recent study compared 24 patients with histologic evidence of PVOD with or without PCH and 24 randomly selected patients with idiopathic, familial, or anorexigen-associated PAH (71). Among the 16 patients with PVOD who received PAH-specific therapy, 7 (43.8%) developed pulmonary edema. These patients were treated mainly with continuous intravenous epoprostenol, but also with oral therapies, bosentan, and calcium channel blockers. Clinical outcomes were worse in patients with PVOD than those with PAH.

PVOD/PCH remains a difficult disorder to categorize because it shares characteristics with IPAH but also has a number of distinct differences. Given the current evidence, it was decided that PVOD/PCH should be a distinct category but not completely separated from PAH. Therefore, in the current classification, PVOD/PCH is designated as 1'.
**Group 2: PH Owing to Left Heart Disease**

Left heart disease probably represents the most frequent cause of PH (78). Left-sided ventricular or valvular diseases may produce an increase in left atrial pressure, with passive backward transmission of the pressure leading to increased PAP. In this situation, PVR is normal or near normal (<3.0 Wood units) and there is no gradient between mean PAP and pulmonary wedge pressure (transpulmonary gradient <12 mm Hg). In the Venice classification, 2 broad subcategories were recognized in this group based on the presence or absence of valvular disease: PH owing to left atrial or ventricular disease and PH owing to left-sided valvular disease (mitral and/or aortic). In the Venice classification, this category was referred to as PH with left heart disease. The new heading for this classification more appropriately denotes cause and effect for this group of heterogeneous disorders on the development of PH. In addition, with increasing recognition of left-sided heart dysfunction with preserved ejection fraction, the subcategories of Group 2 have been modified and now include 3 distinct etiologies: left heart systolic dysfunction, left heart diastolic dysfunction, and left heart valvular disease.

Importantly, in some patients with left heart disease, the elevation of PAP is out of proportion to that expected from the elevation of left arterial pressure (transpulmonary gradient >12 mm Hg) and PVR is increased to >3.0 Wood units. In patients referred to cardiac transplant clinics, PH with PVR >3.0 Wood units is reported in 19% to 35% of patients (78,79). Some patients with left heart valvular disease or even left heart dysfunction can develop severe PH of the same magnitude as that seen in PAH (80–82). The elevation of PAP and PVR is due to either the increase of pulmonary artery vasomotor tone and/or pulmonary vascular remodeling (83,84). No studies using medications approved for PAH have been performed in this patient population, and the efficacy and safety of PAH medications remain unknown.

**Group 3: PH Owing to Lung Diseases and/or Hypoxia**

In this category, the predominant cause of PH is alveolar hypoxia as a result of lung disease, impaired control of breathing, or residence at high altitude. The prevalence of PH in all of these conditions remains largely unknown. In the present classification, the structure of this group was for the most part unchanged. The heading has been modified, again to denote cause and effect on the development of PH. The primary modification in this group was to add a category of lung disease characterized by a mixed obstructive and restrictive pattern. This new subgroup includes chronic bronchiectasis, cystic fibrosis (85), and a newly identified syndrome characterized by the combination of pulmonary fibrosis, mainly of the lower zones of the lung, and emphysema, mainly of the upper zones of the lung (86). In the syndrome of combined pulmonary fibrosis and emphysema, the prevalence of PH is almost 50% (86).

In patients with parenchymal lung disease, PH is generally modest (mean PAP 25 to 35 mm Hg) (87). However, in some patients, PAP elevations can be more substantial (mean PAP 35 to 50 mm Hg) (88). In such patients, particularly those with only moderate pulmonary mechanical impairment, this is considered “out-of-proportion” PH. In a recent retrospective study of 998 patients with chronic obstructive pulmonary disease who underwent right heart catheterization, only 1% had severe pulmonary hypertension (mean PAP >40 mm Hg) (89). The authors described an unusual pattern of cardiopulmonary abnormalities in the patients with more severe PH, including mild to moderate airway obstruction, severe hypoxemia, hypocapnia, and a very low diffusing capacity for carbon monoxide. As with PH out of proportion to left heart disease, large randomized, controlled studies of medications approved for PAH are not available for PH out of proportion for parenchymal lung disease.

**Group 4: Chronic Thromboembolic PH**

In the Venice classification, Group 4 was very heterogeneous and included obstruction of pulmonary arterial vessels by thromboemboli, tumors, or foreign bodies. However, depending on the origin of the obstruction, the clinical presentation and radiologic findings are often different, and management is unique to each etiology. Chronic thromboembolic pulmonary hypertension (CTEPH) represents a frequent cause of PH. The incidence of CTEPH is uncertain, but it occurs in up to 4% of patients after an acute pulmonary embolism (90,91). In contrast, other obstructive etiologies are very rare. It was decided, therefore, to maintain only CTEPH in Group 4. In the Venice classification, CTEPH was divided into 2 subgroups: proximal CTEPH, accessible to pulmonary thromboendarterectomy, and distal CTEPH, which is not accessible to surgery. In practice, however, this distinction may be quite unclear, and it may vary depending on individual centers. Currently there is no consensus among experts about the definitions of proximal and distal CTEPH (92). Thus, it was further decided to maintain in Group 4 only a single category of CTEPH and not to attempt to distinguish between proximal and distal forms.

Importantly, it is strongly recommended that patients with suspected or confirmed CTEPH be referred to a center with expertise in the management of this disease to consider the feasibility of performing pulmonary thromboendarterectomy, currently the only curative treatment. The decision depends on the location of the obstruction (central vs. more distal pulmonary arteries), the correlation between hemodynamic findings and the degree of mechanical obstruction assessed by angiography, comorbidities, the willingness of the patient, and the experience of the surgeon (93,94). Patients who are not candidates for surgery may benefit from PAH-specific medical therapy (95,96); however, the
use of these medications in CTEPH requires further evaluation in randomized, controlled trials (97).

**Group 5: PH With Unclear or Multifactorial Etiologies**

Group 5 consists of several forms of PH for which the etiology is unclear or multifactorial.

### 5.1. The first subgroup comprises several hematologic disorders. PH has been reported in chronic myeloproliferative disorders including polycythemia vera, essential thrombocytopenia, and chronic myeloid leukemia (98). Chronic myeloproliferative disorders can cause PH by various potential mechanisms. High cardiac output, auto or surgical asplenia, direct obstruction of pulmonary arteries by circulating megakaryocytes (99), CTEPH (100), POPH, and congestive heart failure may all play a role. Splenectomy as a result of trauma or as a treatment for hematologic disorders may increase the risk of developing PH (101). CTEPH (102,103) and several cases of PAH (102,104) with medial hypertrophy, internal fibrosis, and plexiform lesions in the pulmonary vasculature have been reported in association with splenectomy.

### 5.2. The second subgroup includes systemic disorders that are associated with an increased risk of developing PH (105). Sarcoidosis is a common systemic granulomatous disease of unknown origin. PH is an increasingly recognized complication of sarcoidosis (106), with a reported prevalence of 1% to 28% (107). PH is often attributed to the destruction of the capillary bed by the fibrotic process and/or to the resultant chronic hypoxemia (108). However, the severity of PH does not always correlate well with the degree of parenchymal lung disease and blood gas abnormalities, suggesting that other mechanisms may be contributing to the development of PH (105). In this setting, such mechanisms could include extrinsic compression of large pulmonary arteries by mediastinal and hilar adenopathy, and direct granulomatous infiltration of the pulmonary vasculature, especially the pulmonary veins, which sometimes mimic PVOD (109). More rarely, POPH secondary to hepatic involvement with sarcoidosis can be associated with the pathogenesis.

Pulmonary Langerhans cell histiocytosis is an uncommon cause of infiltrative lung disease associated with destructive changes in the lung parenchyma. Severe PH is a common feature in patients with end-stage pulmonary Langerhans cells histiocytosis (110). In some patients, PH is probably related to chronic hypoxemia and/or abnormal pulmonary mechanics; in others, especially those patients with more severe elevation of PAP, PH is unrelated to lung parenchymal injury. Histopathologic examination has shown severe diffuse pulmonary vasculopathy involving predominantly intralobular pulmonary veins and, to a lesser extent, muscular pulmonary arteries (111). These vascular changes consist of medial hypertrophy and intimal fibrosis.

Lymphangioleiomyomatosis is a rare multisystem disorder predominantly affecting women, characterized by cystic lung destruction, lymphatic abnormalities, and abdominal tumors. PH is relatively uncommon in patients with lymphangioleiomyomatosis (112,113). Chronic hypoxemia and pulmonary capillary destruction caused by cystic lung lesions probably represent the predominant causes of PH.

Neurofibromatosis type 1, also known as von Recklinghausen disease, is an autosomal dominant disease that can be recognized by characteristic “cafe au lait” skin lesions and by cutaneous fibromas. The disease is occasionally complicated by systemic vasculopathy. Recently it was reported that the neurofibromatosis type 1 gene modulates protein kinase B, an important regulator of cell proliferation. Several cases of PH have recently been reported in the setting of von Recklinghausen disease (114–117). The mechanism of PH is unclear. Lung fibrosis and CTEPH are thought to play a role. In rare cases, histologic examination found both arteries and veins narrowed by medial and/or intimal hypertrophy and fibrosis (117,118).

Lastly, some rare cases of PH have been observed in antineutrophil cytoplasmic antibodies-associated vasculitis; the clinical presentation is similar to PAH, but histologic data are not available (119).

### 5.3. The third subgroup comprises metabolic disorders. PH has been reported in a few cases of type Ia glycogen storage disease, a rare autosomal recessive disorder caused by a deficiency of glucose-6-phosphatase (120–122). The mechanism of PH is uncertain but has been associated with portocaval shunts, atrial septal defects, or severe restrictive pulmonary function defects. Thrombosis may also play a role in this setting. In 1 case, autopsy findings revealed the presence of plexiform lesions (123).

Gaucher disease is a rare disorder characterized by a deficiency of lysosomal B glucosidase, which results in an accumulation of glucocerebroside in reticuloendothelial cells. Typical manifestations include hepatosplenomegaly and bone marrow infiltration. In a study of 134 patients with Gaucher disease who were systematically screened by echocardiography, PH was not uncommon (124). In this setting, several potential mechanisms for PH have been suggested, including interstitial lung disease, chronic hypoxemia, capillary plugging by Gaucher cells, and splenectomy (124,125). One case of histologic findings similar to idiopathic PAH has been reported (126).

The association between thyroid diseases (hypothyroidism and hyperthyroidism) and PH has been reported in a number of studies (127,128). In a recent prospective study using echocardiographic evaluation, more than 40% of patients with thyroid diseases had PH (129). One instance of PVOD confirmed by histology was observed in a patient with Hashimoto thyroiditis (130). Interestingly, a recent prospective study of 63 consecutive adult patients with PAH found a 49% prevalence of autoimmune thyroid disease, including both hypothyroidism and hyperthyroidism, suggesting that these conditions may be linked by a common immunogenetic susceptibility (131).
5.4. The last subgroup in category 5 includes a number of miscellaneous conditions. In tumor obstruction, a tumor grows into the central pulmonary arteries, with additional appositional thrombosis leading to a progressive obstruction of proximal pulmonary arteries and PH. Such cases are due principally to pulmonary artery sarcomas, occur rarely, and are usually rapidly fatal (132,133). The differential diagnosis with CTEPH can be difficult. Computed tomography and magnetic resonance imaging angiography are the most useful diagnostic modalities for differentiation between tumor and thrombotic material (132,134,135).

Oclusion of the microvasculature by metastatic tumor emboli represents another rare cause of rapidly progressive PH (136). The initial laboratory evaluation shows hypoxemia, often severe, with a clear lung field (137). Computed tomography scanning does not show proximal thrombi but often shows thickening of septa. In contrast, the V/Q lung scan is generally abnormal with multiple subsegmental perfusion defects. Pulmonary microvascular cytology sampling through a pulmonary artery catheter in the wedge position is an important diagnostic tool (137). The majority of reported cases occur in association with breast, lung, or gastric carcinomas.

Patients with mediastinal fibrosis may present with severe PH owing to compression of both pulmonary arteries and veins (138,139). V/Q scan, computed tomography, and pulmonary angiography are very useful for accurate diagnosis, but findings can mimic proximal thrombotic obstruction. The predominant etiology is histoplasmosis (139), although mediastinal fibrosis has been reported with other fungal organisms, with tuberculosis (140), and in patients with sarcoidosis.

Lastly, PH has been reported in patients with end-stage renal disease (ESRD) maintained on long-term hemodialysis. Based on echocardiographic studies, the prevalence of PH in this patient population is estimated at up to 40% (141). There are several potential explanations for the development of PH in patients with ESRD. Hormonal and metabolic derangement associated with ESRD might lead to pulmonary vascular constriction. The PAP may also be increased by high cardiac output (resulting from the arteriovenous access itself and often concomitant anemia) as well as fluid overload. In addition, diastolic and systolic left heart dysfunctions are frequent in this setting (142).

Conclusions

In updating the classification of PH, we incorporated recent findings and sought to clarify areas of ambiguity. We believe that this version is both more comprehensive and more comprehensible and hope that it will also be more useful to clinicians, pending further research into this diverse disease.

Author Disclosures

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Key Words: pulmonary hypertension □ clinical classification □ pulmonary arterial hypertension.
Diagnosis and Assessment of Pulmonary Arterial Hypertension

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The diagnosis and assessment of pulmonary arterial hypertension is a rapidly evolving area, with changes occurring in the definition of the disease, screening and diagnostic techniques, and staging and follow-up assessment. The definition of pulmonary hypertension has been simplified, and is now based on currently available evidence. There has been substantial progress in advancing the imaging techniques and biomarkers used to screen patients for the disease and to follow up their response to therapy. The importance of accurate assessment of right ventricular function in following up the clinical course and response to therapy is more fully appreciated. As new therapies are developed for pulmonary arterial hypertension, screening, prompt diagnosis, and accurate assessment of disease severity become increasingly important. A clear definition of pulmonary hypertension and the development of a rational approach to diagnostic assessment and follow-up using both conventional and new tools will be essential to deriving maximal benefit from our expanding therapeutic armamentarium. (J Am Coll Cardiol 2009;54:S55–66) © 2009 by the American College of Cardiology Foundation

Definition of Pulmonary Hypertension (PH)

PH has been defined as a resting mean pulmonary arterial pressure (mPAP) >25 mm Hg, or an mPAP with exercise >30 mm Hg. The subgroup of PH known as pulmonary arterial hypertension (PAH) adds the criterion that the pulmonary arterial wedge pressure must be ≤15 mm Hg. Some definitions have also included pulmonary vascular resistance (PVR), requiring that it be ≥2 or 3 Wood units. Potential weaknesses of the current definition include the fact that the level, type, and posture of exercise have not been specified. Furthermore, the normal exercise pulmonary arterial pressure (PAP) varies with age (1).

Clarification of the definition based on available evidence was an important initial objective of the 4th World Symposium on Pulmonary Hypertension, which took place in Dana Point, California, in early 2008. Members of the Working Group on Diagnosis and Assessment of PAH reviewed the literature and identified 47 studies describing 72 populations of healthy volunteers that were examined for PAH during rest and physical exercise (2–51). Normal resting mPAP was approximately 14 ± 3.3 mm Hg. The upper limit of normal (ULN) was approximately 20.6 mm Hg. During slight exercise (heart rate [HR] 100 to 110 beats/min), the ULN for mPAP was 32 (supine) and 30 mm Hg (upright). During submaximal exercise (HR 130 to 135 beats/min), the ULN was 31 (supine) and 35 mm Hg (upright), and during maximal exercise (HR 160 beats/min), it was 37 (supine) and 35 mm Hg (upright). If only studies were considered that strictly excluded exercise-induced hypertension, the data were not significantly different (1).

According to age group, there were only minor differences in PAP at rest; however, during slight and submaximal exercise, mPAP was significantly higher in older subjects (>50 years old). During slight exercise, the ULN was 29 and 30 mm Hg for people age <30 and 30 to 50 years,
respective,

...whereas for those age >50 years, the ULN was 45 mm Hg. During submaximal exercise, the ULN for subjects age <30, 30 to 50, and >50 years were 33, 36, and 47 mm Hg, respectively.

**Recommendations.** Based on this literature review, we recommend simplifying the definition of PH, as follows:

- The exercise and PVR criteria should be eliminated.
- A resting mPAP of 8 to 20 mm Hg should be considered normal, based on available evidence.
- The proposed new definition of PH is a resting mPAP ≥25 mm Hg.

Further studies are needed to better determine the natural history of patients with mPAP 21 to 24 mm Hg.

**Screening and Diagnosis**

**Noninvasive estimation of PAP—echocardiography.** With the introduction of Doppler echocardiography, approximate evaluation of PAP became feasible. Although this method is helpful in detecting or excluding significant PH, its intrinsic and operator-dependent limitations make early PH diagnosis and screening challenging (52,53). In the presence of a tricuspid insufficiency peak gradient (TIPG) ≥30 mm Hg, some investigators have used arbitrary criteria for noninvasive diagnosis of PH (54). During a meeting on PH held in Evian, France, in 1998, mild PH was arbitrarily defined as a tricuspid jet velocity (TJV) 2.8 to 3.4 m/s, which corresponds to TIPG 31 to 46 mm Hg and to PAP 36 to 51 mm Hg, if a fixed right atrial pressure (RAP) estimate of 5 mm Hg is used (55). It seems reasonable to consider TJV >2.8 m/s and TIPG ≥31 mm Hg at rest as elevated, except in elderly and/or very obese patients (56) (Table 1).

Two large French studies have attempted prospective verification of abnormal echocardiographic results, both taking into account the presence of dyspnea. The ItinerAIR study (57) enrolled 599 patients with scleroderma. In patients with TJV >3.0 or with dyspnea and TJV 2.5 to 3.0 m/s, right heart catheterization (RHC) was performed, regardless of symptoms. Among the 33 patients who met those criteria, 14 had mild to moderate PH on RHC at rest, and an additional 4 developed mPAP >30 mm Hg at exercise. These results were compatible with the 45% false-positive results seen with echo-Doppler screening. The second trial assessed the prevalence of PAH in patients with human immunodeficiency virus infection (58); 10% of patients presented with dyspnea, and 247 were included in the screening program and were eligible for echocardiography. Among 18 patients with TJV >2.5 m/s, only 5 were found to have PAH on RHC. The results should be interpreted with caution because the sensitivity of echocardiography for the detection of PH in this setting is not known.

Doppler echocardiography may be performed during exercise to estimate PAP. One report found an excellent correlation with catheter measurements of PAP performed simultaneously (r = 0.98) (59). Exercise echocardiography has been used to assess systolic PAP >40 mm Hg in several groups of patients (60), including those with chronic lung disease, heart transplantation (61), atrial septal defect (62), and susceptibility to high-altitude lung edema (63), as well as asymptomatic carriers of a PH gene mutation (60). In all groups, PAP significantly increased during exercise compared with control subjects (63). One group has arbitrarily defined systolic PAP <40 mm Hg, calculated after assuming fixed RAP of 5 mm Hg, as a normal hemodynamic response to exercise (60). A multicenter trial that used exercise echocardiography to assess genetic predisposition to PAH among family members of index patients with idiopathic pulmonary arterial hypertension (IPAH) found more pronounced rise of Doppler-derived systolic PAP in family members compared with healthy control subjects (64).

**New modalities in noninvasive screening.** Cardiac magnetic resonance (CMR) may offer more reliable data both at rest and during exercise or acute vasodilator testing, but so far it cannot be used as a screening test. Biomarkers, such as

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Reference Ranges for Normal Systolic Pressure Gradients Assessed With Doppler Between RV and RA (TIPG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>n</td>
</tr>
<tr>
<td>&lt;20</td>
<td>856</td>
</tr>
<tr>
<td>20–29</td>
<td>669</td>
</tr>
<tr>
<td>30–39</td>
<td>650</td>
</tr>
<tr>
<td>40–49</td>
<td>494</td>
</tr>
<tr>
<td>50–59</td>
<td>344</td>
</tr>
<tr>
<td>≥60</td>
<td>199</td>
</tr>
</tbody>
</table>

Data have been extracted from a table in McQuillan et al. (56) and used with permission. CI = confidence interval; RA = right atrium; RV = right ventricle; TIPG = tricuspid insufficiency peak gradient.
brain natriuretic peptide (BNP) and N-terminal pro-brain natriuretic peptide (NT-proBNP), may be useful in early detection of PAH. In an otherwise healthy, young, and mildly symptomatic person with border-line echocardiographic results, elevated BNP levels may justify follow-up.

**Prevalence and characteristics of PH in selected associated conditions.** The prevalence of PAH varies substantially depending on the type, etiology, and underlying condition. Table 2 is a summary of the best available data from a variety of sources (57,65–95).

**Genetic assessment and counseling.** Genetic testing is discussed in another section of this supplement (95). When testing and counseling are performed for genetic mutations, they should be done as part of a comprehensive program that includes discussion of the risks, benefits, and limitations of the test results. When used for genetic testing and counseling, molecular testing for the mutation should be performed only in a clinically approved and certified molecular genetics laboratory.

**Staging and Follow-Up Assessment**

**Prognostic parameters.** Although the tools used to diagnose and assess patients with PAH have improved, the number and complexity of therapeutic interventions have increased, making it even more challenging to accurately predict prognosis. The American College of Chest Physicians Evidence-Based Clinical Practice Guidelines on PAH, published in 2004, provide an excellent review of prognosis in PAH (96). Following is an update to that review.

**Survival in PAH. Historical perspective.** The natural history of IPAH was described in the National Institutes of Health (NIH) registry, which followed up 194 patients

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**Table 2 Prevalence and Characteristics of PH in Associated Conditions**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Prevalence</th>
</tr>
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<tbody>
<tr>
<td>PAH</td>
<td>15/million (65)</td>
</tr>
<tr>
<td>IPAH</td>
<td>5.9/million (65)</td>
</tr>
<tr>
<td>FPAH</td>
<td>Vanderbilt: 107 U.S. families (300 PAH patients among 2,300 individuals; includes 1 kindred with 19 women and 2 men) (James Loyd, MD, personal communication, February 2008); Columbia: 100 U.S. families (353 PAH patients among 3,400 individuals) (personal communication from Robyn J. Barst, MD on behalf of Jane Morse, MD [deceased], February 2008); Utah: 28 U.S. families (C. Gregory Elliott, MD, personal communication, February 2008) Possible overlap between registries</td>
</tr>
<tr>
<td>APAH-scleroderma</td>
<td>Prospective echocardiographic study of scleroderma and MCTD in 50 U.S. community practices: prevalence of PH (estimated RVSP ≥40 mm Hg) of combined previously screened and unscreened patients = 26.7% (66). 8 Canadian CTD centers: PH detected on echocardiography (RVSP &gt;30 or 35 mm Hg depending on center) in 21% of limited and 26% of diffuse scleroderma patients (67). Prospective study in French registry, 599 scleroderma patients without severe lung dysfunction: 29 had known PH, 33 more suspected by echocardiography (VTR &gt;3.0 m/s or &gt;2.5 m/s with unexplained dyspnea); 18 of 33 had PH (mPAP 30 ± 9 mm Hg) on RHC. Thus, 47 of 599 (8%) had confirmed PAH. Patients with known PH had mPAP 49 ± 17 (67). 18.3% (36 of 97) moderate–severe PH suspected in patients who had screening echocardiography (retrospective); confirmed by RHC in 89% (68); PH in 12 of 67 (17.9%) patients without ILD vs. 24 of 110 (21.8%) patients with ILD (69).</td>
</tr>
<tr>
<td>Portopulmonary hypertension</td>
<td>PAH in 1% to 6% of patients with portal hypertension (70–72). 8.2% (101 of 1,235) of candidates for OLT had echocardiographic RVSP &gt;50 mm Hg; of these 90 had RHC mPAP &gt;25 mm Hg; of these 66 had PAH (others had PAH explained by high cardiac output or PAWP), so prevalence of PAH in OLT candidates is 66 of 1,235 (5.3%) (73).</td>
</tr>
<tr>
<td>Sickle cell disease</td>
<td>32% of sickle cell patients have TRV ≥2.5 m/s and 9% ≥3.0 m/s (80,81,95). Further hemodynamic studies may be necessary to better characterize pulmonary hypertension in sickle cell disease.</td>
</tr>
<tr>
<td>HIV</td>
<td>0.5% estimate (74.75)</td>
</tr>
<tr>
<td>CTEPH</td>
<td>0.8% of patients after first pulmonary embolism (76). At 1 year after pulmonary embolism, 44% have evidence of RV dysfunction and PH: 5.1% (4 of 78) had confirmed CTEPH (77). In another study of 223 PE patients, 3.1% at 1 year and 3.8% at 2 years had CTEPH after PE (78).</td>
</tr>
<tr>
<td>Sickle cell disease</td>
<td>32% of sickle cell patients have TRV ≥2.5 m/s and 9% ≥3.0 m/s (80,81,95). Further hemodynamic studies may be necessary to better characterize pulmonary hypertension in sickle cell disease.</td>
</tr>
<tr>
<td>Thyroid diseases</td>
<td>PH can be detected in up to 41% of patients with thyroidism (82) and 49% of those with hypothyroidism (83), and usually responds to treatment of the thyroid disease (84,85).</td>
</tr>
<tr>
<td>Schistosomiasis</td>
<td>20.6% of 34 patients with S. mansoni portal hypertension had mPAP &gt;20 mm Hg (11.8% &gt;25 mm Hg) (86). In an endemic area of Brazil, those with PH by echocardiography had a higher prevalence of S. mansoni (80%) than those without PH (69%) (25% of the screened population of 246 people had PH by the echocardiographic criteria) (87). In 2 Brazilian PH centers, 30% of 123 cases were associated with schistosomiasis (50% IPAH and 10% CTD) (88).</td>
</tr>
<tr>
<td>Myeloproliferative disorders</td>
<td>Literature does not provide meaningful data (89,90).</td>
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<tr>
<td>Post-splenectomy</td>
<td>11.5% of IPAH patients have had splenectomy (91). 8.6% of CTEPH patients have had splenectomy (vs. 2.5% among IPAH patients in this study) (92). Splenectomy is associated with an odds ratio of 13 for development of CTEPH after pulmonary embolism (93).</td>
</tr>
</tbody>
</table>

In CTD patients who are newly screened with echocardiography, the degree of PAH, if present, detected at RHC is usually mild, and the future course is unknown. Based on estimated prevalence of scleroderma 210 to 276/million (68) and U.S. population of 302 million, the number of scleroderma cases is 250 × 302 = 75,500, so approximately 7,550 (10%) people would be expected to have PAH by right heart catheterization criteria. In African Americans, 0.1% have sickle cell disease (hemoglobin S) and 8% have sickle cell trait (hemoglobin SS, thalassemia), and the mortality of PH in sickle cell disease reflects the severity of chronic lung disease and the number of PE. In IPAH–scleroderma Prospective echocardiographic study of scleroderma and MCTD in 50 U.S. community practices: prevalence of PH (estimated RVSP ≥40 mm Hg) of combined previously screened and unscreened patients = 26.7% (66). 8 Canadian CTD centers: PH detected on echocardiography (RVSP >30 or 35 mm Hg depending on center) in 21% of limited and 26% of diffuse scleroderma patients (67). Prospective study in French registry, 599 scleroderma patients without severe lung dysfunction: 29 had known PH, 33 more suspected by echocardiography (VTR >3.0 m/s or >2.5 m/s with unexplained dyspnea); 18 of 33 had PH (mPAP 30 ± 9 mm Hg) on RHC. Thus, 47 of 599 (8%) had confirmed PAH. Patients with known PH had mPAP 49 ± 17 (67). 18.3% (36 of 97) moderate–severe PH suspected in patients who had screening echocardiography (retrospective); confirmed by RHC in 89% (68); PH in 12 of 67 (17.9%) patients without ILD vs. 24 of 110 (21.8%) patients with ILD (69).
enrolled at 32 centers from 1981 to 1985 (97). The estimated median survival was 2.8 years, with 1-, 3-, and 5-year survival rates of 68%, 48%, and 34%, respectively. Survival in associated PAH has also been evaluated. Stupi et al. (98) reported a 2-year survival of 40% among patients with scleroderma and isolated PAH. Several series have suggested that patients with scleroderma-associated PAH have a worse prognosis than those with IPAH, with hazard ratios for death ranging from 2.32 to 2.9 (99,100). In contrast, patients with PAH occurring in association with congenital heart disease have a better prognosis. Hopkins et al. (101) described an actuarial survival in patients with Eisenmenger syndrome who did not receive transplantation of 97%, 89%, and 77% at 1, 2, and 3 years, respectively, compared with 77%, 69%, and 35% at 1, 2, and 3 years for patients with IPAH. A national registry in France showed a 1-year survival rate of 88% in the incident cohort (65).

**THE IMPACT OF MEDICAL THERAPY.** An open-label, randomized controlled trial (RCT) involving 81 IPAH patients showed a survival advantage with epoprostenol therapy over a period of 12 weeks (102). Subsequent longer-term case series have compared survival on epoprostenol therapy with historical control subjects or with predictions based on an equation described in the NIH registry study discussed above (97). Shapiro et al. (103) reported 1-, 2-, and 3-year survival rates in IPAH patients treated with epoprostenol of 80%, 76%, and 49%, respectively. Sitbon et al. (104) followed up 178 patients and reported survival rates at 1, 2, 3, and 5 years of 85%, 70%, 63%, and 55%. McLaughlin et al. (105) reported on 162 patients with IPAH treated with epoprostenol. Observed survival rates at 1, 2, 3, 4, and 5 years were 88%, 76%, 63%, 56%, and 47%, respectively. A recent meta-analysis of RCTs in PAH, conducted by Galie et al. (106), suggested that active treatments were associated with a reduction in mortality of 43% (relative risk: 0.57; 95% confidence interval: 0.35 to 0.92; p = 0.023).

The effects of anticoagulant therapy on survival in patients with IPAH have been evaluated in 2 studies. Fuster et al. (107) reported an apparent survival benefit of warfarin in a retrospective, uncontrolled, single-center study of 120 patients. Rich et al. (108) described a better outcome among patients treated with warfarin who were not calcium-channel blocker responders. The 1-, 3-, and 5-year survival rates in patients treated with warfarin were 91%, 62%, and 47%, respectively, compared with 52%, 31%, and 31% in those not treated with warfarin.

McLaughlin et al. (109) reported that first-line therapy with bosentan, with the subsequent addition of or transition to other therapy as necessary, resulted in Kaplan-Meier survival estimates of 96% at 12 months and 89% at 24 months, compared with predicted survival rates from the NIH registry formula of 69% and 57%, respectively. Sitbon et al. (110) compared survival in patients with WHO (World Health Organization) functional class (FC) III IPAH treated with bosentan with historical data from similar patients treated with epoprostanol. Kaplan-Meier survival estimates at 1 and 2 years were 97% and 91%, respectively, in the bosentan cohort, and 91% and 84% in the epoprostanol cohort.

**Testing to predict prognosis in PAH.** Hemodynamics. The NIH registry (97) suggested that 3 hemodynamic variables were associated with increased risk of death: increased PAP, increased RAP, and decreased cardiac index. Sandooval et al. (111) reported on 61 IPAH patients and found that increased mean RAP, decreased cardiac index, and decreased mixed venous oxygen saturation were predictive of survival. Other studies evaluated prediction of survival in IPAH based on hemodynamics (112–115). With 1 exception (112), lower cardiac output (CO) or cardiac index seems to have been predictive. The prognostic value of baseline hemodynamics may be impacted by epoprostenol therapy. Sitbon et al. (104) found that lower mPAP and higher mean RAP had negative prognostic implications by univariate analysis, whereas only mean RAP was predictive by multivariate analysis. In 162 patients with IPAH treated with epoprostenol, McLaughlin et al. (105) found that only mean RAP was predictive of survival in a univariate analysis.

**Response to acute vasodilator therapy.** Sitbon et al. (116) reported the results of a retrospective analysis of 557 IPAH patients tested acutely with intravenous epoprostenol or inhaled nitric oxide. Using a definition of a ≥20% decrease in mean PAP and PVR, only 70 patients (12.6%) showed vasoreactivity. Long-term calcium channel blocker responders were defined as patients in New York Heart Association (NYHA) FC I or II with sustained hemodynamic improvement after at least 1 year without the addition of other PAH-specific therapy. Of the 70 patients showing acute vasoreactivity, only 38 (6.8% of the overall study group) had a favorable long-term response to calcium-channel blocker therapy.

**Echocardiography.** An early study of patients with IPAH in the late 1980s showed the severity of pericardial effusion to be predictive (117). Hinderliter et al. (118) analyzed the echocardiograms of 79 of 81 IPAH patients who participated in an RCT of epoprostenol (102) and found pericardial effusions in 43 patients. Larger effusions correlated with more severely impaired exercise performance and right atrial (RA) dilation. Effusion size was correlated with death or a composite of death or lung transplantation at 1 year. Longer follow-up showed that the presence of a pericardial effusion and RA area index were predictive of survival (119). A series of 53 IPAH patients showed the value of the right ventricular (RV) index (120). These studies suggest that pericardial effusion, indexed RA area, and RV index have prognostic value.

More recent studies have suggested that novel echocardiographic parameters might be of value. Forfia et al. (121) reported that tricuspid annular plane systolic excursion (TAPSE) predicted survival in PH. In a cohort of 63 patients with PH, a TAPSE <1.8 cm was associated with
greater RV systolic dysfunction. In patients with PAH (n = 47), survival estimates at 1 and 2 years were 94% and 88%, respectively, in subjects with a TAPSE ≥1.8 cm, and 60% and 50%, respectively, in subjects with a TAPSE <1.8 cm. Gurudevan et al. (122) reported that in 50 patients with suspected chronic thromboembolic PH, the systolic velocity of the tricuspid annulus had an inverse relationship with mPAP and PVR. Mahapatra et al. (123) found that a measure of pulmonary vascular capacitance, as determined by Doppler echocardiography, is a predictor of mortality in patients with IPAH and adds prognostic value to conventional risk markers.

**EXERCISE CAPACITY.** Measurements of exercise capacity in PAH have generally included 6-min walk distance (6MWD) and bicycle ergometry cardiopulmonary exercise testing (CPET). Miyamoto et al. (124) compared these measures in 27 patients with IPAH and found good correlation between maximum oxygen consumption and 6MWD. The 6MWD is simpler to perform and reproducible. Barst et al. (102) used 6MWD as the primary outcome measure in an RCT of chronic epoprostenol therapy in 81 patients with IPAH. The 6MWD was less in the nonsurvivors versus the survivors from both treatment groups, and it was found to be an independent predictor of survival. Miyamoto et al. (124) studied 43 patients with IPAH. Patients walking fewer than 332 m had a significantly lower survival rate than those walking farther than 332 m. In a study of long-term intravenous epoprostenol therapy in patients with IPAH, Sitbon et al. (104) reported that a 6MWD of ≤250 m was associated with a poor outcome. Humbert et al. (65) reported the results of a French registry on PH, showing that the 6MWD correlates with NYHA FC in all forms of PAH. A number of important studies in PAH have used the 6MWD as the primary outcome measure (102,125–132) or an important part of the primary end point (133). Wensel et al. (134) studied 86 patients with IPAH, 70 of whom were able to undergo CPET, and found that maximum oxygen consumption was an independent predictor of survival.

**FC.** In the NIH registry of patients with IPAH (before modern therapy), the risk of death was higher among patients in NYHA FC III or IV than among those in FC I or II (97). Median survival was nearly 6 years among those in NYHA FC I or II, 2.5 years among those in FC III, and 6 months among those in FC IV. A study of 91 patients with PAH, all treated with epoprostenol, showed that patients in WHO FC IV, compared with FC I, II, and III, had a significantly decreased survival (99). In a study of 178 patients with IPAH treated with epoprostenol, Sitbon et al. (104) reported that survival was lower for those starting therapy in NYHA FC IV compared with FC III, and that the persistence of FC III or IV after 3 months of therapy was associated with poor survival.

**BIOMARKERS—BNP.** Nagaya et al. (135) measured BNP in 60 patients with IPAH at diagnostic catheterization, together with atrial natriuretic peptide, norepinephrine, and epinephrine. According to multivariate analysis, baseline plasma BNP was an independent predictor of mortality. Patients with a supramedian baseline BNP (≥150 pg/ml) had a significantly lower survival rate than those with an inframedian level, according to Kaplan-Meier survival curves (p < 0.05). The BNP in survivors decreased significantly during the follow-up (217 ± 38 pg/ml to 149 ± 30 pg/ml, p < 0.05), whereas that in nonsurvivors increased (365 ± 77 pg/ml to 544 ± 68 pg/ml, p < 0.05).

Fijalkowska et al. (136) found that NT-proBNP levels correlated with 6MWD, cardiac index, PVR, and RAP, but not with PAP in 55 patients. The NT-proBNP levels were also related to the ratio of the diastolic area of the RV and left ventricle (LV) and to pericardial effusion during echocardiography.

Williams et al. (137) evaluated NT-proBNP, 6MWD, hemodynamics or tricuspid gradient, and survival in 109 patients with systemic sclerosis (SSc). Sixty-eight subjects had PAH, and 41 did not. In patients with normal PAP, 1-year survival was 100%, compared with 83.5% in those with SSc-PAH (p < 0.05). Patients without PAH had a mean NT-proBNP level of 139 pg/ml; those with SSc-PAH had a significantly higher mean NT-proBNP level of 1,474 pg/ml (p = 0.0002). Among patients with PAH, for every order of magnitude increase in NT-proBNP level, there was a 4-fold increased risk of death (p = 0.002 for baseline level and p = 0.006 for follow-up level). Baseline NT-proBNP levels correlated positively with mPAP and PVR and inversely with 6MWD.

Andressen et al. (138) assessed plasma NT-proBNP in 61 consecutive patients with pre-capillary PH. Compared with age-matched control subjects (n = 10), NT-proBNP was significantly greater in those with PAH (n = 16), chronic pre-capillary PH associated with other diseases (n = 26), and chronic thromboembolic disease (n = 19), and was correlated with hemodynamic variables and functional capacity. In 17 medically treated patients, a significant decrease in NT-proBNP levels correlated with improved hemodynamics. Baseline NT-proBNP was an independent predictor of mortality.

Park et al. (139) examined BNP levels in 20 PAH patients as a marker of response to epoprostenol therapy. A decrease in BNP level of ≥50% during the first 3 months on epoprostenol was strongly predictive of event-free survival (p = 0.003).

**Summary of prognostic parameters.** A variety of disease characteristics and diagnostic measurements have been found to be predictive of prognosis in patients with PAH, including etiology, therapeutic interventions, hemodynamics (cardiac index and RAP), echocardiographic findings (pericardial effusion, RV-Tei index, TAPSE), exercise capacity (6MWD, peak oxygen consumption), NYHA FC, and BNP levels.
Follow-up assessment. The follow-up of patients with PAH has never been standardized, and has not been included in current guidelines (140–142). Two different follow-up strategies can be identified: a clinical strategy and a goal-oriented strategy. It is not known which strategy is superior.

CLINICAL STRATEGY. This strategy is based principally on the symptoms and signs reported on clinical examination. If the FC is considered satisfactory (WHO FC I and II) and no signs of right heart failure are detected, no changes in therapy are proposed (143). Noninvasive examinations can also be included—for example, electrocardiography, chest radiography, echocardiography, and 6MWD—but no pre-specified goals are considered, and results are used for confirming clinical stability or deterioration. In this case, the intervals between patient evaluations are variable, ranging from 3 to ≥6 months.

GOAL-ORIENTED STRATEGY. Goal-oriented therapy has been proposed to optimize treatment (144–146). The objective is to correct parameters considered prognostically relevant and to escalate treatment until a threshold is reached. Goal-oriented therapy requires: 1) identification of the parameters to be measured; 2) establishment of levels to be achieved for each parameter; and 3) intervals of assessments. In an example described by Hoeper (144), patients were assessed by 6MWD or CPET; goals were 6MWD >380 m, peak oxygen consumption >10.4 ml/min/kg, and peak systolic blood pressure >120 mm Hg during exercise; intervals were 3 to 6 months. Treatments were escalated to triple combination therapy if required. This strategy led to better survival compared with historical control subjects. A similar strategy, but with different parameters and goals, has been used at the University of Bologna (146). Parameters considered relate to symptoms (World Health Organization FC), functional capacity (6MWD), and RV function as assessed by RHC. The goals are FC I or II, 6MWD ≥500 m in patients <50 years old and ≥380 m in older patients, with RAP ≤10 mm Hg, and cardiac index ≥2.5 l/min/m². Assessments are performed at baseline and after 3 to 4 months of first-line treatment. If the goals are reached, the patient is followed up at 3- to 4-month intervals with a noninvasive approach. In case of subsequent deterioration, hemodynamic confirmation is required. If the first-line treatment is not sufficient to reach the pre-specified goals, combination therapy is initiated and a subsequent evaluation is performed after 3 to 4 months. Noninvasive follow-up is carried out in cases of goal fulfillment. In cases with nonsatisfactory results, triple combination therapy is initiated and patients are considered for transplantation.

RV function in PAH. The usual cause of death in PAH is RV failure. Both diastolic (147,148) and systolic (148) dysfunction are likely contributors to RV failure. Diastolic RV dysfunction is thought to be related to RV hypertrophy and/or chronic pressure overload, with prolonged isovolumetric relaxation time (148,149) being a prominent feature. With systolic RV dysfunction, isovolumetric contraction time is prolonged and ejection time is shortened. An echocardiographic, Doppler-derived index of combined RV systolic and diastolic function correlates with symptoms (149) and seems to be a predictor of survival in patients with PAH (148). Evidence of advanced clinical disease in patients with PAH is apparent, with elevated jugular venous pressure, presence of a third heart sound (S3), and peripheral edema. Combined with increasing symptoms, decreased RV contractility, elevated RAP, and decreased CO, these findings suggest decompensated RV function.

It is difficult to assess the inotropic properties of prostanooids because of the strong vasodilative effects of these substances. Epoprostenol has a positive inotropic effect on the RV (150), but this may be caused by baroreflex activation. In isolated cardiomyocytes, treprostinil per se had no positive inotropic effects, but it significantly amplified the positive inotropic effects of catecholamines (151). This effect of prostanooids, along with inhibition of platelet aggregation and smooth muscle cell contraction and proliferation (152,153), may be in part responsible for its clinical benefit. A PAH treatment strategy based on measures that better reflect RV function may be superior to the traditional strategies outlined in consensus guidelines.

Future Directions

Treatment escalation. EXERCISE CAPACITY. Aerobic exercise capacity in PAH may be largely dependent on maximum CO and on the maximum flow output of the RV. Gas exchange function of the lungs in PAH is often normal or near normal. Rest- and exercise-induced hypoxemia are caused by a decreased mixed venous oxygenation caused by low CO (154) or right-to-left intracardiac shunting. The 6MWD has served well as a primary end point in most of the RCTs of new therapies in PAH (140) and is a potent predictor of functional state and survival (104,105,124). Two important questions remain concerning exercise capacity in PAH: 1) What is the minimal change in 6MWD effectively perceived by the patients as improvement or deterioration? (In patients with chronic obstructive pulmonary disease it was 54 m [155].) 2) What is the contribution of respiratory and skeletal muscle weakness as well as joint, bone, neurological, and psychological factors to aerobic exercise capacity in PAH?

ECHOCARDIOGRAPHY. Echocardiography can be important in the evaluation of RV function. The most relevant measures are RV and LV surface areas, the eccentricity index on parasternal short axis views, the Tei index (the ratio of the sum of isovolumic contraction and relaxation times divided by ejection time), TAPSE, inferior vena cava dimensions and inspiratory collapsibility, the presence and magnitude of a pericardial effusion, RV and LV diastolic function estimated on tissue Doppler imaging of tricuspid and mitral annuli, and systolic function on tissue Doppler
imaging measure of tricuspid annular systolic velocity S wave (117,119,121,148,156–159). All are, to a variable degree, sensitive to prognosis (117–119,121,148,157) and therapeutic interventions (156,160).

**INVASIVE HEMODYNAMICS.** Right heart catheterization with the measurement of PAP, CO, RAP, and pulmonary arterial wedge pressure remains the gold standard for the diagnosis of PAH (140,161). Possible areas for improvement include the measurement of PAP at several levels of flow to derive pulmonary vascular pressure–flow relationships that would improve the assessment of pulmonary vascular function and that might prove useful for diagnosis of latent PAH and for understanding the effects of therapy (162,163).

**Imaging: a developing magnetic resonance imaging era?** Magnetic resonance imaging is useful in the noninvasive assessment of the right heart in PH (164–167). It provides excellent spatial resolution, and virtually any plane of cross section can be obtained. Direct assessment of cardiac volumes, muscular mass, and function is possible. Precise flow measurements in the heart and great vessels can be made using velocity-encoded imaging. Interobserver variability is low, which makes magnetic resonance imaging a potential tool for follow-up assessment (168,169). The SERAPH (Sildenafil versus Endothelin Receptor Antagonist for Pulmonary Hypertension) study used CMR to compare the effect of bosentan and sildenafil on RV hypertrophy (170).

Three-dimensional flow mapping of the central pulmonary artery may allow assessment of mPAP (171).

The first attempt to assess the value of CMR for monitoring mortality risk in patients with PAH was made by Van Wolferen et al. (172). Assessed at baseline, RV stroke volume index >26 ml/m², RV end-diastolic volume <83 ml/m², and LV end-diastolic volume >41 ml/m² each indicated better survival. In a multivariate analysis, only 6MWD added independently to the prognostic message provided by these CMR variables. Importantly, progressive dilatation of the RV, as well as a decrease in LV diastolic volume and a further decrease in RV stroke volume at 1-year follow-up, were related to worse long-term outcome (172) (Fig. 1).

**RV and PA/RV interactions.** Chronic PH results from an increase in PVR, which is a simple measure of opposition to the mean component of flow. However, given the low resistance/high compliance nature of pulmonary circulation, the pulsatile component of hydraulic load is also critical. Several studies have documented the relationship between pulsatile pressure and flow (impedance) (173–176). These studies have shown that pulsatile load is increased in chronic PH. This abnormal pulsatile load may have detrimental effects on ventricular–vascular coupling, unfavorably loading the still-ejecting RV. Impedance is a measure of the opposition to the pulsatile components of flow. The role of pulmonary arterial input impedance has been under-
Cardiomyocyte hypertrophy has been considered an adaptive response to increased load, such as hypertension or pressure overload, because it normalizes the increase in wall stress induced by mechanical overload (179). Prolongation of this hypertrophic response leads to contractile dysfunction and heart failure. The distinctions between physiologic and pathologic hypertrophy are many (180).

There is emerging interest in determining the effect of therapy on RV function. Phosphodiesterase-5A expression is increased in the RVs of patients with PAH, and animal models suggest that inhibition of the enzyme results in inotropic activity (181). Magnetic resonance imaging-based studies have shown that acute sildenafil treatment promotes RV relaxation (147). Several other studies in animal models have shown improved RV systolic and diastolic function in response to acute and chronic treatment with prostacyclin analogs, phosphodiesterase-5A inhibitors, and endothelin-receptor antagonists (181,182). Further studies are needed to translate these observations into therapies for people with PH.

Summary

As new therapies have been developed for PAH, screening, prompt diagnosis, and accurate assessment of disease severity have become increasingly important. A clear definition of PH and the development of a rational approach to diagnostic assessment and follow-up using both conventional and new tools will be essential to deriving maximal benefit from our expanding therapeutic armamentarium.

Author Disclosures

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Interventional and Surgical Modalities of Treatment in Pulmonary Hypertension

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Most patients with chronic thromboembolic pulmonary hypertension are operable, and pulmonary endarterectomy is the treatment of choice. Pulmonary endarterectomy should not be delayed for medical therapy, and risk stratification helps to define patients likely to achieve the best outcome. Inoperable patients should be referred for trials of medical agents. Atrial septostomy is promising but underutilized, although better ways of ensuring an adequate, lasting septostomy still need to be determined. Indications for the procedure are unchanged, and it should be considered more frequently. Bilateral sequential lung or heart–lung transplantation is an important option for selected patients, and potential candidates who are class IV or III but not improving should be referred early to a transplantation center. Currently, there is a need for right ventricular assist devices with flow characteristics suited to the circulation of patients with pulmonary arterial hypertension. Right ventricular synchronization therapy has not yet been tested. Novel shunts (e.g., Potts anastomosis) also hold promise. All surgery for pulmonary hypertension should be performed in centers with experience in these techniques. (J Am Coll Cardiol 2009;54:S67–77) © 2009 by the American College of Cardiology Foundation

Chronic thromboembolic pulmonary hypertension (CTEPH) can be defined as pulmonary arterial hypertension (PAH) (mean pulmonary arterial pressure [mPAP] >25 mm Hg) with persistent pulmonary perfusion defects. CTEPH is an underdiagnosed cause of PAH and carries a poor prognosis if untreated (1–3). Acute or recurrent pulmonary emboli may be the initiating event. These are followed by intraluminal thrombus organization, fibrous obstruction of affected proximal arteries, and vascular remodeling in patent distal pulmonary arteries (4,5). CTEPH, therefore, is a disease with a mechanical component potentially amenable to surgery, and a variable degree of small vessel arteriopathy. Pulmonary endarterectomy (PEA) is a potential cure, and the treatment of choice for CTEPH (6,7); therefore, differentiation between CTEPH and PAH is paramount.

Diagnosis and Assessment of Operability

Since the 3rd World Symposium on Pulmonary Hypertension held in Venice in 2003 (8), the diagnostic algorithm for CTEPH has not significantly changed. The main diagnostic clue is the presence of 1 or more persistent perfusion defects on ventilation–perfusion scanning. Hemodynamic measurements aid in predicting prognosis and periotoperative risk assessment. Multiplanar pulmonary angiography is the gold standard for the confirmation of chronic thromboembolic disease and is recommended for the assessment of operability (6). Multislice computed tomographic scanning (Fig. 1) and magnetic resonance imaging have become valuable complementary investigations (3,9,10). Maximum information regarding pulmonary arterial morphology is required to assess surgical risk and long-term outcome; this information can best be obtained with pulmonary angiography. Patients should be referred for evaluation by a multidisciplinary team experienced in PEA.

There is inadequate evidence that PAH-specific medical therapy is an alternative to surgery (11), and the operation should not be delayed in favor of medical therapy. The best outcomes after surgery are associated with surgeon and center experience; concordance between pre-operative pulmonary vascular resistance (PVR) and anatomic disease; pre-operative PVR <1,000 to 1,200 dynes/s/cm⁻⁵; absence of select comor-
Abbreviations and Acronyms

AS = atrial septostomy  BLTx = bilateral lung transplantation  CI = cardiac index  CO = cardiac output  CTEPH = chronic thromboembolic pulmonary hypertension  DHCA = deep hypothermic circulatory arrest  ECLS = extracorporeal life support  ECMO = extracorporeal membrane oxygenation  HLTx = heart–lung transplantation  IPAH = idiopathic pulmonary arterial hypertension  IVC = inferior vena cava  LV = left ventricle/ventricular  mPAP = mean pulmonary arterial pressure  mRAP = mean right atrial pressure  NYHA = New York Heart Association  PAH = pulmonary arterial hypertension  PEA = pulmonary endarterectomy  PH = pulmonary hypertension  PVR = pulmonary vascular resistance  RAP = right atrial pressure  RV = right ventricular  6MWD = six-min walk distance  SOT = systemic arterial oxygen saturation

bidities, such as splenectomy or ventriculoatrial shunt; and significant post-operative decrease in PVR (7,12–14).

Surgery does not benefit all CTEPH patients (3). In high-risk patients with a likelihood of having significant distal small vessel disease pre-operatively and limited proximal angiographic pulmonary artery obstruction, a trial of PAH-specific medical therapy may be a reasonable option (3,11). However, the role of medical therapy in CTEPH remains to be further tested and defined.

Currently there is no pre-operative classification system that might allow for better risk stratification (15). In conjunction with PVR, exploratory variables such as pre-operative diffusion capacity, upstream resistance, select biomarkers, and precise assessment of right ventricular (RV) dysfunction may have a role in a future classification system (16–21).

The San Diego surgical classification (22) is based on operative findings of pulmonary artery obstruction, and therefore is not suitable for pre-operative risk stratification. Currently, the recommendation for surgery is based on a careful analysis by an experienced multidisciplinary team.

PEA

The PEA procedure with deep hypothermic circulatory arrest (DHCA) to ensure a bloodless field while providing cerebral protection has been described elsewhere (7,13). Optimal intraoperative visibility of pulmonary artery branches is the prerequisite for complete endarterectomy and maximal RV afterload reduction. The best operative outcome is dependent on a complete endarterectomy and significant early reduction of PVR to <500 dynes/s/cm\(^2\) (13). Different techniques for minimizing risk of circulatory arrest have been published (23,24); however, the mortality rates seem worse compared with larger series that included DHCA. Specialists at the University of California, San Diego, reported a 4.4% mortality and 0% neurological morbidity in 500 consecutive PEA operations using DHCA (13). At present, there is no compelling rationale for changing the PEA techniques used by major centers around the world.

Post-operative management. Right heart dysfunction caused by residual pulmonary hypertension (PH) and pulmonary vasoconstriction after extracorporeal circulation, and reperfusion edema in endarterectomized segments of the lung can pose significant challenges after PEA (7,25). However, optimal post-operative care has not been defined, and treatment protocols vary among expert centers.

In most patients, RV afterload reduction by removal of obstructive material from the pulmonary vasculature will result in an immediate and significant decrease of pulmonary artery pressures, increase in cardiac index (CI), improved gas exchange, and diuresis. Medical treatment includes cautious fluid administration, maintaining low right atrial pressure (RAP), and administration of vasoconstrictive drugs, if systemic hypotension caused by vasodilatation is present. Reduction of the CI to pre-operative levels may be necessary to minimize flooding of the lungs (reperfusion edema). Although the achievement of adequate gas exchange is a basic tenet of post-operative care after PEA, and pulmonary reperfusion response can be a serious problem, there is no consensus with respect to ventilatory protocols, even in specialized centers. It is uncertain whether mechanical ventilation with high tidal volumes and a limited degree of positive end-expiratory pressure, or nonaggressive pressure-controlled ventilation with high positive end-expiratory pressure, provides better results with regard to gas exchange and hemodynamic effects. Nevertheless, early
extubation 1 or 2 days post-surgery is possible with both protocols in a majority of patients (26).

In a small number of patients, post-operative right heart failure develops because of severe persistent PH caused by incomplete endarterectomy or small vessel disease combined with the effects of extracorporeal circulation, hypothermia, and ischemia (26). Attempts should be made to optimize right heart function with inotropic agents and reduce RV afterload. The role of treatment with specific pulmonary vasodilators in this challenging situation is unclear, although there is limited information that inhaled iloprost might be useful (11,27).

Early reoclusion prophylaxis using intravenous or subcutaneous heparin and subsequent lifelong anticoagulation is mandatory for all post-PEA patients. The routine pre-operative insertion of an inferior vena cava (IVC) filter to reduce the risk of peri-operative or recurrent pulmonary embolism remains a matter of debate. A randomized trial to compare optimal surveillance anticoagulation with or without IVC filter is warranted.

Outcome. The concept of PEA has been transferred from the University of California, San Diego, to an increasing number of centers internationally. A recent report from San Diego included a cohort of 1,100 patients with post-PEA mortality rates of 4.7% (28). With increasing experience, PEA centers should strive for post-operative mortality rates <10%. Since results depend on experience with the procedure, the number of centers per region may have to be limited.

Although the outcome of PEA in patients with CTEPH has not been evaluated in randomized controlled studies, long-term results with respect to survival, functional status, exercise capacity, quality of life, RV function, hemodynamics, and gas exchange are favorable for most patients (7,13,29–31). Maximum benefits of surgery may take 6 months or more. To exclude recurrent disease or residual symptomatic PH, patients should be systematically followed up with hemodynamic re-evaluation 6 to 12 months after surgery. In cases of residual or recurrent PH, specific pulmonary vasodilatory treatment might be beneficial (11,27,32), although further randomized controlled studies are needed.

Consensus

- CTEPH is defined as symptomatic PAH (mPAP >25 mm Hg) with persistent perfusion defects.
- CTEPH has a mechanical component judged amenable to surgery as well as variable small vessel disease.
- Pulmonary endarterectomy is the treatment of choice for CTEPH.
- Once CTEPH is diagnosed, patients should be referred for surgical evaluation by an experienced multidisciplinary team.
- In candidates found to be operable:
  - pulmonary endarterectomy is efficacious and carries a clear survival benefit;
  - there is inadequate evidence that medical therapy (PAH-specific therapy) is an alternative to surgery;
  - surgery should not be delayed in favor of medical therapy;
  - current best practice results in operative mortality rates of 4% to 7%.
- Pre-operative risk stratification requires better definition.
- The best outcomes at present are associated with:
  - surgeon and center experience;
  - concordance between PVR and anatomic disease;
  - pre-operative PVR <1,000 to 1,200 dynes/s/cm⁻⁵;
  - absence of select comorbidities (e.g., splenectomy, ventriculoatrial shunts);
  - post-operative PVR <500 dynes/cm/s⁻⁵.
- Benefits of surgery may not be immediate: full benefits may take ≥6 months.
- Patients should be systematically followed up with hemodynamic re-evaluation at 6 to 12 months after surgery because response may be partial or disease may recur.
- It is unknown whether the current PAH-specific medications are effective in post-operative persistent PH; studies are required.
- In CTEPH patients judged to be inoperable, a randomized, placebo-controlled, double-blind trial with bosentan showed modest improvement in hemodynamics but no change in 6-min walk distance at short follow-up (33).
- No consensus could be reached regarding the role of IVC filters in CTEPH candidates.

Recommendations

- Pre-operative risk stratification requires further development. Variables other than PVR (pre-operative diffusion capacity, pulmonary artery occlusion technique, emerging biomarkers, precise assessment of RV dysfunction) may have a role.
- The collaboration of major centers with experience in PEA is recommended to pool data prospectively for comprehensive analysis of risk factors and best-practice guidelines.
- A pre-operative classification system should be developed for future use.
- A randomized trial of optimal surveillance anticoagulation with or without an IVC filter is warranted.
- The role of medical therapy in patients deemed to be inoperable needs to be further tested and defined.

Atrial Septostomy (AS) in Severe PAH

Rationale. In advanced idiopathic pulmonary arterial hypertension (IPAH), either normal RV function or compensated hypertrophy is critical for survival (34). Patients with Eisenmenger syndrome have better survival rates than patients with IPAH (35,36) and the concept that AS successfully decompresses the failing RV and left ventricle (LV) is well accepted. Atrial septostomy represents a strat-
nergy for the treatment of RV failure where medical therapy is failing and there is limited access to lung donors. It allows right-to-left shunting, as permitted by the presence of tricuspid regurgitation, and increased systemic output, which allows increased systemic oxygen transport, in spite of a decrease in systemic arterial oxygen saturation (SOT) (37,38).

**Global experience.** Based on the worldwide literature, we presented an updated analysis at the Dana Point meeting comprising 223 cases, including children, with a mean age of 28 ± 17 years. Seventy percent of patients were female, 82% had IPAH, and mean New York Heart Association (NYHA) functional class was 3.6 ± 0.4 (J. Sandoval, unpublished written communication, February 2008). Other etiologies were PAH associated with surgically corrected congenital heart disease (8%), collagen vascular disease (5%), distal CTEPH not amenable to surgery (3%), and miscellaneous (3%). Congestive heart failure (43%), syncope (38%), or both (19%) were the principal indications for the procedure, with bridge to transplantation in 14% of cases. Of patients who underwent AS, 96 were nonresponsive to maximal medical treatment, including intravenous prostacyclin infusion (n = 57), bosentan (n = 18), sildenafil (n = 8), beraprost (n = 6), subcutaneous treprostinil (n = 4), inhaled iloprost (n = 3), or combination therapy (n = 10). The simultaneous use of pharmacologic therapy and AS in these reports, as well as the evidence for the safe administration of intravenous epoprostenol, subcutaneous treprostinil, or bosentan in the setting of PAH associated with Eisenmenger syndrome (39–41), support the safety of a combination of medical and surgical treatment.

**Procedures.** Two techniques have been used. Stepwise balloon dilatation is the procedure of choice. In stepwise balloon-dilation AS, the interatrial orifice is created by puncture with a Brockenbrough needle, then dilated using progressively larger balloon catheters. A 10% decrease in arterial oxygen saturation (SaO₂%) and an increase in LV end-diastolic pressure to 18 mm Hg preclude further dilatation (42). No prospective hemodynamic evaluation has been performed. There are no guidelines for the optimal size of the defect. Anecdotally, a defect size of 8.5 ± 2 mm is said to increase cardiac output (CO) by 20% to 25%. The defect may close and require a repeat procedure.

The choice between balloon-dilation AS or blade balloon AS depends on center expertise and should include both interventional cardiology and PH expertise. Alternative techniques with a custom-made fenestrated Amplatzer (AGA Medical, Golden Valley, Minnesota) device or a butterfly stent at the end of the procedure to keep the AS patent have met with modest success (43,44).

**Immediate outcome after AS.** In most reports, AS was performed in severe PAH with RV failure with an overall procedure-related mortality of 16% (45). Recommendations to minimize risk have been established (Table 1). In our analysis of 223 cases, mortality was 7.1% at 24 h and 14.8% at 1 month. Factors significantly associated with procedure-related mortality at 1 month are shown in Table 2. The most common cause of death within 24 h was refractory hypoxemia. Less common were progressive right heart failure, procedural complications, multiple organ failure, hemoptysis, and withdrawal of dialysis. From a total of 186 reported surviving patients with early follow-up, syncope and right heart failure improved in 88%, and 12% were unimproved. In all, 16.6% were transplanted. The 6-min walk distance (6MWD) improved 30% to 100% (46–48).

**Immediate hemodynamic response.** In our updated analysis of 223 cases, hemodynamic results before and after AS were reported in 117 patients. There was a significant decrease in mRAP (14.6 ± 8 mm Hg to 11.6 ± 6.3 mm Hg; p < 0.0001), SaO₂% (93.3 ± 4.1% to 83 ± 8.5%), and NYHA functional class (3.49 ± 0.6 to 2.1 ± 0.7), accom-

### Table 1

<table>
<thead>
<tr>
<th>Recommendations for Minimizing Procedure-Related Mortality of Atrial Septostomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Only perform in a center experienced in pulmonary hypertension.</td>
</tr>
<tr>
<td>2. Contraindications to AS (unchanged from 2003) are severe right ventricular failure on cardiopulmonary support, mRAP &gt; 20 mm Hg, PVR &gt; 55 U/m², resting D₂O saturation &lt; 90% on room air, and LVEDP &gt; 18 mm Hg.</td>
</tr>
<tr>
<td>3. Pre-procedure, optimize cardiac function with adequate right heart filling pressure and additional inotropic support if needed.</td>
</tr>
<tr>
<td>4. During procedure:</td>
</tr>
<tr>
<td>a. Supplemental oxygen</td>
</tr>
<tr>
<td>b. Appropriate sedation to prevent anxiety</td>
</tr>
<tr>
<td>c. Monitoring variables (LAP, SaO₂%, and mRAP)</td>
</tr>
<tr>
<td>d. Tailor the defect to ~10% decrease in SaO₂ saturation.</td>
</tr>
<tr>
<td>5. Post-procedure, optimize oxygen delivery with transfusion of packed red blood cells or darbepoetin before and after.</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard Ratio (95% Confidence Interval)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAP &gt; 20 mm Hg</td>
<td>30.5 (3.8–244)</td>
<td>0.001*</td>
</tr>
<tr>
<td>NYHA functional class</td>
<td>8.53 (0.89–61.2)</td>
<td>0.062*</td>
</tr>
<tr>
<td>RHF</td>
<td>5.97 (0.75–47.2)</td>
<td>0.089</td>
</tr>
<tr>
<td>Mean RAP, mm Hg</td>
<td>1.19 (1.1–1.29)</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Septostomy type, blade</td>
<td>1.19 (0.30–4.6)</td>
<td>0.800</td>
</tr>
<tr>
<td>Age &gt; 18 yrs</td>
<td>1.12 (0.29–4.34)</td>
<td>0.865</td>
</tr>
<tr>
<td>Mean LAP, mm Hg</td>
<td>1.11 (0.86–1.43)</td>
<td>0.420</td>
</tr>
<tr>
<td>Mean PAP, mm Hg</td>
<td>1.01 (0.98–1.05)</td>
<td>0.321</td>
</tr>
<tr>
<td>Age, yrs</td>
<td>0.99 (0.96–1.03)</td>
<td>0.966</td>
</tr>
<tr>
<td>Baseline SaO₂%</td>
<td>0.97 (0.83–1.14)</td>
<td>0.773</td>
</tr>
<tr>
<td>Mean SAP, mm Hg</td>
<td>0.96 (0.92–1.01)</td>
<td>0.148</td>
</tr>
<tr>
<td>SaO₂% after procedure</td>
<td>0.90 (0.84–0.96)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Gender, female</td>
<td>0.73 (0.18–2.8)</td>
<td>0.635</td>
</tr>
<tr>
<td>Baseline CI, l/min/m²</td>
<td>0.38 (0.09–1.6)</td>
<td>0.189</td>
</tr>
<tr>
<td>Syncope</td>
<td>0.14 (0.03–0.66)</td>
<td>0.013*</td>
</tr>
</tbody>
</table>

*p values are statistically significant.

CI = cardiac index; NYHA = New York Heart Association; PAP = pulmonary arterial pressure; SAP = systemic arterial pressure; RHF = right heart failure; RPF = right portal pressure; RV = right ventricle; RAP = right atrial pressure; PVRI = pulmonary vascular resistance index; SaO₂% = arterial oxygen saturation.
Panoni by an increase in mean left atrial pressure (5.7 ± 3.3 mm Hg to 8.1 ± 4.0 mm Hg; p < 0.0001) and CI (2.04 ± 0.69 l/min/m² to 2.62 ± 0.84 l/min/m²).

Hemodynamic improvement is contingent on baseline mRAP (42,45,49) (Table 3). In patients with an mRAP <10 mm Hg, the decrease in mRAP was not significant (−10.6% from an already-low baseline reading), yet there was a 22.5% increase in CI. In patients with an mRAP >20 mm Hg (in whom procedural mortality is highest), mRAP and SaO₂% decreased 25% and 15%, respectively, and CI increased 38% from baseline. Patients with a baseline mRAP between 11 and 20 mm Hg had an intermediate response but a better risk/benefit ratio (47). These measurements represent the resting state and are likely to be different with exercise, explaining the increase in 6MWD (46–48). Post-AS hemodynamics during exercise have not been established.

An increase in PVR, low mixed venous PO₂, and refractory hypoxemia after AS have been successfully managed with inhaled iloprost (50). The increase in CO with no change in PAP or PVR suggests that the mechanism may be RV decompression and improved LV filling. Clinical improvement occurs despite resting desaturation and further desaturation with exercise.

Mechanisms for hemodynamic and clinical benefit include decompression of the RV at rest, prevention of further RV dilation and dysfunction during exercise, and an increase in CO and SOT, both at rest and during exercise (via right-to-left shunt). The increase in SOT and delivery also produces beneficial effects on peripheral oxygen utilization and decreased muscle sympathetic nerve activity (42,51–53).

### Table 3 Hemodynamic Effects of Atrial Septostomy Related to Baseline Resting Mean Right Atrial Pressure

<table>
<thead>
<tr>
<th>Variable</th>
<th>mRAP &lt;10 mm Hg (n = 42)</th>
<th>mRAP 11–20 mm Hg (n = 49)</th>
<th>mRAP &gt;20 mm Hg (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mRAP, mm Hg</td>
<td>Before: 6.6 ± 2.4, p 0.214</td>
<td>After: 14.8 ± 2.8, p 0.0001</td>
<td>Before: 26.6 ± 4.4, p 0.0001</td>
</tr>
<tr>
<td>mPAP, mm Hg</td>
<td>Before: 62 ± 16, p 0.329</td>
<td>After: 66.4 ± 17, p 1.000</td>
<td>Before: 6.4 ± 20, p 0.102</td>
</tr>
<tr>
<td>mLAP, mm Hg</td>
<td>Before: 5.0 ± 2.7, p 0.005</td>
<td>After: 5.3 ± 3.6, p 0.0001</td>
<td>Before: 7.9 ± 3.0, p 0.029</td>
</tr>
<tr>
<td>SaO₂%</td>
<td>Before: 93.8 ± 4, p 0.0001</td>
<td>After: 93.0 ± 4, p 0.0001</td>
<td>Before: 93.1 ± 4, p 0.0001</td>
</tr>
<tr>
<td>CI, l/min/m²</td>
<td>Before: 2.36 ± 0.6, p 0.0001</td>
<td>After: 2.04 ± 0.7, p 0.0001</td>
<td>Before: 1.55 ± 0.5, p 0.0001</td>
</tr>
<tr>
<td>mSAP, mm Hg</td>
<td>Before: 83 ± 15, p 0.931</td>
<td>After: 84.5 ± 14, p 0.005</td>
<td>Before: 78 ± 20, p 0.254</td>
</tr>
<tr>
<td>NYHA functional class</td>
<td>Before: 3.25 ± 0.6, p 0.0001</td>
<td>After: 3.63 ± 0.5, p 0.0001</td>
<td>Before: 3.71 ± 0.5, p 0.0001</td>
</tr>
</tbody>
</table>

mLAP = mean left atrial pressure; mPAP = mean pulmonary arterial pressure; mSAP = mean systemic arterial pressure; other abbreviations as in Tables 1 and 2.

Long-term hemodynamics and survival. Evaluation of long-term hemodynamics showed a higher CI and lower RAP in patients at repeat catheterization after a mean of 2 years post-septostomy (36). Echocardiography at 5.5 months after AS showed a significant decrease in right atrial and RV systolic and diastolic areas (54).

Of the 223 cases presented at Dana Point, follow-up information was available for 128. For these 128 patients, median survival was 60 months and mean survival time was 52.3 months. The mean survival after AS (excluding procedural deaths) was 63.1 months. Mortality after septostomy (excluding procedure-related mortality) was dictated by older age (hazard ratio [HR]: 1.04), scleroderma (HR: 8.32), NYHA functional class (HR: 4.71), NYHA class III and IV (HR: 6.24), CI (HR: 0.179), left atrial pressure (HR: 0.737), and SOT (HR: 0.99). The impact of baseline mRAP on survival, which is relevant before septostomy, disappears once the procedure has been performed. A diagnosis of scleroderma negatively impacts survival after AS.

Post-septostomy survival of patients with severe PH and right heart failure seems at least comparable to that achievable with current pharmacologic agents. However, given that a significant proportion of patients were receiving medications before and after the procedure, it is difficult to separate the relative benefits.

**Summary.** Atrial septostomy stands as an additional promising strategy in the treatment of severe PAH. It can be performed successfully in selected patients with advanced pulmonary vascular disease. In patients with PAH who have undergone successful AS, the procedure has resulted in a significant clinical improvement, beneficial and long-lasting hemodynamic effects at rest, and a trend toward improved survival. Procedure-related mortality is still high but seems to be decreasing since recommendations to minimize risk were implemented. However, because the disease process in PAH is unaffected, AS is considered a palliative procedure.

Indications for AS include: 1) failure of maximal medical therapy, persisting RV failure, and/or recurrent syncope; 2) as a bridge to transplantation; and 3) when no other therapeutic options exist (8,55).

**Consensus**

- The concept that AS decompresses the RV is accepted.
- Uptake has been limited. Impediments may be lack of a training pathway or the terminology “palliative procedure.”
- Patients known to benefit from AS have IPAH with syncope or persistent RV failure or have received failed medical therapy.
- Atrial septostomy has a role in health care systems without drug access.
- Atrial septostomy has been used to bridge to lung transplantation and might prolong survival for patients on a waiting list.
- Stepwise balloon dilatation is the procedure of choice.
• Data are sparse with Amplatzer devices, blade septostomy, butterfly stents, and cutting-edge balloons.
• Selection guidelines are unchanged from 2003: do not undertake AS if baseline O2 saturation is <90% on room air or LV end diastolic diameter is >18 mm Hg.
• Procedural deaths relate to inadvertent overly large defect or a decrease in O2 saturation >10%. Deaths are more common if RAP is >20 mm Hg.
• Procedural mortality is approximately 5%.
• The defect created should be tailored to the end O2 saturation.
• Use in children could be increased.
• Spontaneous closure of the defect may require a repeat procedure.
• Survival after AS is superior to survival predicted by the National Institutes of Health.
• The benefit on survival differs from that of single-drug therapy in that it is immediately apparent.
• It is unknown how early in the course of PAH AS may be useful.

Recommendations

• The lack of data on exercise and long-term hemodynamics after AS needs to be addressed.
• The role of AS as a bridge to transplantation should be delineated because it may delay transplantation.
• A combination approach of early AS with drug therapy seems attractive in class IV patients.
• A trial of monotherapy patients (stable or deteriorating class III to IV) randomized to AS or no procedure is recommended.

Lung Transplantation

Heart–lungen transplantation (HLTx) or bilateral lung transplantation (BLTx) is the final option for selected patients in whom medical therapy fails (56,57). The most common indication is IPAH; less common indications are scleroderma, histiocytosis, and sarcoidosis. Use of BLTx and HLTx for PAH has decreased worldwide as the result of PAH-specific medical treatments (58). Nevertheless, patients in class IV or who remain in class III despite combination therapy should be referred for transplantation assessment.

Patient selection. Published consensus guidelines are used by major transplantation centers, yet many patients with IPAH are still referred at a late stage, when medical therapy has failed and multi-organ failure is present. Because the lack of optimal donor organs is a global problem, death rates on the waiting list are high for patients with end-stage PAH. Post-operative mortality is significantly higher if transplantation is performed in the setting of right heart, renal, and hepatic failure, and marginal organs may also be used.

Potentially eligible class IV patients with IPAH should be referred immediately for transplantation assessment. The etiology of PAH, the functional and hemodynamic status, and the course of the disease in the particular patient must be considered to allow optimal timing of the transplantation listing. Patients with improvements after combination medical therapy over the first 3 months and, in particular, who move to class II, can be withdrawn from the waiting list and closely followed up. In patients remaining in class III on combination therapy, transplantation assessment and listing should not be delayed.

Patients with pulmonary veno-occlusive disease or pulmonary capillary hemangiomatosis have the worst prognosis because medical treatment is ineffective (56); these patients must be referred for transplantation assessment at the time of diagnosis. Despite medical treatment, patients with scleroderma-associated PAH have a worse prognosis than do patients with IPAH (59), and the transplantation option should be discussed earlier. In contrast, patients with congenital right-to-left shunts and Eisenmenger syndrome have higher survival rates (37,38). Criteria for transplantation listing for these patients are difficult to define. However, the decision for transplantation for these patients should not be postponed until renal and hepatic failure occur, when patients can become unacceptable candidates.

The 6MWD and peak myocardial oxygen consumption predict survival. Listing algorithms for transplantation in PAH should incorporate hemodynamics because RAP >15 mm Hg and a CI <2.0 l/min/m² are primary determinants of poor survival (56).

Type of transplantation. In patients with IPAH, BLTx or HLTx are performed in most centers around the world. Single-lung transplantation for IPAH has been abandoned because of high rates of pulmonary edema and poor outcomes.

In patients with congenital cardiac abnormalities and Eisenmenger syndrome (particularly atrial septal defect), isolated lung transplantation combined with repair of cardiac defects is possible. HLTx provides survival advantages in this group of PAH patients, in whom it should be considered the procedure of choice (60). A HLTx in PAH is technically easier and preserves airway blood supply. The median sternotomy preserves respiratory mechanics, and the post-operative course is simpler.

In other etiologies of PAH, the choice between BLTx and HLTx remains open, and factors such as organ donation shortage, local allocation protocols, and center experience and preference play a role. Patients with PAH requiring 2 or 3 donor organs may be disadvantaged in many systems. Because a definite survival benefit for HLTx in IPAH has not been shown, it may not be appropriate to use the donor organs for 1 recipient rather than 2 if there is no major cardiac disease other than RV dysfunction. There are several advantages and disadvantages of BLTx or HLTx, but none has proven to be a determinant of survival. Post-operative RV dysfunction or high blood flow to the
new lungs with an increased LV pre-load can contribute to primary graft failure (60). These conditions can be stabilized by careful post-operative management, and RV function has been shown to recover following afterload reduction by transplantation in PAH. Airway ischemia is more common after isolated lung transplantation compared with HLTx, but it was not a significant problem in a recent series of BLTx (57). In HLTx, a shorter ischemic time is mandatory; this may reduce the possible donor pool. Primary cardiac graft failure and accelerated long-term coronary artery disease are drawbacks of HLTx.

**Outcome.** In selected patients with end-stage PAH, the quality of life, exercise capacity, and long-term survival are profoundly improved by lung transplantation. Survival rates at 3 months after BLTx or combined HLTx for PAH are, however, the lowest among all lung transplant recipients (58). Heart–lung recipients with Eisenmenger syndrome and IPAH had significantly better overall survival than patients with other congenital abnormalities. Poor pre-operative patient status, including multiorgan failure, the complexity of the operation with routine use of extracorporeal circulation, and post-operative hemodynamic instability caused by RV or LV dysfunction after BLTx may result in inferior early survival. However, 5- and 10-year survival rates after BLTx for IPAH are similar to those seen with transplantation for other etiologies (58). Lung transplant recipients with IPAH who survived to 1 year had a significantly better survival at 10 years than transplant recipients with chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis.

Retrospective studies from individual centers showed superior early and long-term results after BLTx or combined HLTx for PAH (57,61). In a retrospective study from Pittsburgh (30 recipients who underwent transplantation between 1994 and 2006), actuarial survival was 86% at 1 year, 75% at 5 years, and 66% at 10 years (57). Early referral to a specialized multidisciplinary lung transplantation center familiar with the unique problems of PAH patients and the complex transplantation procedure is key.

**Consensus**

- Lung transplantation is the final effective treatment for selected patients with IPAH.
- The procedure of choice is BLTx or HLTx. Single-lung procedures have been abandoned. Living lobar transplants put donors at risk.
- In choosing BLTx versus HLTx, organ donation rates, local allocation protocols, and unit preference play a role.
- Patients with PAH require 2 or 3 organs and may be disadvantaged in some allocation systems.

**Recommendations**

- Although drug therapy may delay transplantation, a class IV patient who fulfills criteria should be referred for transplantation assessment.
- Patients with veno-occlusive disease must be referred for transplantation assessment at diagnosis.
- Listing algorithms for transplantation in PAH must incorporate hemodynamics because hemodynamics are the primary determinant of survival, in addition to functional class, exercise capacity, and failure to respond to other therapies (Guidelines of the International Society for Heart and Lung Transplantation define RAP >15 mm Hg, CO <2.0 l/min) (58).

**Extracorporeal Support**

Extracorporeal life support (ECLS) has been successfully used for cardiorespiratory support in neonates and children (62). Common indications for ECLS in adults are respiratory failure and acute respiratory distress syndrome (63). Possible indications for ECLS in PAH are acute RV failure and hypoxemia caused by massive pulmonary embolism (63), bridge to lung transplantation, support after lung transplantation (64–66), and treatment of severe reperfusion edema after PEA for CTEPH (67).

Extracorporeal life support is considered for patients with PH and potentially reversible right heart failure in whom conventional support is failing, including optimized ventilation and fluid management, prone positioning, inhaled nitric oxide, prostanoid agents, and pharmacologic therapy for right heart function (62).

The modes of ECLS are veno-venous or venoarterial systems using the internal jugular or common femoral veins and/or common femoral, common carotid, or right axillary arteries for cannulation (62,68). Venovenous ECLS is useful for carbon dioxide removal, oxygenation, and RV afterload reduction. Venoarterial ECLS is preferred for RV decompression and after lung transplantation (although having high mortality rates) because it provides more effective oxygenation.

Bleeding, neurological, infectious, and thromboembolic complications limit widespread use of ECLS (62,67). Controlled randomized studies are not available and are unlikely to be performed. However, ECLS can be a lifesaving option in critically ill PAH patients and should be at hand in specialized PH centers.

Extracorporeal membrane oxygenation (ECMO) is a valuable tool in lung transplantation, providing the potential to bridge patients to transplantation, to replace cardiopulmonary bypass with at least equal results, and to overcome severe post-operative complications. Favorable survival rates can be achieved despite the fact that ECMO is used in the more complex patient population undergoing lung transplantation (69).

**Conclusions**

- When conventional support for the RV is ineffective, ECLS may take several forms. Established modalities are:
Right ventricular assist devices with pulsatile flow cause
venoarterial: preferred for RV decompression and support and after lung transplantation (although high mortality if needed), providing more effective oxygenation.

Consensus

- Extracorporeal life support can be lifesaving in critically ill patients with RV failure, but no data are available from randomized controlled trials.
- Many questions remain regarding indications, timing, and cannulation choice.

Ventricular Assist Devices

Mechanical circulatory support for the RV has been used for patients refractory to medical therapy. The usual goal of mechanical circulatory support involving the RV is to bridge the patients to lung transplantation.

Patients with end-stage right heart failure secondary to IPAH have fared poorly with pulsatile assist systems in anecdotal reports. The PAP typically increases markedly with pulsatile mechanical support because of the high energy imparted to blood by a pulsatile mechanical device, even with pumps that are pneumatically driven and set to deliver the lowest possible dP/dT. The high dP/dT of pulsatile ventricular assist devices results in damage to the pulmonary microcirculation, with increased PVR and PAP often resulting in intraparenchymal pulmonary hemorrhage, hemoptysis, and death. The challenge is to increase flow through a circulatory bed with inherently high resistance and high impedance attributable to derangements of smooth muscle proliferation and vascular collagen accumulation. Such patients may be best served by a support device that incorporates a gas exchange function in addition to a circulatory function, taking blood from the venous circulation and delivering it directly to the left atrium or arterial circulation, thereby excluding the diseased RV and lungs from the patient’s circulatory system. Unfortunately, ECMO provides only short-term support in adults, and an inflammatory response associated with the large prosthetic surfaces of an oxygenator has limited its success.

Nonpulsatile flow axial or centrifugal flow devices—for example, roller pumps inserted percutaneously—may have promise, with the potential for vascular remodeling, but they are largely untested.

Consensus

- There is little literature on RV assist devices.
- Right ventricular assist devices are effective in RV failure secondary to LV failure.
- Right ventricular assist devices with pulsatile flow cause high distension pressure, and flow characteristics are less adjustable than with nonpulsatile axial or centrifugal flow devices.
- In addition to short-term survival benefit, long-term support may permit vascular remodeling.
- There is a strong rationale for the development of such devices.

Other Interventional Modalities

Novalung®. The Novalung® interventional Lung-Assist (iLA) membrane ventilator device (Novalung, GmbH, Germany) is a pumpless extracorporeal lung-assist device used in acute lung failure. It is driven by the patient’s CO and does not require extracorporeal pump assistance. A membrane gas exchange system with optimized blood flow is integrated in an arteriovenous bypass established by vascular cannulation. Novalung® has been applied in 4,000 patients for artificial lung assistance with easy use and low cost (70).

Stem cell transplantation. Circulating endothelial progenitor cells instigate angiogenesis in PAH and home to the location of endothelial damage, providing endothelial repair. Autologous endothelial progenitor cell transplantation may be explored in PAH in the future.

RV synchronization. In PAH, significant interventricular dyssynchrony occurs because of a right bundle branch block with prolonged RV systolic contraction time, compared with the LV (71). This is probably caused by a decrease of electrical conductivity over the RV caused by high pre-stretch of the RV myocardial fibers and the large force these fibers must generate to shorten. Dyssynchrony is known to impede LV diastolic filling (72).

In animal studies, opening the pericardium facilitates LV filling, increases LV end-diastolic volume and output, and reduces septal bowing. Because a relationship exists between pericardial pressure and RAP, and RAP is increased in patients with severe PAH, diastolic interaction may theoretically be improved.

Consensus

- Right ventricular resynchronization therapy has potential and is likely to be safe enough for a pilot study in humans with IPAH.

Novel shunts for PAH: Potts anastomosis or “de novo ductus arteriosus,” a hypothetical exercise. The rationale for creating new right-to-left shunts is that PAH patients with Eisenmenger physiology or patent foramen ovale survive longer than IPAH patients. Atrial septostomy is accepted as a mechanism for unloading the RV and reducing RV area, syncope, ascites, and edema. This occurs despite an 85% resting desaturation and a further 68% desaturation with exercise. A potential alternate decompression technique is to create a Potts anastomosis (side-to-side anastomosis from left pulmonary artery to descending aorta) (73).
Seven children with varying etiology (IPAH or corrected transposition) and suprasystemic PA pressure failing drug therapy underwent Potts anastomosis (73). There was 1 technical death. Follow-up at 26 ± 22 months in the remainder showed a tripling of 6MWD, improved functional class, freedom from syncope, upper limb \( \text{SaO}_2 \) 97%, lower limb 80%, and pulmonary artery and aortic pressures equalized (74,75). In theory, this technique might be applicable in adults with IPAH, although some operative risk is likely.

Purely hypothetical is the idea of creating a de novo ductus arteriosus with conduit, which might perhaps be valved to prevent aortic backpressure surges into the pulmonary artery. Possible materials might be derived from LV assist device circuitry or arteriovenous fistulae formation for dialysis, radial artery graft (used in coronary artery bypass surgery), valved saphenous vein grafts, or new polymers.

**Consensus.** There is no consensus, given the paucity of data. The idea, however, is quite an exciting one, particularly if it can be applied in adults in whom other therapies have failed.

**Author Disclosures**

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Key Words: surgical modalities • treatment in PAH • interventional modalities.
Updated Evidence-Based Treatment Algorithm in Pulmonary Arterial Hypertension

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Uncontrolled and controlled clinical trials with different compounds and procedures are reviewed to define the risk-benefit profiles for therapeutic options in pulmonary arterial hypertension (PAH). A grading system for the level of evidence of treatments based on the controlled clinical trials performed with each compound is used to propose an evidence-based treatment algorithm. The algorithm includes drugs approved by regulatory agencies for the treatment of PAH and/or drugs available for other indications. The different treatments have been evaluated mainly in idiopathic PAH, heritable PAH, and in PAH associated with the scleroderma spectrum of diseases or with anorexigen use. Extrapolation of these recommendations to other PAH subgroups should be done with caution. Oral anticoagulation is proposed for most patients; diuretic treatment and supplemental oxygen are indicated in cases of fluid retention and hypoxemia, respectively. High doses of calcium-channel blockers are indicated only in the minority of patients who respond to acute vasoreactivity testing. Nonresponders to acute vasoreactivity testing or responders who remain in World Health Organization (WHO) functional class III, should be considered candidates for treatment with either an oral phosphodiesterase-5 inhibitor or an oral endothelin-receptor antagonist. Continuous intravenous administration of epoprostenol remains the treatment of choice in WHO functional class IV patients. Combination therapy is recommended for patients treated with PAH monotherapy who remain in WHO functional class III. Atrial septostomy and lung transplantation are indicated for refractory patients or where medical treatment is unavailable. (J Am Coll Cardiol 2009;54:S78–84) © 2009 by the American College of Cardiology Foundation

In 1891, Ernst von Romberg, a German physician, described an autopsy subject as having “pulmonary vascular sclerosis”; however, it is only since 1995 with the introduction of intravenous epoprostenol that disease-specific targeted medical therapies for pulmonary arterial hypertension (PAH) have become available. Furthermore, significant advances in the treatment of PAH have occurred during the past 15 years. Currently 9 medical therapies have either received regulatory approval or are under regulatory review. These agents target the prostacyclin pathway, the nitric oxide pathway, and the endothelin pathway. Combination trials have demonstrated additive or synergistic benefit by targeting 2 or all 3 of these pathways.

Until the 1980s, attempts to reduce pulmonary arterial pressure were performed with nonselective (pulmonary and systemic) vasodilators. Favorable and sustained results were convincingly shown only with the use of high doses of calcium-channel blockers (CCBs) and only in the minority of patients who responded to acute vasoreactivity testing (1–6). In addition, oral anticoagulant treatment was considered effective on the basis of retrospective or uncontrolled studies (1,7–9). In the 1990s, treatment with continuous IV administration of epoprostenol was shown in 3 nonblinded randomized clinical trials (RCTs) to improve symptoms, exercise capacity, and hemodynamic status in PAH and to improve survival in idiopathic pulmonary arterial hypertension (IPAH)/heritable pulmonary arterial hypertension (HPAH) (10–12). During that period, favorable results of several uncontrolled series of PAH patients who underwent atrial septostomy or lung transplantation were also reported (13–16).

Twenty RCTs with 9 new compounds as monotherapy have been completed in PAH patients (10–12,17–31). In addition, 6 RCTs testing combinations of agents (e.g., endothelin-
receptor antagonists [ERAs] and phosphodiesterase [PDE]-5 inhibitors, or prostanoid and ERA or PDE-5 inhibitors) have been completed (32–37). Approximately 5,000 patients have participated in these studies aimed at developing effective treatments for PAH.

The conclusions derived from clinical trials over the past 15 years have provided us with an evidence-based treatment strategy. The purpose of the present report is to review the RCTs performed in PAH and to propose an evidence-based updated treatment algorithm that incorporates currently available therapies. This algorithm can be used worldwide, subject to the availability of specific drug therapies.

Uncontrolled Clinical Studies in PAH

Anticoagulants. The evidence for favorable effects of oral anticoagulant treatment in patients with IPAH, HPAH, or PAH associated with anorexigen is based on retrospective analyses from 7 studies, of which 5 were positive and 2 were negative (1,7–9). The survival of anticoagulated patients selected on the basis of clinical judgment was improved, as compared with a concurrent population that was not treated with oral anticoagulants. Three-year survival improved from 21% to 49% in the series reported by Fuster et al. (7); and the 3- and 5-year survival rates increased from 31% to 47% and from 31% to 62%, respectively, in the series reported by Rich et al. (1). These studies were not randomized, and one can argue that the lower survival of the control groups could be related to comorbidity that precluded the use of anticoagulation in the untreated patients. In addition, only IPAH, HPAH, and anorexigen-related PAH patients were included in the studies. In recent RCTs, approximately 70% of patients were treated with oral anticoagulants (10–12,17–37). Interestingly, the highest prevalence of oral anticoagulant treatment was seen in the trials involving mainly IPAH and HPAH patients in World Health Organization (WHO) functional class III and IV, whereas the lowest prevalence was observed in a trial of patients with scleroderma. It should be emphasized that there is no evidence of any difference in the efficacy of oral anticoagulant therapy on the basis of functional class severity.

Diuretics, digoxin, and oxygen. The symptomatic and clinical benefits of diuretic treatment in right heart failure preclude the need for controlled trials to demonstrate efficacy in PAH. In recent RCTs with new treatments, approximately 50% to 70% of patients were treated with diuretics (38,39). The lack of trials with specific classes of diuretics in PAH and individual variability in responses leave the choice of the type and dose of drug to be used in individual cases to the experience of the physician.

Short-term intravenous (IV) administration of digoxin in IPAH produces a modest increase in cardiac output and a significant reduction in circulating norepinephrine (40); no data are available on the effects of long-term treatment. Accordingly, the use of digitalis in PAH patients is based primarily on the judgment of the physician rather than on scientific evidence of efficacy. Digoxin was administered to approximately 25% to 50% of patients in recent RCTs in PAH (38).

No consistent data are currently available on the effects of long-term oxygen treatment in PAH. Although improvement in pulmonary hypertension (PH) with low-flow supplemental oxygen has been reported in some PAH patients (41), this has not been confirmed in controlled trials. In a controlled study in patients with Eisenmenger syndrome, nocturnal oxygen therapy had no effect on hemodynamic variables, quality of life, or survival (42); in contrast, a previous study suggested increased survival (43).

CCBs. Favorable clinical and prognostic effects of high doses of oral CCB drugs in acutely vasoreactive patients with IPAH have been shown in single-center, non-randomized, uncontrolled studies (1–6). In these studies, the control group consisted of nonresponders, who might have a poorer prognosis, as compared with acutely vasoreactive individuals (3). Furthermore, the demonstration of a consistent reduction of pulmonary artery pressure by acute pharmacologic testing in vasoreactive patients raises ethical questions concerning the appropriateness of performing placebo-controlled clinical trials in these patients.

A definition of “a positive acute vasoreactive response” to predict long-term response with high-dose oral CCBs was proposed at the 3rd World Symposium on Pulmonary Hypertension in 2003 (5). With this definition—reduction of mean pulmonary arterial pressure ≥10 mm Hg to reach a mean pulmonary arterial pressure ≤40 mm Hg with a normalized or increased cardiac output with acute pulmonary vasodilator challenge with either inhaled nitric oxide or intravenous epoprostenol—<10% of IPAH patients have a positive acute vasoactive response.

Favorable results of long-term administration of high doses of oral CCBs have also been shown in children with IPAH (4,6). In contrast, the effects of high-dose CCBs on associated forms of PAH have not yet been clearly demonstrated (41). Acute vasodilator testing is recommended for all PAH patients, even though patients with IPAH and anorexigen-induced PAH are more likely to respond. Furthermore, although functional class IV patients are less likely to respond than functional class II and III patients, some functional class IV patients might respond favorably to acute vasodilator testing and might benefit from CCBs; however, it is recommended that these patients be evaluated in a specialized PH center. Empirical treatment with CCBs without a positive response
with acute vasodilator testing using either inhaled nitric oxide or IV epoprostenol is contraindicated (41).

**Surgical and interventional procedures.** Lung transplantation or atrial septostomy might be indicated in select patients who progress despite optimal medical therapy or for whom medical therapy is not available. Lung transplantation and atrial septostomy are discussed in detail in another article in this supplement (44).

### Controlled Clinical Trials in PAH

**Synthetic prostacyclin and prostacyclin analogues.** The efficacy of continuous IV administration of epoprostenol (synthetic prostacyclin) has been evaluated in 3 unblinded, controlled clinical trials: 2 in IPAH/HPAH (10,11), and 1 in PAH associated with the scleroderma spectrum of diseases (12). Although IV epoprostenol improves symptoms, exercise capacity, and hemodynamic status in both clinical conditions, survival was increased only in IPAH and HPAH.

Five RCTs with 3 prostacyclin analogues as monotherapy have been performed in PAH patients (19,45). The effects of continuous subcutaneous administration of treprostinil were assessed in a pilot RCT in which the improvement in exercise capacity was not statistically significant (45). In the 2 pivotal RCTs, improvements were reported in symptoms, exercise capacity, and hemodynamic status (19). Continuous IV administration of treprostinil seems to be safe and effective on the basis of 2 small, open-label, uncontrolled studies in patients with PAH (46,47).

The orally active prostacyclin analogue beraprost was evaluated in PAH patients in 2 RCTs, 1 in Europe (20) and 1 in the U.S. (23). In the first study, an increase in exercise capacity was seen after 3 months. In the second, which lasted 12 months, improvement in exercise capacity was observed at 3 and 6 months but not thereafter (23). No hemodynamic improvements were observed in the 12-month study, and clinical events were reduced only at the 6-month evaluation.

Inhaled iloprost as monotherapy was evaluated in 1 RCT that enrolled patients with both PAH and chronic thromboembolic PH (21). Overall, this study showed an increase in exercise capacity and improvement in symptoms, pulmonary vascular resistance, and clinical events in PH patients. Continuous IV administration of iloprost was shown to be effective in a small, open-label, uncontrolled series of patients with PAH and chronic thromboembolic PH (48).

**Endothelin receptor antagonists.** Nine RCTs using 1 of 3 ERAs as monotherapy have been performed in PAH patients. The orally active endothelin receptors A and B (ET\(_A\)/ET\(_B\)) ERA bosentan was evaluated in 4 RCTs in PAH patients (17,22,27,30,49), including 1 RCT performed in a cohort of patients with the Eisenmenger syndrome (27) and 1 RCT performed in a cohort of patients with only mildly symptomatic PAH (30). Overall, bosentan improved exercise capacity, functional class, hemodynamic status, echocardiographic and Doppler variables, and time to clinical worsening (17,22,27,31,49). Small increases in the dose of warfarin might be required to maintain therapeutic international normalized ratio (INR) when bosentan is coadministered with warfarin.

Sitaxsentan, an orally active ET\(_A\) selective ERA, has been assessed in PAH patients in 2 RCTs, both of which demonstrated improvement in exercise capacity (assessed by the 6-min walk test) and hemodynamic status (25,28,50). In 1 of the 2 studies (25), the primary end point (peak oxygen consumption as assessed by cardiopulmonary exercise testing) was not statistically significant. Coadministration of sitaxsentan and warfarin requires the reduction of the warfarin dose up to 80% to maintain a therapeutic INR, due to a drug–drug interaction.

Ambrisentan, an orally active ET\(_A\) selective ERA, has been evaluated in 3 RCTs (29,51,52). Results showed improvements in exercise capacity and clinical events that seem similar to the results observed with the other 2 ERAs.

On the basis of the results of RCTs using ERAs, the incidence of elevated hepatic transaminases ≥3 times the upper limit of normal seems to be approximately 10% with bosentan, approximately 4% with sitaxsentan, and approximately 2% with ambrisentan. The patient populations in the various RCTs differed, and these numbers should be considered only as approximations and may not be comparable.

**PDE-5 inhibitors.** Two RCTs with 2 different PDE-5 inhibitors have been performed in PAH patients (26,31). Used as monotherapy, both sildenafil and tadalafil improved exercise capacity and hemodynamic status in approximately 50% of enrolled patients; tadalafil also improved clinical events (31).

The optimal agent for PAH monotherapy remains unclear.

**Combination therapy.** More recently, combination treatment has been evaluated to address the multiple pathobiologic mechanisms present in PAH. The combination of oral bosentan and IV epoprostenol was investigated in 1 small study, with inconclusive results (32). Five additional RCTs have evaluated combination therapy in PAH. The addition of inhaled iloprost to background oral bosentan demonstrated improved hemodynamic status and clinical events in 1 RCT (35); however, these results were not confirmed in an open trial (34). In another study, the addition of oral sildenafil to background IV epoprostenol demonstrated improved exercise capacity, hemodynamic status, and clinical events; furthermore, in post hoc analysis, the addition of oral sildenafil to background IV epoprostenol increased survival versus IV epoprostenol alone (37). In the pivotal tadalafil RCT, approximately 50% of the patients had oral tadalafil added to background oral bosentan; in that study overall, tadalafil improved exercise capacity, hemodynamic status, and clinical events (31). Inhaled treprostinil has also been studied as add-on therapy to either background bosentan or background sildenafil; in both combinations, the addition of inhaled treprostinil improved exercise capacity (36). These studies support the efficacy of combination treatment in patients who remain symptomatic on monotherapy. The optimal combination on the basis of overall risk–benefit considerations remains unknown.

Although there seems to be an interaction between sildenafil and bosentan (increased bosentan and decreased sildenafil
levels) (53), the clinical relevance of this is unclear. Similarly, although the interaction between tadalafil and bosentan is less than that between sildenafil and bosentan (i.e., tadalafil exposure decreased with minimal changes in bosentan exposure) (54), the clinical relevance is also unknown. Tadalafil has also been evaluated in the presence of ambrisentan, with no clinically relevant pharmacokinetic interactions reported (55). There is no clinically relevant pharmacokinetic interaction between ambrisentan and sildenafil, with no dose adjustment of ambrisentan or sildenafil recommended compared with administration of either drug alone. There is a minimal interaction reported between sitaxsentan and sildenafil, with no changes in sitaxsentan plasma concentrations in the presence of sildenafil and only modest increases in sildenafil plasma concentrations (57). Overall, no dose adjustments have been recommended for patients treated with 1 of the aforementioned ERAs in combination with either sildenafil or tadalafil.

**Early intervention.** For functional class II or III patients, the role of early aggressive intervention (i.e., IV epoprostenol as first-line treatment), either as monotherapy or in conjunction with a PDE-5 inhibitor and/or an ERA, remains unknown. Although the first RCTs in PAH focused primarily on functional class III and IV patients, results from a more recent RCT evaluating the efficacy of bosentan in only mildly symptomatic PAH patients support early intervention (30). In addition, prespecified subgroup analyses of the sildenafil, tadalafil, and ambrisentan RCTs did not show any significant differences in the therapeutic efficacy of these drugs between patients in WHO functional classes II and III (30). The apparent lack of “catch-up” in placebo-treated patients supports early intervention in PAH (41). Future studies seem warranted.

**General comments on controlled clinical trials.** Although these studies have similar designs, treatment duration, and end points, analyses of baseline WHO functional class and etiology profiles show substantial differences. Accordingly, comparisons might be misleading. Improvement of exercise capacity as assessed by the 6-min walk test has been observed in all of these studies, albeit to different degrees. In evaluating the clinical relevance of exercise capacity improvements, additional elements, such as baseline functional class, effects on combined clinical events (e.g., hospital stays, mortality, rescue therapies),

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**Figure 1 PAH Evidence-Based Treatment Algorithm**

Drugs within the same grade of evidence are listed in alphabetical order and not order of preference. Not all agents listed are approved or available for use in all countries. Strengths of recommendations are defined in Table 1. *To maintain oxygen at 92%. +Investigational, under regulatory review. APAP = associated pulmonary arterial hypertension; ERA = endothelin receptor antagonist; HAPAH = heritable pulmonary arterial hypertension; IPAH = idiopathic pulmonary arterial hypertension; IV = intravenous; PAH = pulmonary arterial hypertension; PDE-5 = phosphodiesterase type 5; SC = subcutaneous; WHO = World Health Organization.
and hemodynamic effects, should be considered. As mentioned previously, a survival benefit has been demonstrated in only 1 controlled, third-party–blinded study of IV epoprostenol in patients with severe IPAH/HPAH (11). Because, on the basis of these results, IV epoprostenol is considered rescue therapy, subsequent RCTs assessing mortality as an end point could not ethically be performed. Furthermore, severely ill subjects requiring IV epoprostenol treatment were excluded in recent RCTs, resulting in a low mortality in these study populations. A recent meta-analysis performed on all RCTs in PAH patients published through October 2008 reports a 43% decrease in mortality and a 61% reduction in hospital stays in patients treated with targeted therapies versus patients randomized to placebo (39). These results, achieved after an average treatment period of 14.3 weeks, support the efficacy of the currently approved PAH treatments.

### Evidence-Based Treatment Algorithm

A treatment algorithm based on a consensus of the PH community evaluating the clinical trials presented in this review is presented in Figure 1. The recommendations in this guideline are based on a grading system in which the strength of the recommendation results from the interaction of 2 components: the quality of the evidence, and the net benefit of the therapy (Tables 1 and 2). Because treatments have been evaluated primarily in IPAH, HPAH, and PAH associated with scleroderma or anorexigen use, extrapolation of these recommendations to other PAH subgroups should be done with caution.

### Conclusions

The suggested initial approach after the diagnosis of PAH is to treat patients with oral anticoagulant drugs if no contraindication exists, diuretics in cases of fluid retention, and supplemental oxygen in cases of hypoxemia, even though RCTs with these compounds are lacking. Patients should be referred without delay to centers experienced in acute vasoreactivity testing and the treatment of pulmonary vascular diseases. Acute vasoreactivity testing should be performed in all patients with PAH, although patients with IPAH, HPAH, and PAH associated with anorexigen use are the most likely to exhibit a positive response. Vasoreactive patients, as defined in the preceding text, should be treated with optimally tolerated doses of CCBs; maintenance of response, defined as WHO functional class I or II with near-normal hemodynamic status, should be confirmed by repeat right heart catheterization and clinical assessment after 3 to 6 months of treatment. Nonresponders to acute vasoreactivity testing or responders who remain in WHO functional class III should be considered candidates for treatment with either a PDE-5 inhibitor or an ERA. Among prostanooids, treprostinil is administered subcutaneously, intravenously, or by inhalation; iloprost can be given intravenously or by inhalation; beraprost is administered orally, and epoprostenol is administered intravenously.

The choice of drug is dependent on a variety of factors, including the approval status, route of administration, side-effect profile, patient preference, and the physician’s experience and clinical judgment. Continuous IV epoprostenol remains first-line therapy for PAH patients in WHO functional class IV, because of its demonstrated survival benefit in IPAH/

### Table 1

#### Quality of Evidence, Net Benefit, and Strength of Recommendation

<table>
<thead>
<tr>
<th>Variables</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Quality of the evidence</strong></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>Evidence is based on good randomized controlled trials or meta-analyses.</td>
</tr>
<tr>
<td>Fair</td>
<td>Evidence is based on other controlled trials or randomized controlled trials with minor flaws.</td>
</tr>
<tr>
<td>Low</td>
<td>Evidence is based on nonrandomized, case-control, or other observational studies.</td>
</tr>
<tr>
<td>Expert opinion</td>
<td>Evidence is based on the consensus of the carefully selected panel of experts in the topic field.</td>
</tr>
<tr>
<td></td>
<td>There are no studies that meet the criteria for inclusion in the published reports review.</td>
</tr>
<tr>
<td><strong>Net benefit</strong></td>
<td></td>
</tr>
<tr>
<td>Substantial</td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td></td>
</tr>
<tr>
<td>Small/weak</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Conflicting</td>
<td></td>
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<tr>
<td>Negative</td>
<td></td>
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<tr>
<td><strong>Strength of recommendation</strong></td>
<td></td>
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<tr>
<td>A</td>
<td>Strong recommendation</td>
</tr>
<tr>
<td>B</td>
<td>Moderate recommendation</td>
</tr>
<tr>
<td>C</td>
<td>Weak recommendation</td>
</tr>
<tr>
<td>D</td>
<td>Negative recommendation</td>
</tr>
<tr>
<td>I</td>
<td>No recommendation possible (inconclusive)</td>
</tr>
<tr>
<td>E/A</td>
<td>Strong recommendation on the basis of expert opinion only</td>
</tr>
<tr>
<td>E/B</td>
<td>Moderate recommendation on the basis of expert opinion only</td>
</tr>
<tr>
<td>E/C</td>
<td>Weak recommendation on the basis of expert opinion only</td>
</tr>
<tr>
<td>E/D</td>
<td>Negative recommendation on the basis of expert opinion only</td>
</tr>
</tbody>
</table>

### Table 2

#### Relationship of Strength of the Recommendations Scale to Quality of Evidence and Net Benefits

<table>
<thead>
<tr>
<th>Quality of Evidence</th>
<th>Substantial</th>
<th>Intermediate</th>
<th>Small/Weak</th>
<th>None</th>
<th>Conflicting</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>A</td>
<td>A</td>
<td>B</td>
<td>D</td>
<td>I</td>
<td>D</td>
</tr>
<tr>
<td>Fair</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>I</td>
<td>D</td>
</tr>
<tr>
<td>Low</td>
<td>B</td>
<td>C</td>
<td>C</td>
<td>I</td>
<td>I</td>
<td>D</td>
</tr>
<tr>
<td>Expert opinion</td>
<td>E/A</td>
<td>E/B</td>
<td>E/C</td>
<td>I</td>
<td>I</td>
<td>E/D</td>
</tr>
</tbody>
</table>

See Table 1 for definition of designations.
HPAH, with extrapolation to associated PAH patients in WHO functional class IV. Combination therapy should be considered for patients who fail to show improvement or who deteriorate with monotherapy. The goal in treating PAH patients is to improve WHO functional class III and IV patients to functional class I or II and to improve all functional class II patients to functional class I or at least to maintain functional class II in patients presenting in that functional class. Finally, both atrial septostomy and lung transplantation are indicated in carefully selected patients for refractory PAH or in cases where medical treatments are unavailable. These procedures should be performed only in experienced centers.

Major therapeutic advances for PAH patients have been achieved in the last decade; however, none of the currently approved therapies represents a cure for this progressive disease. The search for such treatments continues, with promising new concepts arising from a better understanding of the pathobiology of pulmonary vascular diseases. Patients and physicians should be encouraged to foster such research by participating in RCTs conducted at specialized PH centers.

**Author Disclosures**

Dr. Barst has received honoraria for serving as a consultant, advisory board member, and/or speaker from Actelion, Eli Lilly, GlaxoSmithKline, Gilead, Novartis, and Pfizer. Dr. Gibbs has received honoraria for advisory boards and/or lecturing from Actelion, Bayer Schering, GlaxoSmithKline, Pfizer, and United Therapeutics, and has been an investigator in trials sponsored by BioMarin and Lung Rx. Dr. Ghofrani has received honoraria and research funds from Actelion, Bayer Schering, Encysive, ErgoNex Pharma, GlaxoSmithKline, Novartis, and Pfizer. Dr. Hooper has received pharmaceutical grants from Actelion, Bayer Schering, and Encysive; travel accommodations and speaker’s honoraria from Actelion, Encysive, GlaxoSmithKline, Lung Rx, Pfizer, and Schering; and has served as a consultant to Actelion, Bayer Schering, Encysive, GlaxoSmithKline, and Lung Rx. Dr. McLaughlin has received honoraria and/or consulting fees from Actelion, Gilead, MondoBIOTECH, and United Therapeutics. The University of Michigan has received research grants from Actelion, Pfizer, and United Therapeutics. Dr. Rubin has received research grants from Actelion, Gilead, the National Heart, Lung and Blood Institute, Pfizer, and United Therapeutics; and has served as a consultant and on advisory boards for Actelion, Gilead, and United Therapeutics. Dr. Galiè has served on advisory boards for Actelion, Eli Lilly, Encysive, Gilead (Myogen), GlaxoSmithKline, mondoBIOTECH, Pfizer, and Schering; and has received lecture fees from Actelion and Schering. His Institute has received grant support from Actelion, Eli Lilly, Encysive, Gilead (Myogen), MondoBIOTECH, Pfizer, Schering, and United Therapeutics.

**References**


Key Words: algorithm - evidence-based treatment - pulmonary arterial hypertension.
Diagnosis, Assessment, and Treatment of Non-Pulmonary Arterial Hypertension Pulmonary Hypertension

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Hannover, Germany; Barcelona, Spain; La Jolla, California; Baltimore, Maryland; Vienna and Graz, Austria; Bologna, Italy; Ann Arbor, Michigan; Brussels, Belgium; Cambridge, United Kingdom; Rochester, Minnesota; Nashville, Tennessee; São Paulo, Brazil; and Warsaw, Poland

The 4th World Symposium on Pulmonary Hypertension was the first international meeting to focus not only on pulmonary arterial hypertension (PAH) but also on the so-called non-PAH forms of pulmonary hypertension (PH). The term “non-PAH PH” summarizes those forms of PH that are found in groups 2 to 5 of the current classification of PAH, that is, those forms associated with left heart disease, chronic lung disease, recurrent venous thromboembolism, and other diseases. Many of these forms of PH are much more common than PAH, but all of them have been less well studied, especially in terms of medical therapy. The working group on non-PAH PH focused mainly on 4 conditions: chronic obstructive lung disease, interstitial lung disease, chronic thromboembolic PH, and left heart disease. The medical literature regarding the role of PH in these diseases was reviewed, and recommendations regarding diagnosis and treatment of PH in these conditions are provided. Given the lack of robust clinical trials addressing PH in any of these conditions, it is important to conduct further studies to establish the role of medical therapy in non-PAH PH. (J Am Coll Cardiol 2009;54:S85–96) © 2009 by the American College of Cardiology Foundation

Previous international meetings on pulmonary hypertension (PH) have focused predominantly on pulmonary arterial hypertension (PAH), a form of PH that is usually severe but overall quite rare. The 4th World Symposium was the first to assign a working group to address in detail the so-called non-PAH forms of PH, that is, those forms of PH that are encountered in patients with chronic obstructive pulmonary disease (COPD), interstitial lung disease (ILD), left heart disease (LHD), venous thromboembolism, and other conditions. It is a paradox in the field of PH that one of the less common forms, that is, PAH, has been extensively studied, whereas fewer data are available on other types of PH, many of which are far more common. At the same time, drugs with proven efficacy in PAH (1–3) are being increasingly used in other forms of PH, despite the virtual absence of clinical trials supporting this approach.

Pulmonary hypertension in chronic lung disease subsumes COPD, ILD, and other diffuse parenchymal lung diseases such as sarcoidosis, connective tissue disease, or pulmonary Langerhans cell histiocytosis. Space limitation prevents a discussion of the rarer diseases, such as sarcoidosis and pulmonary Langerhans cell histiocytosis, which are often associated with clinically relevant PH (4).

Epidemiology, Features, and Importance of PH in COPD

The prevalence of PH in COPD depends on the population under study, the definitions applied, and the tools used to evaluate patients (5). Most hemodynamic studies have been
performed in patients with advanced disease. Three recent studies have provided data in large series of patients, the majority of them in the Global initiative for chronic Obstructive Lung Disease (GOLD) IV stage. In 1 study among 120 patients with severe emphysema (mean forced expiratory volume in 1 s \( [FEV_1] \), 27% predicted) undergoing evaluation for lung volume reduction surgery (6), the incidence of PH, defined as a mean pulmonary artery pressure (mPAP) \( >20 \) mm Hg, was 91%, although in the majority of patients (86%), it was in the mild-to-moderate range (mPAP \( 20 \) to 35 mm Hg). Only 5% of the patients showed an mPAP \( >35 \) mm Hg. The correlation between mPAP and lung function was weak (\( FEV_1, r^2 = 0.11 \) and \( PaO_2, r^2 = 0.03 \)). The mPAP was more closely related to pulmonary capillary wedge pressure (PCWP) (\( r^2 = 0.32 \)), which was mildly elevated in the majority of patients, suggesting the presence of diastolic left ventricular (LV) dysfunction in advanced COPD. However, gas trapping with elevated intrathoracic pressures may be an alternative explanation for the increased PCWP.

In a retrospective analysis of pulmonary hemodynamic studies in 998 COPD patients (7), 27 patients had severe PH, defined as an mPAP \( \geq 40 \) mm Hg. Of the 27, 16 had alternative explanations for PH. Among the remaining 11 (1.1% of the whole group), COPD was the only identifiable cause of PH. This subset of patients had only moderate airway obstruction (FEV\(_1\) 50% predicted), but at the same time, they showed severe hypoxemia, hyperventilation, and a very low diffusion capacity of the lung for carbon monoxide (DLCO). The survival time of these patients was much shorter than in the other patients. These findings indicate that there is a subset of COPD patients with “out of proportion” PH sharing some clinical features with idiopathic PAH.

In a study of 215 patients with severe COPD (FEV\(_1\) 24% predicted) who were candidates for lung volume resection surgery or lung transplantation, PH, defined as an mPAP \( >25 \) mm Hg, was present in 50%, although it was mostly mild (mPAP \( 26 \) to 35 mm Hg) (8). In 9.8% of these patients, PH was considered moderate (mPAP \( 36 \) to 45 mm Hg), and in 3.7%, severe (mPAP \( >45 \) mm Hg). Cluster analysis identified a subset of patients with moderate impairment of airway function, high PAP, and severe arterial hypoxemia, further supporting the concept of the existence of a subgroup of COPD patients with moderate airflow obstruction and “out of proportion” PH.

The overall burden of PH in patients with COPD is substantial. If 1% of patients with advanced COPD have severe PH with mPAP \( >40 \) mm Hg (7), extrapolation from the U.S. or French prevalence figures on COPD suggests a prevalence of severe PH in COPD patients of 3 to 17 per million, similar to the prevalence of PAH (9). However, with an mPAP cutoff of 35 mm Hg rather than 40 mm Hg, the prevalence of out-of-proportion PH in advanced COPD increases by a factor of approximately 5 to 10. When all patients with mPAP \( >20 \) mm Hg are taken into account, the population-based prevalence of PH in COPD could be in the range of 100 to 150 per million.

The hemodynamic features of PH in COPD differ from those seen in PAH. In general, the degree of PH is lower in moderate in magnitude, with mPAP rarely exceeding 35 to 40 mm Hg. Both right atrial pressure and PCWP tend to be normal or mildly elevated (10–13). The rate of progression of PH in COPD is slow, with an annual increase in mPAP of 0.4 to 0.7 mm Hg per year (14,15). Patients with COPD often develop right ventricular (RV) diastolic dysfunction with elevated RV filling pressures resulting in fluid retention and edema, especially during COPD exacerbations. Cardiac output in COPD is usually preserved and may increase during exacerbation episodes. Right ventricular forward failure, that is, low cardiac output as commonly seen in end-stage PAH, is exceedingly rare in COPD-associated PH, and death from right heart failure is a rarity in this group of patients.

Numerous studies have shown that the presence of even mild PH is of prognostic relevance in patients with COPD. A longitudinal 7-year study of 50 patients with COPD showed that survival was inversely related to pulmonary vascular resistance (PVR) (13). In a 15-year follow-up study with 200 patients (16), the presence or absence of PH was one of the strongest predictors of mortality. In a 1981 study of 175 patients with COPD (10), those with mPAP \( >20 \) mm Hg had shorter survival time than those in whom PAP was normal. A more recent study involving 34 patients receiving long-term oxygen therapy observed that mPAP was the best predictor of mortality (17). The 5-year survival rate was 36% in patients with mPAP \( >25 \) mm Hg, whereas in patients with mPAP \( <25 \) mm Hg the survival rate was 62%. In this study neither the FEV\(_1\) nor the degree of hypoxemia or hypercapnia had prognostic value.
Epidemiology, Features, and Importance of PH in ILD

The term “ILD” summarizes a heterogeneous group of lung diseases with similar clinical, radiographic, and physiologic manifestations. The prevalence of PH in patients with ILD varies greatly as a function of the underlying disease and the diagnostic mode used to identify PH. The most extensive data have been published in idiopathic pulmonary fibrosis (IPF).

The incidence and prevalence of PAH in IPF remain unclear, with widely varying estimates. The differences reflect varying patient populations, varying underlying disease severity, and differing diagnostic modalities. In general, studies of patients undergoing assessment for lung transplantation have suggested a higher prevalence of PH. In one early study of ventricular dysfunction and tricuspid regurgitation (TR) in patients evaluated for lung transplantation, 50 of 77 IPF patients had echocardiographic evidence of RV dysfunction (18). Another study retrospectively identified 50 of 77 IPF patients had echocardiographic evidence of RV dysfunction (18). Another study retrospectively identified an estimated systolic pulmonary arterial pressure (SPAP) >35 mm Hg in 84% of 88 patients and >50 mm Hg in 16% (19). The combination of emphysema in the upper lung zones and pulmonary fibrosis in the lower lobes on high-resolution computed tomography (CT) of the chest seems to be associated with a higher prevalence of PH (20,21). Echocardiographic data are difficult to interpret because the operating characteristics of echocardiography to estimate SPAP in patients with advanced lung disease seem to be quite poor (22).

Recent studies have used right heart catheterization (RHC) to accurately measure pulmonary pressures in IPF patients. Two retrospective analyses of IPF patients undergoing RHC reported PH (defined as mPAP >25 mm Hg) in 31.6% (23) and 33.9% (24) of the patients, respectively. In the United Network for Organ Sharing and the Organ Procurement and Transplant Network registries for IPF patients listed for lung transplantation between January 1995 and June 2004 (25), 2,525 of the 3,457 patients listed had RHC results available. Of these, 932 (37.0%) had an mPAP ≥25 mm Hg, whereas 231 (9.1%) had an mPAP >40 mm Hg. Among 70 IPF patients evaluated prospectively (26), PH (resting mPAP >25 mm Hg) was detected in only 6 patients (8.1%) at baseline.

Several groups have emphasized the prognostic importance of PAH complicating IPF. Echocardiographically defined PH (SPAP >50 mm Hg) has been associated with impaired survival (19). Echocardiographically defined PH in IPF patients with superimposed emphysema negatively influences survival (20). In IPF patients undergoing RHC before listing for lung transplantation, the presence of PAH correlated linearly with mortality (23). In a prospective study, an mPAP >17 mm Hg was predictive of mortality (26).

Assessment of PH in Chronic Lung Disease

Dyspnea and fatigue are symptoms of chronic lung disease as well as PH. Thus, patients with chronic lung diseases should be evaluated for PH when the symptoms are more severe than one would expect from lung function data, or when signs of right heart failure develop. Suspicion of PH should be high if clinical deterioration is not matched by a decline in pulmonary function. Profound hypoxemia, hyperventilation, and a low DLCO are indicators of PH.

Once PH is suspected, patients should be evaluated by Doppler echocardiography. A measurable TR velocity, however, is less likely to be observed in patients with COPD than in patients with PAH, ranging between 24% and 77% (27–29). Even if a TR jet is available, echocardiographic estimates of the PA pressure are often inaccurate, and both false-positive and -negative results have been reported. In 2 large series comparing echocardiographic data and findings from RHC in patients with chronic lung disease, the positive predictive values of echocardiography were 32% and 68%, respectively, and the negative predictive values were 93% and 67%, respectively (22,30). The results are somewhat better but still suboptimal in patients with ILD (22).

Plasma levels of brain natriuretic peptide (BNP) or N-terminal pro-brain natriuretic peptide (NT-proBNP) have also been evaluated as predictors of PH in patients with lung disease (31,32), but these biomarkers lack sensitivity, especially for milder forms of PH, and specificity, because elevated levels may also reflect LHD.

Given the limitations of echocardiography and biomarkers, RHC remains the standard for the diagnosis of PH. This is of particular relevance for patients who also suffer from some degree of LV dysfunction, which seems to be a common comorbidity and may contribute to the clinical features of cor pulmonale.

Treatment of PH in Patients With Chronic Lung Disease

It is self-evident that the underlying lung disease should be optimally treated according to relevant guidelines (33,34). Summarizing these recommendations is beyond the scope of this article. We will focus exclusively on the use of PH-targeted medication in patients with lung disease. So far, no large randomized controlled trials (RCTs) addressing the long-term effects of drugs targeting PH have been performed in patients with chronic lung disease. Patients with advanced lung disease, that is, those with total lung capacities <70% predicted and FEV1/forced vital capacity ratios <50% to 60% have been excluded from RCTs in the field of PAH. There is not sufficient evidence showing that drugs approved for the treatment of PAH, that is, endothelin receptor antagonists (ERAs), phosphodiesterase-5 (PDE-5) inhibitors, and prostanoids, are safe and effective in patients with chronic lung disease–associated PH. This is
true for patients with advanced chronic lung disease and mild PH as well as for patients with severe PH in the setting of chronic lung disease, independent of its severity.

Any pulmonary vasodilator has the potential to worsen gas exchange in patients with chronic lung disease, and the effects of these drugs may vary substantially depending on whether the underlying disease has obstructive or restrictive features (35). Short-term studies have been performed in ILD patients with sildenafil (36), bosentan (37), and inhaled iloprost (38), and these drugs had no adverse effects on oxygenation. In contrast, unpublished data suggest that sildenafil may worsen oxygenation in patients with COPD and PH (39), and it has been shown that sildenafil can inhibit hypoxic pulmonary vasoconstriction (40). In another study in COPD patients, sildenafil did not improve stroke volume at rest or during exercise (41). A small RCT with bosentan in patients with COPD and mild PH found a significant deterioration of the PaO₂. At the same time, there was no improvement in exercise capacity, peak oxygen uptake, and health-related quality of life (42). All in all, much more evidence is needed before the use of PH-targeted drugs can be recommended for certain subpopulations of patients with chronic lung disease.

**Working Group Recommendations for PH in Chronic Lung Disease (COPD, ILD, and Other Forms)**

**Diagnosis and assessment of PH in chronic lung disease**

- In patients with chronic lung disease, the presence of PH should be suspected when the symptoms are more severe than expected based on lung function data, or when signs of right heart dysfunction are present. Profound hypoxemia, hyperventilation, or low DLCO values can be indicators of PH (E/A). (Please refer to Barst et al. [43] for an explanation of the evidence-based grading system.)
- Doppler echocardiography remains the most useful noninvasive tool for assessing the presence of PH in patients with chronic lung disease, but both false-positive and false-negative results are not uncommon (E/B).
- Biomarkers such as BNP or NT-proBNP need to be further evaluated. They seem to be useful screening tools for the presence of PH in patients with chronic lung disease, although they lack sensitivity and specificity (E/B).
- If the presence of PH is going to affect the management of a patient with chronic lung disease, confirmation by RHC is recommended (E/A).
- Patients with chronic lung disease and severe PH (i.e., mPAP >35 mm Hg and/or signs of right heart failure) should be referred to a center with expertise in PH (E/A).
- The use of RHC is strongly recommended in clinical trials studying patients with chronic lung disease and PH to categorize the patients under study and to identify subpopulations likely to benefit from PH-targeted drug therapy (E/A).

**Treatment of PH in chronic lung disease**

- The underlying lung disease should be optimally treated according to the respective guidelines, including the use of long-term oxygen therapy in patients with chronic hypoxemia (E/A).
- There is no sufficient evidence that the drugs currently used for PAH are safe and effective in patients with PH associated with chronic lung disease (E/A).
- Patients with PH and chronic lung disease should be treated in the setting of clinical trials whenever possible (E/A).
- PH in various lung diseases should be studied separately, because patients with COPD and PH may respond differently to medical therapy than patients with ILD and PH (E/A).
- Registries are needed to obtain data from patients with very rare conditions (E/A).
- The use of drugs currently approved for PAH in patients with chronic lung disease is not recommended until further data are available (E/B).

**Clinical trial strategy for PH in chronic lung disease.** It is unlikely that patients with end-stage lung disease are going to derive a substantial benefit from PH treatment. In contrast, patients with mild-to-moderate chronic lung disease but severe PH may be good candidates for clinical trials with drugs targeting the pulmonary vascular component. Because one would not expect all subpopulations of patients with chronic lung disease to respond similarly to PH medications, it is crucial that the patients under study be carefully evaluated and characterized: this will include the use of RHC to define the severity of PH and the hemodynamic profile.

A 2-step approach is recommended to evaluate drugs targeting PH in the setting of chronic lung disease.

- **Step 1.** Safety, proof-of-concept, and preliminary efficacy: important safety parameters include vital signs and blood gases (both PaO₂ and PaCO₂). Preliminary efficacy can be assessed by hemodynamics, exercise capacity (i.e., 6-min walk test), peak oxygen uptake, and ventilator efficacy measured during cardiopulmonary exercise testing, and improvement of oxygenation at rest and during exercise (E/A).
- **Step 2.** Long-term safety and efficacy: prevention of clinical worsening (i.e., morbidity and mortality), improvements in exercise capacity and quality of life (E/A).

**Chronic thromboembolic pulmonary hypertension (CTEPH).** CTEPH results from obstruction of the pulmonary vascular bed by nonresolving thromboemboli. It has been estimated that there are 2,500 new cases of CTEPH each year in the U.S. (44). In a prospective study following survivors of acute pulmonary embolism, 3.8% of patients developed CTEPH within 2 years (45). However, up to 40% of patients with CTEPH have not had a clinically apparent acute pulmonary embolic episode (46–48). Sple-
nectomy, ventriculoatrial shunt for the treatment of hydro-cephalus, chronic central intravenous lines, inflammatory bowel disease, and osteomyelitis seem to be risk factors for developing CTEPH (49). However, the absence of such a history does not rule out CTEPH. Novel interesting concepts are derived from the observation of abnormal (lysis-resistant) fibrinogen variants underlying clot nonresolution in CTEPH (50).

CTEPH differs from PAH by its major vessel involvement of the vascular remodeling process (51), which can be approached surgically by pulmonary endarterectomy (PEA); this has evolved over 4 decades to become the treatment of choice (52,53). The outcomes of PEA with regard to functional status, quality of life, hemodynamics, and RV function have been very favorable, including normalization of hemodynamics and exercise capacity in frequent cases. However, small vessel arteriopathy is variably present in CTEPH (54,55), and small vessel lesions are an important determinant of the outcome after PEA.

**Diagnosis of CTEPH.** The recommended strategy for diagnosis and evaluation of CTEPH is shown in Figure 1 (55).

The perfusion lung scan is the examination of choice for ruling out CTEPH. A normal or low-probability perfusion scan in a patient with PH effectively rules out CTEPH. Patients with operable CTEPH typically have at least 1 segmental or larger perfusion defect with normal or near-normal ventilation (56,57). However, the complete absence of perfusion to 1 lung should raise suspicion for other processes, such as malignancy, mediastinal fibrosis, congenital absence of the pulmonary artery, or vasculitis.

Contrast-enhanced chest CT findings in CTEPH include the following: chronic thromboembolic material within the central pulmonary arteries, increased bronchial artery collateral flow, variability in the size and distribution of pulmonary arteries, parenchymal abnormalities consistent with prior infarcts, and mosaic attenuation of the pulmonary parenchyma (55). Chest CT scanning is also useful in ruling out significant underlying fibrotic or emphysematous disease, obstructing tumors, mediastinal fibrosis, or lymphadenopathy that could mimic chronic thromboembolic disease. Although a negative CT scan does not rule out the diagnosis of CTEPH, new-generation multirow CT scanners are expected to provide improved diagnostic accuracy.

Pulmonary angiography is still considered the standard diagnostic tool in the evaluation of CTEPH. Characteristic angiographic findings include pouching, webs or bands with or without post-stenotic dilation, intimal irregularities, abrupt narrowing, or total occlusion of segmental or larger branches (58). When performed by experienced individuals, angiography is safe, even in patients with severe hemodynamic impairment (59).

Right heart catheterization for full hemodynamic assessment is mandatory in the workup of CTEPH. The presence of PH indicates a hemodynamic consequence of chronic thromboemboli. In addition, assessment of the degree of right-sided heart failure by measuring right atrial pressure, cardiac output, and mixed venous O₂ saturation is important in determining severity of disease and risk from surgical intervention.

Other techniques with reported utility in evaluating CTEPH include pulmonary angiography (60), assessment of pulmonary artery pulse pressure and reflectance (61,62), and magnetic resonance imaging (63,64).

**Treatment of CTEPH.** Patients with CTEPH should receive lifelong anticoagulation adjusted to a target international normalized ratio between 2.0 and 3.0 to prevent recurrence of thromboembolic events. Figure 2 is a treatment algorithm for CTEPH.

**SURGICAL THERAPY.** The goal of PEA is to improve pulmonary hemodynamics, exercise capacity, symptoms, and survival. The procedure may be curative in appropriately selected patients. For details regarding patient selection and the procedure itself, please refer to the paper by Keogh et al. (65) in this issue of the Journal.

**MEDICAL THERAPY.** Although surgical intervention with PEA is the preferred treatment in appropriate candidates, pharmacotherapy may be beneficial in certain contexts: 1) in patients with predominantly distal disease that is not surgically accessible; 2) when surgery is contraindicated because of prognostically significant comorbidity; 3) in patients who are at high risk because of extremely poor hemodynamics before PEA (bridging to PEA); and 4) in patients with persistent or residual PH after PEA (54).

**Patients with inoperable disease or with persistent or recurrent PH after PEA.** Elevation of PVR out of proportion to what is attributable to mechanical thrombus obstruction is occasionally seen and signals a significant and in some cases inoperable extent of peripheral vasculopathy. High PVR is associated with poor outcome in terms of both survival and persistent PH. In addition, approximately 10% to 15% of patients show persistent or residual PH after PEA surgery, with or without concomitant diminished functional capacity; these individuals may benefit from adjunctive medical treatment (66).

Several open-label studies with prostanoids, ERAs, and PDE-5 inhibitors have been performed in patients with CTEPH, and most suggest hemodynamic and clinical improvement (67–70). Some open-label studies suggest improved survival with medical therapies compared with historical controls (71,72). However, only 1 large RCT has so far been performed in patients with inoperable CTEPH: the BENEFIT (Bosentan Effects in iNopErable Forms of chronic Thromboembolic pulmonary hypertension) study (73). This first randomized controlled study in inoperable CTEPH patients showed that treatment with bosentan significantly reduced PVR and NT-proBNP at week 16, but the 6-min walk distance remained unchanged, and there was no treatment effect on time to clinical worsening. Further studies are required to determine whether medical therapy offers a substantial benefit in various CTEPH populations.
Pre-PEA bridging therapy. The concept of introducing medical treatment as a therapeutic bridge between CTEPH diagnosis and PEA was initially proposed for continuous intravenous epoprostenol (74,75). A significant proportion of CTEPH patients undergoing PEA are hemodynamically unstable in the pre-operative period, to the point where risks from surgery in general are significantly heightened. Under these circumstances, effective medical therapy may improve pre-operative hemodynamics and stability, furthering post-operative stability. On the other hand, delay in surgery may be detrimental. Thus, the use of medical therapy and the timing of surgery should be discussed with the surgeon to make sure that risks are balanced.

Working Group Recommendations for CTEPH

Diagnosis and assessment of CTEPH

- Perfusion scintigraphy should be performed in all patients with unexplained PH because a normal or near-normal perfusion pattern virtually excludes CTEPH (E/A).
- Patients with a history or findings suggesting CTEPH should be evaluated at a center with expertise in this condition. A surgeon experienced in PEA surgery should be available at this center, or a close collaboration with such a surgeon should exist (E/A).
- Pulmonary angiography to assess operability should be performed at the center where surgery would be performed or at centers with an established cooperation with a surgical center (E/A).

Treatment of CTEPH

- Surgical PEA is the preferred treatment of CTEPH because it is potentially curative (E/A).
- The decision of whether or not a patient is a candidate for PEA surgery should involve a multidisciplinary team that includes at least 1 surgeon with substantial experience in this procedure (E/A).
- In severely compromised patients with surgically accessible disease but for whom surgery must be delayed, pre-operative medical therapy with prostanoids, ERAs, or PDE-5 inhibitors may be used to improve hemodynamics and clinical performance before surgery, but this approach needs to be adjusted with the responsible surgeon (E/B).
- Patients with predominantly peripheral (i.e., inoperable) disease may be candidates for medical therapy and should be considered for enrollment in clinical trials whenever possible (E/A).
- Preliminary data suggest that drugs currently approved for PAH may have beneficial effects in patients with CTEPH, but as long as there are no robust data from RCTs, the decision of whether or not to treat CTEPH...
patients with these drugs should be restricted to centers experienced in the management of this disease (E/B).

Clinical trial strategy for CTEPH. There is no doubt that drugs used in PAH, such as prostanoids or PDE-5 inhibitors, improve hemodynamics in CTEPH. The question is whether administration of these drugs also improves meaningful clinical end points such as exercise capacity, quality of life, time to clinical worsening, and survival. At least 2 different scenarios require further investigation:

- Patients with operable disease but severe hemodynamic impairment (i.e., PVR >~1,000 dyne·s·cm⁻²): these patients have an elevated perioperative risk, and it is unclear whether a limited period of medical treatment to improve hemodynamics also improves outcome when compared with immediate surgery. This problem is extremely difficult to study because a blinded placebo-controlled study might be considered unethical. Thus, this question should be addressed in a carefully designed open study involving only centers with broad experience in the surgical and medical management of CTEPH patients (E/B).
- Patients with peripheral (inoperable) disease and those with persistent or recurrent PH after PEA surgery are good candidates for clinical trials. The questions that need to be answered are whether medical therapy improves exercise capacity and quality of life and whether it delays time to clinical worsening (E/A).

PH Associated With LHD

LHD is one of the most common causes of PH. It may be caused by chronic heart failure attributable to LV dysfunction of systolic or diastolic origin or by valvular diseases, predominantly mitral valve disorders (76). Up to 60% of patients with severe LV systolic dysfunction and up to 70% of patients with isolated LV diastolic dysfunction may develop PH, and the presence of PH is associated with a poor prognosis in these patient populations (77–79).

Pulmonary hypertension with PVR >2 mm Hg/l/min (Wood units) has been reported in up to 50% of patients referred to transplant clinics (79). Pulmonary hypertension and RV dysfunction carry a poor prognosis for patients with chronic heart failure (80). In one study, the mortality rate after 28 months was 57% in patients with left heart failure and moderate PH, compared with 17% in patients without PH (81). Patients with a transpulmonary gradient ≥16 mm Hg have an increased risk of post-operative RV failure after heart transplantation (82).

The pathogenesis of PH in LHD is complex. There is a passive (pulmonary venous) component in response to increased left atrial pressure (83). In some patients, a superimposed active component caused by pulmonary arterial vasoconstriction and vascular remodeling may lead to a further increase in PAP.
Diagnosis and assessment of PH associated with LHD

In many cases, the presence of LHD is obvious, given the patient’s history and the echocardiographic findings. Sometimes, however, it can be extremely difficult to distinguish between PH caused by diastolic LV dysfunction and PAH. Risk factors of LV dysfunction are outlined in Table 1.

Echocardiographic findings suggestive of diastolic LV dysfunction include the presence of a dilated left atrium, atrial fibrillation, abnormal mitral inflow pattern, and LV hypertrophy (84). Although echocardiography provides important information, invasive measurements of PCWP or LV end-diastolic pressure may be required to document the presence of elevated LV filling pressures (84). However, resting PCWP and LV end-diastolic pressure can be normal despite LV diastolic dysfunction, especially when patients have been treated with diuretics. Exercise or volume challenge has been proposed to identify occult LV dysfunction, but these tools have not been fully standardized and require further evaluation.

An elevated transpulmonary gradient (mPAP–mean PCWP) >12 mm Hg is suggestive of active PH, that is, a pre-capillary component of PH (84). Figure 3 depicts a suggested diagnostic strategy to discriminate between pulmonary arteriopathy and PH related to diastolic heart failure or “heart failure with normal ejection fraction.”

Vasoreactivity tests using compounds with inotropic and/or systemic and/or pulmonary vasodilator activities have been recommended in heart transplant candidates to determine whether this procedure is feasible in patients with LHD and PH. However, there is no internationally accepted standard protocol for this procedure, and the criteria for operability or nonoperability, respectively, need to be further evaluated and standardized.

### Table 1 Risk Factors Favoring Diagnosis of Diastolic Heart Failure

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<tr>
<th>Clinical features</th>
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<tr>
<td>Age ≥65 yrs</td>
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<td>Elevated systolic blood pressure</td>
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<td>Elevated pulse pressure</td>
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<td>Obesity</td>
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<td>Hypertension</td>
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<td>Coronary artery disease</td>
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<td>Diabetes mellitus</td>
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<td>Atrial fibrillation</td>
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<th>Echocardiography</th>
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<tr>
<td>Left atrial enlargement</td>
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<tr>
<td>Concentric remodeling (relative wall thickness &gt;0.45)</td>
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<tr>
<td>Left ventricular hypertrophy</td>
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<tr>
<td>Elevated left ventricular filling pressures (grade II to IV diastolic dysfunction)</td>
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<th>Interim evaluation (after echocardiography)</th>
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<td>Symptomatic response to diuretic drugs</td>
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<td>Exaggerated increase in systolic blood pressure with exercise</td>
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<td>Re-review of chest radiograph consistent with heart failure</td>
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**Figure 3 Diagnostic Approach to Distinguish Between PAH and PH Caused by Diastolic Left Heart Disease**

See Table 1 for risk factors for diastolic heart failure. DHF = diastolic heart failure; Dx = diagnosis; EF = ejection fraction; HF = heart failure; NTG = nitroglycerine; OMT = optimized medical therapy; PAH = pulmonary arterial hypertension; PCWP = pulmonary capillary wedge pressure; PH = pulmonary hypertension; PVR = pulmonary vascular resistance; RCT = randomized controlled trial; RHC = right heart catheterization; WU = Wood units.
Treatment of PH associated with LHD. Optimal correction of the underlying substrate is a necessary first step in management because treatment of LHD may decrease PH (85). Careful attention must be paid to optimize LV filling pressures (86). In valvular heart disease such as mitral stenosis, post-operative reduction of PCWP may lead to a rapid reduction of PAP in both passive and active PH (87). However, the reduction may be incomplete, despite the normalization of the PCWP, in cases with PH caused by chronic pathological obstructive changes. This component may require weeks to months to regress, even after successful valve surgery (87). In the majority of patients, an almost complete normalization of PH is expected.

There is an ongoing debate regarding whether drugs used in PAH, that is, ERAs, PDE-5 inhibitors, or prostanoids, may be useful in patients with LHD and PH, especially when PH becomes severe and dominates the clinical picture. Theoretical concerns about this approach include the possibility of causing pulmonary edema when pulmonary vasodilators are administered to patients with elevated LV filling pressures. This potentially adverse effect, however, may be offset when the concomitant treatments reduce LV afterload and LV filling pressures.

Virtually no data from long-term RCTs address the population of patients with LHD and PH. Inhaled nitric oxide and prostanoids exert favorable acute hemodynamic effects in these patients (88–90). However, it is of concern that a study of epoprostenol in heart failure showed a higher mortality in the active treatment group (91). Large trials with ERAs in patients with LHD have also failed to show beneficial long-term effects, although these trials did not look specifically at the subset of patients with LHD and PH (92,93). The PDE-5 inhibitor sildenafil improves systolic and diastolic LV function as well as systemic vasoreactivity in experimental models of heart failure, making it a promising agent for future studies in patients with LHD and PH (94,95). Results of recent small studies suggest that sildenafil may improve exercise capacity and quality of life in patients with PH caused by LHD (96–98). However, long-term data from carefully designed clinical studies are required before sildenafil can be recommended for patients with LHD and PH because there have been instances in which drugs used to treat left heart failure had positive effects on surrogate end points but decreased survival, as was the case with PDE-3 inhibitors (99,100).

Working Group Recommendations for PH Associated With LHD

Diagnosis and assessment of PH in LHD

- Left heart disease is one of the most common causes of PH. The presence of LHD is obvious in many patients, but it may be occult in others, especially when isolated LV diastolic dysfunction is present. Features suggestive of LV diastolic dysfunction or diastolic left heart failure include an older age, atrial fibrillation, and an enlarged left atrium (E/A).
- Right and left heart catheterization may be required to distinguish between PAH and PH associated with LHD (E/A).
- In patients with elevated PCWP and an elevated transpulmonary gradient, reduction of LV afterload with nifedipine or other vasodilators is useful to identify an active pulmonary arterial component of PH (E/A).
- In patients with LHD and PH considered for heart transplantation, pulmonary vasoreactivity testing with inhaled nitric oxide or prostanoids may be used to assess reversibility and operability, although the criteria for operability or nonoperability require further evaluation (E/A).

Treatment of PH in LHD

- Treatment of LHD according to established criteria is the basis for a successful approach to patients with LHD and PH (E/A).
- Patients with end-stage heart failure and PH may be candidates for heart transplantation (in milder, largely reversible forms of PH) or heart–lung transplantation (if PH is severe and fixed) (E/A).
- The use of prostanoids, ERAs, and PDE-5 inhibitors in patients with LHD and PH is not recommended until robust data from long-term clinical trials are available (E/A).

Clinical trial strategy for PH in LHD. Drugs that reduce LV afterload as well as PVR may have beneficial effects in patients with moderate or severe PH in the setting of milder forms of heart failure or LV diastolic dysfunction with preserved ejection fraction. Short-term beneficial effects on hemodynamics and exercise capacity have been shown with several drugs, including PDE-5 inhibitors. These drugs need to be rigorously studied to assess long-term safety and efficacy in patients with LHD and PH and to identify subpopulations of patients most likely to derive a benefit. These trials should address morbidity and mortality end points to yield meaningful results (E/A).

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REFERENCES


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End Points and Clinical Trial Design in Pulmonary Arterial Hypertension

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New and emerging therapies might provide benefit in patients with pulmonary arterial hypertension. Their efficacy and safety will be compared with existing combination therapies in randomized clinical trials. Appropriate end points for these trials need to be identified: these will include exercise testing, the composite end point of time to clinical worsening, and hemodynamic markers, including advanced imaging modalities and biomarkers. Quality-of-life questionnaires are useful and important secondary end points; pulmonary arterial hypertension-specific questionnaires are currently being developed. Advantages and disadvantages of various trial designs, including placebo-controlled monotherapy or add-on trials, noninferiority studies, and withdrawal trials are also discussed. (J Am Coll Cardiol 2009;54:S97–107) © 2009 by the American College of Cardiology Foundation

When the 2nd World Health Organization (WHO) conference on Pulmonary Hypertension met in Evian in 1998, the only approved medical treatment for pulmonary arterial hypertension (PAH) was intravenous epoprostenol. By the time of the 3rd World Symposium on PAH, which took place in Venice in 2003, new therapies had emerged that extended and improved the quality of our patients’ lives. Since the Venice meeting, an increasing number of agents, including prostanoids, endothelin receptor antagonists, and phosphodiesterase-5 inhibitors, are providing more options for treatment. With these new agents comes a new challenge: to design clinical trials that will guide us in making the best therapeutic choices on the basis of currently available therapies and those that are still in development. The views presented here are a summary from the Task Force on End Points and Clinical Trial Design that met at the 4th World Symposium on Pulmonary Hypertension, held in Dana Point, California, in 2008.

Patient Populations in Randomized Clinical Trials of PAH

A key question concerns the types of PAH patients that can reasonably be considered together in a single randomized controlled trial (RCT). To date, most pivotal studies in PAH have grouped together several subcategories of patients. Because of the relative rarity of this disease (1–4), this strategy has allowed investigators to maximize recruitment. Nevertheless, it is clear that disparate PAH populations are not biologically identical and often not even similar. Subgroup analyses of these trials, moreover, have been limited because of the small numbers of subjects, and no studies to date have prespecified subgroup analyses. Such post-hoc subgroup analysis should be viewed as hypothesis-generating. Thus, we still have only rudimentary data in

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subpopulations regarding the pathogenesis and natural history of the disease and clinical response to current PAH–targeted therapies.

Although more than 50% of the patients included in randomized trials of PAH have had idiopathic PAH, many trials have included a significant number of patients with other forms of PAH, such as that associated with connective tissue disease and congenital heart disease. Although regulatory agencies have been approving drugs for the global indication of PAH on the basis of data from such trials since 2001, a paucity of data in PAH subpopulations makes it difficult to extrapolate specific therapeutic approaches targeted to them. What we have learned from these trials is that most drugs for PAH, with the possible exception of sildenafil, seem to be more efficient in patients with idiopathic PAH than in those with nonidiopathic PAH and, furthermore, that patients with connective tissue disease consistently demonstrate a lesser response to treatment (5–9). With 2 notable exceptions, the intravenous (IV) epoprostenol in scleroderma study (10) and the BREATHE-5 (Bosentan Randomised trial of Endothelin Antagonist THERapy) trial in patients with congenital heart disease (11), most studies in subpopulations have been uncontrolled, and long-term data in these groups are scarce. The following options can be considered:

- Group all PAH patients together. This strategy has the advantage of facilitating patient recruitment, but the patients might not be biologically similar, and etiology might modify treatment effect.
- Do separate studies in each PAH subgroup. However, this approach is resource intensive, and the results of studies done in selected populations might not be generalizable to a broader population.
- Generalize in some cases, and do prototype studies in designated subgroups.

Recommendations. The pivotal studies in PAH treatment have included mainly subgroups within Group I. These patients share sufficient pathophysiologic and pathobiologic similarities that they might reasonably be grouped together in future studies (9,12,13). Recognizing that some compromise is necessary, the U.S. Food and Drug Administration (FDA) has not required significant effects within each Group I subgroup and is likely to continue to approve global indications on the basis of such trials.

Patients in Groups II through V (i.e., those with non-PAH pulmonary hypertension [PH]) should be studied in individual subgroups. The BREATHE-5 trial, in patients with Eisenmenger syndrome, a Group I subgroup, showed that such subgroup study is feasible and can be productive, despite logistical complications (11).

Patients with sickle cell disease, portopulmonary hypertension, or chronic thromboembolic pulmonary hypertension (CTEPH), who were underrepresented in the pivotal trials, represent unique challenges that compel separate investigative approaches (14,15). Substantial numbers of patients have sickle cell disease, although this is a heterogeneous group and might be difficult to study. As for patients with portopulmonary hypertension, they have been excluded from all studies, with the exception of the ALPHABET (Arterial Pulmonary Hypertension and Bera-prost European Trial) (16), because of concerns for hepatic toxicity and the fact that the treatment algorithm incorporates consideration of liver transplantation early in the disease. Nonoperable CTEPH patients present a large collective. Although CTEPH patients have rarely been included in studies (6), the recent BENEFiT (Bosentan Effects in iNOpEnable Forms of chronic Thromboembolic pulmonary hypertension) trial, which enrolled 157 patients with CTEPH, demonstrated the feasibility of such a study design (17). However, there remains an urgent need for evidence-based decision-making for the pharmacologic treatment of these patients.

It is possible that enrolling such subpopulations into clinical trials on the basis of a presumed pathogenesis might not be productive and that a lack of rigorous entry criteria could dilute treatment effect or even, in some cases, be unsafe. Therefore we need to continue to expand our knowledge of PH in these non-PAH groups and exercise restraint in enrolling these patients in RCTs until we know more about their disease.

End Points in RCTs of PAH

Primary and secondary end points. Primary end points in PAH trials, as in other RCTs, must meet 3 criteria; they should be: 1) clinically relevant; 2) sensitive to treatment effect; and 3) measurable and interpretable. Secondary end points complement the primary end points by providing a more global view of the benefit of the drug being tested and by clarifying its risk-to-benefit ratio. Secondary end points may be of 2 types: 1) those that, like primary end points, are clinically relevant and may be taken into consideration for drug indications; and 2) “feel-good” end points, which are not likely to lead to a new indication or a change in labeling but might provide reassurance about the primary end point along with new information about the disease. Some secondary end points might be exploratory analyses (i.e., although they might demonstrate biologically plausible
effects, they remain hypothesis-generating and will need to be confirmed by additional studies).

**Exercise testing.** Exercise capacity is one of the most important prognostic indicators in PAH. Several exercise protocols have been used in PAH assessment protocols (Table 1) (5,6,16,18–24). Of these, the 6-min walk (6MW) test has been accepted by regulatory agencies and is most commonly used as a primary end point in RCTs. The 6MW test correlates fairly well with peak aerobic capacity (25). It also has prognostic significance in PAH (26), and it can be performed simply and inexpensively. However, within-subject variability has been seen in the 6MW test, with repeat testing on the same day resulting in a 66-ft improvement in a study of patients with chronic obstructive pulmonary disease (27), an 18-m improvement on 2 successive days in post-myocardial infarction patients (28), and a 4.2% change in patients with interstitial lung disease after 1 week (29). In addition, the 6MW test might be less discerning in patients who are less ill (30). Drug effects on the 6MW test tend to be slow to manifest and modest (10% to 15%) and might not provide an accurate reflection of how patients really feel. Therefore, regulatory authorities are open to assessments of exercise capacity beyond the 6MW test, and these have been and continue to be explored (31).

Cardiopulmonary exercise testing (CPET) measures metabolic gas exchange at rest and during exercise. It quantitates aerobic capacity and ventilatory inefficiency in order to determine the severity of PAH (32) and might provide more sensitive exercise assessment than the 6MW test early in the course of the disease (33). It might also provide a more complete physiologic assessment of the pulmonary vasculature (32). The CPET is expensive and has proven to be technically challenging to perform and interpret in the setting of a multicenter RCT. Nevertheless, because exercise capacity measured by CPET (peak oxygen consumption) is prognostic in PAH patients (34), it has been used as a primary end point in 2 clinical trials (20,35). In the STRIDE-1 (Sitaxsentan To Relieve Impaired Exercise) multicenter trial, however, effects on peak oxygen consumption did not correlate with effects on the 6MW exercise test (20,35). Treadmill and cycle ergometry have been successfully used as end points in PAH trials (38–40).

**Recommendation.** Although the 6MW test has been the most common primary end point in clinical trials and its use has led to the approval of many agents by regulatory authorities, several questions remain, including: 1) What is a clinically relevant improvement in 6MW test? 2) How should variables that are known to affect the 6MW test, such as age and height, be factored into this end point? 3) Is the 6MW test still a sensitive exercise test as we study patients earlier in the course of the disease?

Regardless of the type of assessment, some evaluation of change in exercise capacity will continue to be an important outcome measure in ongoing trials, if only as a secondary end point, and additional and more sensitive methodologies should be explored.

**Time to clinical worsening.** Recent studies in PAH have been similar in design, with exercise capacity as a primary end point, and with a blinded phase ranging from 3 to 6 months and, in 1 case (20), to 12 months. Because of the low event rate, mortality alone would not be an adequately powered end point in this setting. Therefore, a composite end point, time to clinical worsening (TtCW), has been developed and is generally included as a secondary end point.

Different definitions of TtCW have been used in different studies (5,20,22,24,35,41–44), making comparison difficult (Table 2). However, common components have been time-from-randomization to: 1) all-cause mortality; 2) hospital stay for PAH; 3) need for interventional procedures (transplantation or balloon atrial septostomy); and 4) clinical progression of PAH. Some definitions of TtCW have also included a combination of deterioration of 6MW test distance from a baseline of 10% to 20%, an increase in New York Heart Association/World Health Organization (NYHA/WHO) functional class, symptoms of right heart failure, and/or escalation of medical treatment. In considering a single definition of TtCW, the following definitions are possible:

- Only all-cause mortality and hospital stays for PAH. Despite the fact that thresholds for hospital stay might differ according to geographic areas and health sys-

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**Table 1: Functional Capacity Assessments**

<table>
<thead>
<tr>
<th>Test</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>6-min walk</strong></td>
<td>• Inexpensive&lt;br&gt;• Technically simple&lt;br&gt;• Useful in large groups of patients</td>
<td>• Subject to patient effort&lt;br&gt;• Unable to measure gas exchange, ventilatory efficiency</td>
</tr>
<tr>
<td><strong>Formal exercise testing (treadmill, cycle ergometry)</strong></td>
<td>• Most familiar</td>
<td>• Unable to measure gas exchange, ventilatory efficiency</td>
</tr>
<tr>
<td><strong>CPET</strong></td>
<td>• Provides objective measurements of peak metabolism and rate of exertion of ventilatory and circulatory reserves&lt;br&gt;• Reproducible</td>
<td>• Technically more difficult&lt;br&gt;• Subjective</td>
</tr>
<tr>
<td><strong>NYHA/WHO classification</strong></td>
<td>• Simple, large body of scientific literature</td>
<td></td>
</tr>
</tbody>
</table>
tems, hospital admission for PAH progression is usually considered to be an objective measure of deterioration (45).

- Adding interventional procedures to death and hospital stay. However, the availability of such procedures might also vary from country to country, and in any case, they are very rarely performed in the setting of RCTs.
- Inclusion of an additional event, defined as progression of PAH that does not require hospital stay. This category might include progression of NYHA/WHO functional class, symptoms of right heart failure, and the need for additional therapies, as determined by the physician/investigator.

Because TtCW should be used only in blinded trials, disease progression might be a subjective and therefore a weak end point. To address this, the physician/investigator could be required to provide “measurable” parameters for supporting the definition of PAH progression or the decision to increase medical therapy.

As with all composite end points, the U.S. FDA has expressed concern about TtCW. Although they have accepted it as a primary end point, they have suggested that it could be more useful if it were possible to assign a numerical value to each component, on the basis of community input.

They have also suggested that total events is a broader, more inclusive, and therefore stronger end point than time to first event.

**Recommendations.** We recommend that a uniform definition of TtCW be used in future pivotal (phase III) RCTs in PAH. In the definition of TtCW, hard events would include:

- All-cause mortality
- Nonelective hospital stay for PAH (with predefined criteria, usually for initiation of intravenous prostanoids, lung transplantation, or septostomy)
- Disease progression defined as a reduction from baseline in the 6MW test by 15%, confirmed by 2 studies done within 2 weeks plus worsening functional class (except for patients already in functional class IV)

We strongly suggest that, in all cases where TtCW is used as a primary end point in an RCT, some adjudication of events should be mandatory.

There might not be sufficient numbers of patients in phase IIb trials to use the TtCW end point. In these kinds of trials, some type of exercise assessment should be used, along with secondary confirmatory end points, to include hemodynamic data for new therapies. In phase III or pivotal trials, however, we recommend TtCW as a primary end point, with assessment of exercise ability as a secondary end point. Trials with TtCW as a primary end point will likely need to be of longer duration than typical 12- to 16-week trials that have used 6MW test as a primary end point. This strategy would be acceptable to regulatory authorities.

**Quality-of-life assessments.** A variety of instruments have been developed to measure outcomes in PAH. Dyspnea is the most frequent complaint for which persons with PAH seek medical attention, and numerous instruments exist to evaluate dyspnea during exercise and activities of daily living (46–52). The Borg score (53), developed in 1982, has been shown to be responsive to interventions aimed at reducing dyspnea as well as to therapeutic intervention in most PAH RCTs.

Global and health-related quality-of-life measurements are also used to evaluate patient perceptions of treatment effect. Those that have been used with PAH patients include the St. George’s Respiratory Questionnaire (54), the Minnesota Living with Heart Failure questionnaire (55), the Chronic Heart Failure Questionnaire (52), and a general-health questionnaire, the Medical Outcomes Study Short Form-36 (56). In general, congestive heart failure–specific instruments have performed better than general health instruments in PAH patients. Two groups have now started to develop a quality-of-life instrument specific to PH (including collagen vascular disease) that will address concerns that have not been addressed in nondisease-specific instruments (57,58).

Regulatory agencies respond differently to these kinds of instruments. The European Medicines Agency might regard them more favorably than the FDA, although both organizations regard them with caution. The FDA has expressed concerns that this end point is too amorphous and needs validation, particularly if it is not consistent with the

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**Table 2 Definition of Time to CW in Different Trials**

<table>
<thead>
<tr>
<th>Component</th>
<th>BREATH-1 &amp; EARLY (5,41)</th>
<th>EARLY (42)</th>
<th>STRIDE-1 (35)</th>
<th>STRIDE-2 (22)</th>
<th>ARIES-1 (43)</th>
<th>ARIES-2 (43)</th>
<th>SUPER-1 (21)</th>
<th>STEP (23)</th>
<th>PACES (44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Hospital stay</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Lung transplantation</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Atrial septostomy</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Symptomatic progression (NYHA/WHO FC)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Lack of improvement or worsening PAH (≤6-min walk)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Need for additional PAH therapy</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

CW = clinical worsening; NYHA/WHO FC = New York Heart Association/World Health Organization functional class; PAH = pulmonary arterial hypertension.
primary end point. The FDA is also concerned that cultural differences, different types of assessments, and patient interpretation might affect the outcome. To date, only 5 RCTs in PAH have used health-related quality-of-life instruments.

**Recommendations.** Some evaluation of quality of life is important. There is a need to develop and validate in multicultural fashion a disease-specific questionnaire that will be acceptable to both the FDA and the European Medicines Agency as a secondary end point in future trials. **Imaging and hemodynamic assessment.** Hemodynamic data are not currently accepted as an end point for regulatory authorities. Nonetheless, these data provide valuable information, because the functional capacity of the right ventricle is a major prognostic determinant in PH, and death from PH usually results from right ventricular (RV) failure (59–62). Therefore, techniques that image RV morphology and functional change in the face of increasing outflow obstruction can greatly advance our understanding of the disease. Such techniques include echocardiography, computed tomography, radionuclide ventriculography, and cardiac magnetic resonance imaging (CMRI).

**NONINVASIVE.** Echocardiography is routinely used for assessing RV size and function and severity of PH. In 1 RCT (63), advanced PAH therapy improved many echocardiographic variables, including left ventricular (LV) area, systolic eccentricity index, RV/LV diastolic areas ratio, pericardial effusion score, RV ejection time, Doppler RV index, LV stroke volume, and cardiac output, and parameters of LV filling. Improvements in exercise capacity were also related to echocardiographic changes. Thus, this technique is sufficiently sensitive to clinical changes to be used as a reinforcing end point. However, standardization of echocardiographic measurements, some of which can be highly technical, would be necessary to consider this as an end point in clinical trials. Two indexes that might be considered include the Tei Index and tricuspid annular plane systolic excursion (64,65). Although there is substantial interest in exercise stress echocardiography, particularly in early disease, this study is difficult to perform and interpret. Similarly, although 3-dimensional echocardiography might improve our understanding of the pathophysiology of RV failure in PAH, it has been minimally evaluated in PAH patients.

CMRI is a safe and reproducible technique that allows both morphologic and functional assessment of the right ventricle (66). CMRI findings in PAH include RV dilatation, RV hypertrophy, interventricular septal flattening or paradoxical motion, and change in RV chamber morphology from a normal crescent shape to a more concentric form. Stroke volume, cardiac output, and distensibility of pulmonary arteries can also be assessed (67–69). CMRI imaging has not been validated to measure pulmonary vascular resistance, but good correlation between right heart catheterization and CMRI suggests that CMRI data could be used as a surrogate for right heart hemodynamic status. Preliminary data suggest that the prognostic importance of CMRI measurements in PAH includes low stroke volume, RV dilatation, and impaired LV filling (70). Furthermore, because this technique is noninvasive, it can be used repeatedly to determine disease progress or response to therapy. Technical improvements in CMRI allow increasingly rapid and robust data acquisition.

In the SERAPH (Sildenafil versus Endothelin Receptor Antagonist for Pulmonary Hypertension) study in patients with idiopathic PAH, changes in RV mass measured by CMRI were a primary end point (71). CMRI is now considered the gold standard for detailed study of the right ventricle and an established modality for the physiologic assessment of PAH patients in cross-sectional and longitudinal follow-up studies of therapy. It will likely be increasingly used as the primary modality for anatomic and functional assessments that will enable more complete and efficient evaluation of patients with PAH (72).

**INVASIVE.** Right heart catheterization, an invasive procedure, provides measurements of systemic blood pressure and saturation, pulmonary arterial pressure and saturation, pulmonary capillary wedge pressure, right atrial pressure, and cardiac output by thermodilution or Fick method if oxygen consumption is measured. The hemodynamic definition of PAH is based on catheter measurements, which are accurate and reproducible and, because the measurements are less noisy than noninvasive imaging, require fewer patients to power clinical trial end points. In addition, the data obtained have been shown to be prognostic (59). However, the fact that these measurements are made at rest rather than during exercise—when patients develop symptoms—limits its usefulness, as do procedural concerns, potentially including a need for dehydration after an overnight fast and sometimes the need for sedation. Some of the measurement techniques also have particular limitations. Despite these limitations, routine right heart catheterization provides a simple description of resting hemodynamic status that can be interpreted in the context of the underlying disease process.

**Recommendations.** Imaging of the right ventricle, either by echocardiography or CMRI, often provides clinically useful information to the experienced clinician caring for an individual patient. Similarly, invasive hemodynamic measures of RV function, particularly right atrial pressure and cardiac index, provide important clinical information and insight into disease progression. RV function has potent prognostic abilities, and it is reasonable to consider indexes of RV function as secondary end points in pivotal clinical trials.

Hemodynamic measures provide a rational basis for decisions on dose range and dosing intervals. At a minimum, these should be included in Phase II trials and should be considered as exploratory secondary end points that advance our understanding of PAH. They will very likely not be primary end points for registration in the foreseeable future.

**Biomarkers.** Echocardiography, CMRI, or right-heart catheterization provides accurate assessment of RV function, but right heart catheterization is invasive, and all these
modalities are costly. Therefore, various biomarkers have been increasingly explored to assess cardiac function in PAH patients. These include the cardiac hormone brain natriuretic peptide (BNP), a highly sensitive and specific marker of LV failure; however, levels of brain and atrial natriuretic peptides also seem to correlate with RV dysfunction (73–75). Many clinicians routinely order an assay for BNP or the inactive N-terminal-pro-fragment, NT-proBNP, in the setting of PAH or congestive heart failure. Measurement of NT-proBNP is preferred over BNP, because it is more stable both in vivo and in vitro than the active BNP molecule (76). Levels of NT-proBNP of approximately 1,400 to 1,700 ng/l cause it is more stable both in vivo and in vitro than the active BNP molecule (76). Levels of NT-proBNP of approximately 1,400 to 1,700 ng/l seem to suggest a poor prognosis (74,77). The NT-proBNP is now commonly used in congestive heart failure and PAH (78). The NT-proBNP levels also correlate with survival (74,77,79). Therefore, it might be reasonable to include NT-proBNP as a secondary end point in RCTs of PAH.

Acute RV strain might also be reflected in troponin elevation in patients with PH or CTEPH. Although the majority of stable patients with PAH do not have abnormal troponin values, measurement of troponin levels could improve prognostication in more advanced patients. In spite of similar hemodynamic status, patients with elevated troponin levels in 1 study had higher heart rates, shorter 6MW distances, lower mixed venous oxygen saturations, and higher NT-proBNP levels; all were statistically significant compared with normal troponin values (80).

Serum uric acid levels are increased in obstructive pulmonary disease (81), Eisenmenger syndrome (82), and other hypoxemic settings (83). Serum uric acid levels increase in proportion to the severity of idiopathic PAH, in which they also have a strong independent association with mortality (84). In functional class III patients with idiopathic PAH, serum uric acid levels and diastolic blood pressure at peak exercise independently predicted survival (p < 0.005) (34). Serum uric acid levels are readily available; however, they are somewhat nonspecific and might be affected by variables such as acute illness, drugs, tissue perfusion, decreased glomerular filtration, and hypoxia. Nevertheless, in PAH, in the absence of other causes of hypoxemia, uric acid levels might contribute to prognostication (84).

Other biomarkers that might potentially be useful in patients with PH are D-dimer levels (85), endothelin-1 (86), nitric oxide (87), prostaglandins (88), and cyclic guanosine monophosphate (89). Although these show promise, studies to date have been too small to be conclusive, and practical considerations currently preclude their routine use. Further studies are needed.

Although biomarker end points are important in clarifying our understanding of PAH, it is unlikely that they will be accepted by regulatory authorities as primary end points in registration trials in the foreseeable future.

Summary of end point recommendations. Although we believe TrtCW to be an important, clinically relevant primary end point in the modern era, we realize that Phase II trials might not be large enough or of sufficient duration to detect a meaningful difference in TrtCW. For Phase II trials, we recommend the 6MW test as a primary end point and encourage the inclusion of hemodynamic status as a secondary end point. For Phase III trials, we recommend TrtCW as the primary end point. A number of the secondary end points discussed in the preceding text might also be studied in a Phase III trial. We suggest choosing a few but caution against including too many secondary end points.

Clinical Trial Design

The RCT environment is becoming increasingly complex. Six therapeutic agents have been approved for PAH in the U.S. and 7 in Europe, and many patients are already receiving some kind of approved therapy when they enroll in RCTs. Therefore, these trials increasingly involve add-on therapy. Several designs have been proposed and/or used for future RCTs in the PAH populations:

- Placebo-controlled monotherapy or add-on trials. Placebo-controlled trials are still considered to be among the strongest designs to evaluate new therapies. However, because many patients are already receiving or have access to some type of approved therapy, placebo-controlled monotherapy trials might be considered unethical (90,91). Nevertheless, because of the lack of evidence regarding the efficacy of advanced therapies in non-WHO Group I populations (e.g., patients with chronic obstructive pulmonary disease or interstitial lung disease, CTEPH, or PH due to diastolic dysfunction of the left ventricle), it is reasonable to consider enrolling these patients in placebo-controlled monotherapy RCTs. Placebo-controlled RCTs might be reasonable as well where the new treatment is added to standard therapy and is compared with placebo added to standard therapy.

- Noninferiority studies. Another approach might be to perform head-to-head comparisons by conducting non-inferiority studies. A noninferiority study aims to demonstrate that the tested drug is not worse than the comparator by more than a prespecified, small amount, known as the noninferiority margin. The size of an acceptable margin depends on the smallest clinically significant difference, expected event rates, the established efficacy advantage of the control over placebo, and regulatory requirements. Noninferiority trials are statistically based on a 1-sided comparison with an active control in the positive direction. This trial design is useful in cases where: 1) bioequivalence cannot be established, as in modified-release products or topical presentations; 2) new products have a potential safety advantage, and therefore a risk-benefit assessment can be made; 3) a direct comparison against the active comparator is needed to assess risk-benefit; 4) no important loss of efficacy compared with the active comparator would be acceptable; and 5) a placebo arm is not possible, and an active control trial is required to demonstrate the efficacy
of the tested drug. In noninferiority studies, new end points can be used only along with primary end points already investigated—in PAH, the 6MW test. They must also replicate the setting of the pivotal study of the comparator (i.e., inclusion and exclusion criteria, patient population). One of the obstacles in conducting these trials is the sample size, which would often need to be >500 patients, which limits the feasibility of such a study, both in terms of cost and patient recruitment. Thus, noninferiority studies have a number of inherent weaknesses, and they have not yet been used in PAH registration studies.

- Withdrawal trials. The current standard of therapy for PAH usually involves initial use of oral endothelin antagonists or phosphodiesterase-5 inhibitors for patients in NYHA/WHO functional classes II and III and parenteral prostanoids for patients in class IV. Several RCTs have shown benefit when a second drug is added to the background of a single drug (23,92–95), but others have not (96). It is not known whether the benefits seen in some of these trials would have been seen had the first drug been withdrawn at the time the second drug was added. Because most agents do not have a progression claim, it might not be unethical to withdraw them, although there are some safety concerns. However, the FDA feels that there should not be a problem in a placebo-controlled setting where an investigator can withdraw a patient who is not doing well.

**Induction therapy.** Pulmonary vascular disease is very aggressive, and late therapy might be disadvantageous. Data show that patients randomized to placebo groups never really catch up: delaying therapy even 12 to 16 weeks might be injurious (97,98). Possible strategies might involve earlier treatment with parenteral prostanoids for patients in most NYHA/WHO classes, simultaneous initiation of 2 or more oral drugs or an oral drug in combination with an inhaled or a subcutaneous drug, simultaneous IV prostanoid therapy and 1 or more nonparenteral PAH drugs, or simultaneous use of 1 PAH drug from each category of pathologic targets. Objections to such strategies concern the potential for drug interactions or toxicity, the costs of PAH drugs, the need for end points other than the 6MW test (99), and the current lack of evidence-based information on drug combinations.

**Recommendations.** Various approaches to combination PAH therapy need to be explored in detail in well-designed RCTs. Head-to-head trials in which 2 drugs would be used both separately and in combination would be highly appropriate in the PAH population and would respond to many of the aforementioned objections. However, it will not be easy to garner industry support for such trials, because of the large numbers of patients required.

**Adaptive design.** Standard monitoring procedures for RCTs specify a primary end point and a test statistic to be used for the primary analysis before the initiation of the trial. The false positive error rate for the null hypothesis and the statistical power to detect the targeted size of treatment effect are also pre-specified. Adaptive monitoring procedures were developed in an attempt to streamline drug development without compromising safety. In contrast to standard procedures, adaptive procedures attempt to allow modification of pre-specified design features in the course of a trial, on the basis of early efficacy and safety findings. For example, at an interim analysis, if the effects of the drug being tested are modest and it seems that the effect size required for statistical significance will not be achieved, some adaptive procedures have been proposed to enable changing the sample size.

The primary goal of clinical research is to obtain a timely and reliable evaluation of the benefit-to-risk profile of an experimental intervention in order to provide benefit to public health (100). With that as a guiding precept, one might post the following objections to the use of these adaptive designs:

- One must be very cautious about using approaches that propose to allow development of hypotheses in the same data set used to confirm them.
- If additional patients are enrolled as the result of modest effects seen at interim analysis, the second stage of the trial would be “artificially” down-weighted, leading to a less efficient clinical trial and making interpretability more difficult.
- Early results are often quite unreliable. Hence, use of interim efficacy and safety data to redesign the trial might lead to making very inappropriate changes.
- Interim results regarding effects on efficacy or safety measures should be available only to the Data Monitoring Committee, to preserve the integrity and credibility of the trial. Adaptive measures that allow redesign of the trial on the basis of interim efficacy or safety data might put the integrity and the credibility of the trial at risk. This breach also reduces the flexibility to use results from external sources that emerge during the trial to alter key design features.
- If the sponsor considers early results to be sufficiently informative or reliable to warrant design changes, would there be an ethical obligation to provide patients access to this information as well?
- The use of the adaptive designs overemphasizes the importance of statistical significance relative to the importance of clinical significance. It is not adequate to rely on statistics to define a clinically significant effect.
- Finally, standard monitoring procedures already provide substantial flexibility to adapt to unexpected findings, while maintaining the integrity and credibility of the trial.

**Conclusions**

At the same time that the number of pharmacologic agents with potential for benefit in PAH continues to grow, new and improved modalities for measuring outcomes in RCTs
continue to emerge. Randomized controlled trials must now be designed that will yield robust and reliable data on the safety and efficacy of these new treatment options. The identification of appropriate end points for these trials is an integral part of that process. Some measure of exercise testing, time to clinical worsening, hemodynamic markers, and quality-of-life assessment will be among those end points. However, although experts can and must make recommendations, ultimately, agreement on optimal end points for RCTs in PAH will involve collaboration between clinicians, investigators, regulatory authorities, and industry.

In the short term, new therapies will undoubtedly continue to extend our patients’ lives and improve their quality of life. Our long-term goal—to reverse remodeling and achieve a cure for this devastating disease—has not changed. We continue to improve our understanding of the disease and to find better ways of treating it, and although our long-term goal is not yet within reach, we have reason to believe that the likelihood of its achievement is substantially increased.

Author Disclosures

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Key Words: clinical trial design • PAH • end points.
Over the past 2 decades, pulmonary arterial hypertension has evolved from a uniformly fatal condition to a chronic, manageable disease in many cases, the result of unparalleled development of new therapies and advances in early diagnosis. However, none of the currently available therapies is curative, so the search for new treatment strategies continues. With a deeper understanding of the genetics and the molecular mechanisms of pulmonary vascular disorders, we are now at the threshold of entering a new therapeutic era. Our working group addressed what can be expected in the near future. The topics span the understanding of genetic variations, novel antiproliferative treatments, the role of stem cells, the right ventricle as a therapeutic target, and strategies and challenges for the translation of novel experimental findings into clinical practice. (J Am Coll Cardiol 2009;54:S108–17) © 2009 by the American College of Cardiology Foundation

Genetic Variations in Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH) is characterized by extensive narrowing of the pulmonary vascular bed, leading to a progressive increase in pulmonary vascular resistance, right ventricular (RV) afterload, and cardiac failure. Vasoconstriction, structural changes in the vessel wall (remodeling), and thrombosis contribute to the increased pulmonary vascular resistance. In advanced disease, this process involves proliferation and hyperplasia of endothelial and smooth muscle cells (SMCs), with an increase in the extracellular matrix. A variety of growth factors and their receptors, neurohormones, and cytokines can produce these morphologic changes. The levels of these mediators are determined, in part, by their respective gene expression. Variations in the genes coding for (or regulating expression/activity of) bone morphogenetic protein receptor type 2 (BMPR2), serotonin (5-HT), serotonin transporters (SERT), prostacyclin (PGI2) receptors, PGI2 synthase, voltage-dependent potassium channel (Kv) 1.5, nitric oxide (NO), endothelin (ET)-1, ET-1 receptors A and B (ET_A and ET_B), and reactive oxygen species (ROS) may be relevant in PAH. Accordingly, understanding the genetic regulation of these proteins, including the roles of genetic polymorphisms and mutations, may provide useful insight into pathogenesis, prognosis, and treatment of PAH.

Genetic polymorphisms with potential relevance to PAH. BMPR2. BMPR2 is a member of the transforming growth factor (TGF)-β family. Studies suggest that BMPR2 suppresses growth in vascular tissue (i.e., SMCs) (1,2). Isolated vascular SMCs from patients with idiopathic pulmonary arterial hypertension (IPAH) show enhanced cell proliferation (3). Several mutations in the coding sequences (13 exons) have been identified in the BMPR2 gene, including deletion/insertion, nonsense, and missense (4,5). Strong evidence has established an association between BMPR2 polymorphisms and familial PAH and IPAH (6–9). Inactivating heterozygous mutations are distributed throughout the BMPR2 gene in at least 70% of patients with a family history of PAH (i.e., familial heritable
vascular SMCs, mediates vasoconstriction and proliferation was also related to radial artery parameters (32). Many of the Smad-responsive genes encode for proteins that inhibit cell growth and induce apoptosis (17). Thus it has been proposed that BMPR2 signaling subserves a growth regulatory function in pulmonary vascular cells, inhibiting the proliferation and possibly enhancing apoptosis in SMCs. Mutations that interfere with BMPR2 signaling would enhance vascular remodeling. Genetic variations in the Smad4 gene have been identified in different forms of cancer (18–21). Two missense mutations in the Smad4 amino-terminal domain, L43S and R100T, result in proteins that are not efficiently translocated to the nucleus and, consequently, produce severely defective transcriptional responses to specific TGF ligands (22).

ET-1, ET<sub>A</sub>, and ET<sub>B</sub>. ET-1 has been implicated in the pathogenesis of multiple vascular abnormalities, including PAH (23). ET-1 is believed to act in a paracrine manner on two G-protein-coupled receptors (GPCRs), ET<sub>A</sub> and ET<sub>B</sub>, with opposite effects (24,25). ET<sub>A</sub>, which is present on vascular SMCs, mediates vasoconstriction and proliferation (26). ET<sub>B</sub> is found predominantly on endothelial cells, where it promotes vasodilation by releasing NO, PGI<sub>2</sub>, or other endothelium-dependent vasodilators (27,28).

Six polymorphisms in the ET<sub>A</sub> receptor gene and 3 in the ET<sub>B</sub> receptor gene have been identified (29), which may explain some of the differential response to drugs. Alleles at the different polymorphic sites were similarly distributed in patients with myocardial infarction (MI) and controls. A C/T substitution located in the nontranslated part of exon 8 of the ET<sub>A</sub> receptor gene was associated with pulse pressure. A G/T polymorphism (ET1 K198N) in the ET-1 gene strongly interacted with body mass index in the determination of blood pressure levels. The T allele was associated with an increase of blood pressure in overweight subjects. An insertion/deletion polymorphism in the untranslated region of exon 1 of the ET-1 gene was correlated with parameters of essential hypertension (30). Polymorphisms of the ET<sub>A</sub> receptor gene was associated with pulse pressure in overweight patients (31). The H323H (C/T) polymorphism of the ET system have also been correlated with parameters of essential hypertension (30). Polymorphisms in the ET<sub>A</sub> receptor gene polymorphism variant is associated with systemic and pulmonary hypertension (33–35) and altered vascular remodeling (36,37). Decreased expression of eNOS in the pulmonary vascular endothelium of patients with most forms of PAH suggests that sustained attenuation of pulmonary vascular NO production is associated with clinically significant alterations in pulmonary vascular tone (38). The eNOS Glu298Asp polymorphism is reported to be a strong risk factor for coronary artery disease and hypertension (39). Moreover, this Glu298Asp polymorphism is associated with reduced basal NO production (40). A new polymorphism in the promoter of the eNOS gene (–786 T/C) significantly reduces its promoter activity (41). This mutation affects coronary arterial vasoreactivity by reducing endothelial NO synthesis.

**GPCRs.** G proteins are essential partners of multiple transmembrane receptors for the activation or inhibition of intracellular signaling cascades. More than one-half of all drugs target GPCRs and either activate or inactivate them. The GPCRs consist of α, β, and γ subunits, which are intracellular signals for stimuli such as hormones and chemokines. These stimuli activate GPCR by inducing or stabilizing a new conformation in the receptor (42). Mutations in genes encoding GPCR can cause loss of function by impairing any of several steps in the normal GPCR/guanosine triphosphatase (GTPase) cycle (43). Polymorphisms in the GPCR signaling pathway have NO. NO dilates pulmonary and systemic vessels and inhibits vascular cell growth. There are 3 isoforms of the enzyme: endothelial NO synthase (eNOS), inducible NO synthase, and neuronal NO synthase, and all are expressed in the lung. Altered eNOS expression has been associated with systemic and pulmonary hypertension (33–35) and altered vascular remodeling (36,37). Decreased expression of eNOS in the pulmonary vascular endothelium of patients with most forms of PAH suggests that sustained attenuation of pulmonary vascular NO production is associated with clinically significant alterations in pulmonary vascular tone (38). The eNOS Glu298Asp polymorphism is reported to be a strong risk factor for coronary artery disease and hypertension (39). Moreover, this Glu298Asp polymorphism is associated with reduced basal NO production (40). A new polymorphism in the promoter of the eNOS gene (–786 T/C) significantly reduces its promoter activity (41). This mutation affects coronary arterial vasoreactivity by reducing endothelial NO synthesis.

**Abbreviations and Acronyms**

- **ANP** = atrial natriuretic peptide
- **BNP** = brain natriuretic peptide
- **BP** = bone morphogenetic protein
- **BMPR2** = bone morphogenetic protein receptor type 2
- **C/T** = C to T substitution
- **eNOS** = endothelial nitric oxide synthase
- **ET** = endothelin
- **ET<sub>A</sub>** = ET-1 receptor A
- **ET<sub>B</sub>** = ET-1 receptor B
- **G<sub>α</sub>** = G<sub>α</sub> subunit
- **GPCR** = G-protein–coupled receptor
- **H<sub>V</sub>** = voltage-dependent potassium channel
- **HCT** = monocrotaline
- **MI** = myocardial infarction
- **NADPH** = nicotinamide adenine dinucleotide phosphate
- **NO** = nitric oxide
- **PAH** = pulmonary arterial hypertension
- **PASMC** = pulmonary artery smooth muscle cell
- **PDGF** = platelet-derived growth factor
- **PET** = positron emission tomography
- **PGI<sub>2</sub>** = prostacyclin
- **PH** = pulmonary hypertension
- **ROS** = reactive oxygen species
- **RV** = right ventricular
- **SERT** = serotonin transporter
- **SMC** = smooth muscle cell
- **TGF** = transforming growth factor
- **VEGF** = vascular endothelial growth factor

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Future Perspectives

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been identified in the G protein α subunit (Gαs) (44) and in the G protein β3 subunit (45). The Gαs polymorphism leads to constitutively active α-subunit, and overexpression of Gαs induces hypertrophy and heart failure. Several studies suggest an association of the α-subunit of G proteins with hypertension (46). A study has demonstrated the association between a common silent polymorphism T393C in GNAS1 and hypertension. T/C substitution at position 393 in exon 5 changes mRNA folding structures (47). The T393C GNAS gene polymorphism was found to be more common in 268 white hypertensive patients than in 231 matched control subjects (41). Recently, a polymorphism in the G protein β3 subunit gene (GNB3) exchanging cytosine to thymidine (C825T) has been discovered in selected patients with essential hypertension and considered as a candidate mutation for both arterial hypertension and atherosclerosis (48). The T allele of the GNB3 polymorphism has been associated with increases in signal transduction.

**NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE (NADPH) OXIDASE SYSTEM.** The ROS play important roles as signaling molecules in vascular cells, and NADPH oxidases contribute to ROS production within the vasculature (49). Enhanced production of ROS, especially \( \cdot \text{O}_2^- \), also decreases NO bioavailability (50).

The NADPH oxidase consists of 4 subunits (p22phox, gp91phox, p47phox, and p67phox), and a substantial proportion of the ROS generated in endothelial cells appear to be intracellular (51). Enhanced vascular NADPH oxidase activity is associated with upregulation of p22phox mRNA in several models of hypertension, including the spontaneously hypertensive rat (52). Several polymorphisms for the p22phox subunit have been described and are associated with coronary artery disease (53,54). A polymorphism in the promoter of the p22phox gene has been identified (−930 A/G) and has been associated with hypertension (55,56).

**5-HT.** 5-HT is a neurotransmitter that is a potent pulmonary vasoconstrictor and smooth muscle cell mitogen (57). Pulmonary vascular lesions in PAH display markedly elevated levels of SERT, and explanted pulmonary vascular SMCs exhibit increased 5-HT uptake, implicating SERT in vascular remodeling. Recent studies have shown that cultured pulmonary artery SMCs from patients with IPAH demonstrate a greater proliferative response to 5-HT in comparison with cells from subjects without PAH (58). The pulmonary vasoconstrictor effects of 5-HT are produced via binding to receptors, and the mitogenic actions of 5-HT are transduced via the SERT pathway (59,60). An insertion/deletion polymorphism in the promoter region of the SERT gene with long (L) and short (S) forms affects SERT expression and function, with the L allele driving a 2- to 3-fold higher rate of gene transcription than the S allele (61). This polymorphism has been associated with PAH (62), as the LL variant is more frequent in patients with PAH. The L-allelic variant of the SERT gene promoter was present in homozygous form in 65% of patients but in only 27% of controls. Moreover, SMCs from the pulmonary artery of PAH patients with the LL polymorphism are highly proliferative in response to 5-HT, compared with cells from IPAH patients without the LL genotype.

**PGI\textsubscript{2}.** PGI\textsubscript{2} is produced by the action of PGI\textsubscript{2} synthase on arachidonic acid in endothelial cells. PGI\textsubscript{2} synthase activity and PGI\textsubscript{2} levels are reduced in patients with PAH, which leads to a relative deficiency of its potent vasodilatory and antiproliferative effects (63). Patients with severe PAH have an imbalance in the local production of PGI\textsubscript{2} and reduced expression of PGI\textsubscript{2} synthase (63,64). In vivo studies in mice have demonstrated that overexpression of PGI\textsubscript{2} synthase protects against hypoxia-induced pulmonary hypertension (PH) (65). Several polymorphisms for the PGI\textsubscript{2} synthase gene have been described. One polymorphism resulting in an altered PGI\textsubscript{2} synthase protein sequence (a nonsense mutation in exon 2) has been observed in a family with essential hypertension and cerebral infarction (66) and 3 missense mutations in the coding sequence (P38L, S118R, and R379S) and 1 in the promoter region of the PGI\textsubscript{2} synthase (R6) (67). The human PGI\textsubscript{2} receptor is a GPCR that plays an important role in vascular homeostasis. Two PGI\textsubscript{2} receptor polymorphisms have been identified in the coding sequence, the V25M and the R212H. Recent genetic analyses have revealed 2 polymorphisms within the coding sequence, V25M and R212H of the PGI\textsubscript{2} receptor. In in vitro experiments, the R212H variant has been associated with a significant decrease in binding affinity for PGI\textsubscript{2} and G-protein activation versus the wild-type receptor (68).

**Kv.** Membrane potential is an important regulator of intracellular free calcium concentration ([Ca\textsubscript{2+}]\textsubscript{i}) and pulmonary vascular tone. The pore-forming α-subunit, Kv1.5, in human pulmonary artery SMCs (PASMCs) plays an important role in regulating membrane potential, vascular tone, and PASMC proliferation (69,70). Inhibition of Kv1.5 expression and function has been implicated in PASMCs from patients with IPAH (71,72). Recently, several genetic variations in the Kv1.5 channel gene (KCNA5) have been identified (73). Remillard et al. (73) showed an association between allele frequency of the single-nucleotide polymorphisms no. 4 (T-937a) and 17 (G2870a) in the KCNA5 gene and NO response in patients with IPAH, suggesting that variations in KCNA5 transcriptional regulation may affect pulmonary vascular reactivity to vasodilators in patients with IPAH.

**NATRIURETIC PEPTIDES.** The natriuretic peptide family comprises 3 major members, atrial or A-type (ANP), brain or B-type (BNP), and C-type, which interact with 3 receptor subtypes, NPR-A, NPR-B, and NPR-C (74). Both ANP and BNP reduce elevated pulmonary vascular tone and attenuate hypoxia-induced PH in mice (74–76). Thus, overexpression of ANP may protect against some...
forms of experimental PH (75). Several genetic variations have been described for the ANP and the BNP genes (77,78). A significant association has been demonstrated between a GT repeat in intron 2 of the NPR-B gene with essential hypertension (79). A recent study showed an association between ANP/NPRA gene polymorphisms and left ventricular structure in human essential hypertension (77). This study showed that the ANP–C664G and the NPRA polymorphisms, both in the promoter region, have a significant effect on left ventricular MI in patients carrying the mutant alleles.

**Pharmacogenomics in PAH.** Clinicians and the lay public accept the notion that not all patients respond to drug therapy in the same fashion. Genetic polymorphisms in drug-metabolizing enzymes, transporters, receptors, and other drug targets have been linked to interindividual differences in the efficacy and toxicity of many medications. Pharmacogenomics and pharmacogenetics can lead to DNA-based tests to improve drug selection, identify optimal dosing, maximize drug efficacy, and minimize toxicity. For some drugs, there are clear implications of genetic information for drug therapy to avoid toxicity and to optimize response (80,81). In addition, understanding genetic contributors to variability in drug response provides a new tool in drug development that carries the hope of decreasing the risk for unexpected toxicities, identifying patients most likely to respond, and streamlining drug development (82). This is a relatively new area of study in PAH, and a large study investigating pharmacogenomics in PAH is now underway.

### Antiangiogenesis Strategies for PAH

**Angiogenesis in PAH.** The role of angiogenesis in PAH remains controversial (83). In support of dysregulated angiogenesis, circulating and platelet levels of vascular endothelial growth factor (VEGF) are increased in PAH and are further increased with prostanoid treatment (84,85). In support of this hypothesis, Tuder et al. (86,87) cite evidence of increased VEGF, VEGF receptor 2, endothelial cell monolocularity, loss of tumor suppressor genes in endothelial cells, and diminished endothelial cell apoptosis.

The converse hypothesis is that angiogenesis is protective in PH. This hypothesis is supported by the demonstration that inhibition of angiogenesis factors (VEGF receptor 2) promotes hypoxia-induced PH, whereas overexpression of proangiogenesis factors (VEGF, angiopoietin-1) reduces and/or reverses monocrotaline (MCT) and hypoxic PH (88,89).

Other angiogenic pathways that may play a role in PAH include the epidermal growth factor receptor (EGFR). MCT-induced PH in rats was attenuated by an EGFR inhibitor (90). Thalidomide inhibits angiogenesis through as yet undetermined pathways and has been used in some patients with polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes (POEMS syndrome) and multiple myeloma with mixed results (91,92). In rats with severe PAH, thalidomide failed to improve PH (93).

Statins decrease angiogenesis in systemic atherosclerotic vascular disease (94). In MCT, hypoxia, and VEGF receptor blockade + hypoxia models, statins inconsistently attenuate PAH (95–98). One clinical study of statins in PAH suggested improvement (99).

**Antiangiogenesis strategies.** Antiangiogenesis strategies can approach the pathway from several different angles. The VEGF is the most well-studied angiogenesis factor, and several antiangiogenesis strategies to date target either VEGF itself or its receptors. Bevacizumab (anti-VEGF antibody) is approved for the treatment of colorectal and nonsmall-cell lung cancers as an adjuvant to conventional chemotherapy. Unfortunately, bevacizumab has been associated with increased risk of vascular events, including acute hypertension and cerebrovascular and coronary events, especially in patients with established disease or risk factors for vascular disease. The mechanism of these complications is not known (100,101).

The oral multireceptor tyrosine kinase inhibitors sunitinib and sorafenib are used in the treatment of renal and gastrointestinal tumors. These agents act to inhibit the VEGF receptor and have also been associated with acute systemic hypertension and cardiac ischemia (102). Sorafenib has been evaluated in a rodent model of PAH (103). Cetuximab (monoclonal antibody that binds to the EGFR) is approved for use in head and neck and colorectal cancers. Panitumumab is another anti-EGFR antibody used in colorectal cancer. Cetuximab has been associated with fatal cardiac arrest in one patient (101).

Angiogenesis may also be a target of inhibitors of mammalian target of rapamycin, which signals through PI3K/AKT. Inhibition of mammalian target of rapamycin with rapamycin decreased hypoxia-induced angiogenesis and neointimal formation in systemic arteries (104,105). In models of PH, rapamycin has been reported to attenuate hypoxic PH and either has had no effect (when combined with a statin) or has attenuated MCT-induced PH associated with decreased pulmonary vascular resistances and inhibition of neointimal formation (98,106–108).

### Unresolved questions

1. In PAH, is angiogenesis protective, harmful, or both?
2. What angiogenic targets should be considered?
3. Is the risk of treatment-induced heart disease a reason to abandon antiangiogenesis strategies in PAH?

**Growth factor inhibitors: role of platelet-derived growth factor (PDGF) signaling in PAH.** In the MCT rat model of PH, thrombotic lesions and platelet dysfunction appear to play significant roles (109). Abnormalities in procoagulant activity and fibrinolytic function due to shear stress may generate a thrombogenic surface, with the subsequent development of thrombotic lesions. Increased plasma levels of fibrinopeptide A- and D-dimers support this hypothesis,
with more recent studies suggesting that the interactions between platelets and vessels contribute to the vascular changes in PAH (109). These perturbations may also accelerate vasoconstriction by releasing thromboxane A2, platelet-activating factor, 5-HT, PDGF, TGF-β, and VEGF.

The PDGF receptor antagonist STI571 (imatinib mesylate) reversed pulmonary vascular remodeling in 2 different animal models of PH (110). Up-regulation of the PDGF receptor β was found in both tissue from experimental models of pulmonary hypertension (108) and in human lungs from patients with PAH (110,111). In several case reports, addition of imatinib to approved PAH drugs was shown to improve pulmonary hemodynamics and functional capacity of patients with severe PAH (112–114). A recently completed phase II clinical trial evaluating the safety and efficacy of imatinib mesylate in PAH failed to meet the primary efficacy end point of improvement in exercise capacity; however, many secondary end points, including pulmonary hemodynamics, were significantly improved. Phase III randomized controlled trials with tyrosine kinase inhibitors in PAH are expected to begin soon.

**Questions for clinical research**

1. In addition to PDGF, how significant are various other growth factors, such as basic fibroblast growth factor, insulin-like growth factor 1, and epidermal growth factor (90), in PAH?
2. Angiogenesis, apoptosis, and proteolysis may all be important in the pathobiology of PAH. Is targeting increased elastase activity using elastase inhibitors (115,116) another possible strategy that warrants exploration?
3. How, if at all, do growth factor inhibitors interact with the disease-specific targeted PAH treatments currently in use?
4. Can early intervention with growth factor inhibitors arrest vascular injury, allowing restoration of endothelial function?

**Endothelial Progenitor Cells/ Stem Cells in Lung Repair**

Regeneration of lung microvasculature may be a novel and effective therapeutic strategy for restoring pulmonary hemodynamics in patients with advanced PAH. Somatic cell-based gene therapy with eNOS (117) or various angiogenic factors, including VEGF and angiopoietin-1 (88,118), can reduce MCT-induced PAH in prevention models, possibly by protecting against endothelial cell apoptosis or inducing microvascular angiogenesis. Delivery of fibroblasts transduced with eNOS significantly improved RV systolic pressure in rats with established PAH, associated with evidence of regeneration of the lung microcirculation and consistent with the now well-accepted role of eNOS and NO in angiogenesis (119–121).

Recently it has been shown that circulating bone marrow–derived endothelial progenitor cells (EPCs) play an important role in repair of endothelial injury and participate directly in postnatal vasculogenesis and angiogenesis in systemic vascular beds (122,123). The administration of EPCs after MCT-induced PAH in rats almost completely prevented the increase in RV systolic pressure seen with MCT alone (122). Delayed administration of progenitor cells after MCT-induced PAH prevented the further progression of PAH, whereas only animals receiving EPCs transduced with human eNOS exhibited significant reversal of established disease.

In contrast with these promising results, other experimental findings indicate that bone marrow–derived stem cells may contribute not only to the maintenance of pulmonary vascular homeostasis, but to the pathogenesis of PAH as well. Acute, severe PAH is a frequent complication of allogenic bone marrow stem-cell transplantation for malignant infantile osteopetrosis (124), and late-onset PAH also occurs in association with graft-versus-host disease after allogenic stem-cell transplantation (125). These conflicting observations suggest that further studies are needed to determine whether stem cells have a beneficial role in PAH, which cell types contribute to the unregulated vessel remodeling, and whether a feasible and affordable strategy for vascular lung repair can be developed.

**Molecular imaging.** Monitoring stem cells in vivo remains problematic due to limitations of conventional histologic assays and imaging modalities. These limitations may be circumvented by novel methods of molecular imaging in vivo, encompassing micro positron emission tomography (PET) analysis and the use of suitable tracers, PET reporter genes, and probes to monitor both changes in tissue perfusion and stem-cell homing and engraftment. Noninvasive imaging reporter genes are useful for many medical and biologic research applications (126,127). The PET reporter genes and probes offer potential for long-term imaging of therapeutic transgenes and cells in patients (128). Integration of molecular cell imaging into studies of PAH-directed cell therapy holds promise to facilitate further growth of the field toward a broadly clinically useful application.

**Clinical impact.** A successful cell therapy for lung repair will require the development of multiple interconnected strategies that will improve stem-cell culturing conditions and enhance the inherent technological content in Good Manufacturing Practice cell factories. This will result in the development of populations of human stem cells that will make feasible both vasculogenesis and paracrine release of trophic mediators for the treatment of patients with PAH.

**Mechanisms of RV Remodeling: Developing Therapeutic Antiremodeling Strategies**

Irrespective of the etiology of the PAH, most patients die from intractable right heart failure. Despite its profound clinical consequences, little is known about RV adaptation and failure within the context of PH. Relatively few mechanistic studies have addressed the role of the right ventricle in this disease and,
specifically, the role of the interaction of the right ventricle with the pulmonary vasculature. Moreover, there is a paucity of information about the interaction between the pulmonary vasculature and the right ventricle (RV–PA coupling). Recent data suggest that exercise limitation in PH may primarily be related to poor RV–PA coupling.

A critical aspect to the future understanding of the nature of RV function/failure is to better delineate the differences and similarities between RV and left ventricular hypertrophy and failure. An understanding of RV hypertrophy and failure signaling will allow for future therapies that will promote the growth of the adult heart (hypertrophy) to produce a stable molecular and cellular response to adverse hemodynamic and/or neurohormonal stress. Accordingly, disrupted intracellular signaling along this signaling axis leads to decompensation, maladaptive remodeling, and RV failure.

**PAH and the heart.** Although the distinctive pathologic abnormality in PAH is the degree and distribution of the pulmonary arteriopathy, the level of pulmonary artery pressure has only modest prognostic significance (129). Rather, it is the ability of the right ventricle to function under this increased load that determines both the severity of symptoms and survival (130). With this in mind, novel and practical ways to assess the presence and extent of subclinical RV failure are desperately needed before the stage of overt RV failure. Moreover, the role of pulmonary vascular stiffening and wave reflectance in increasing RV hydraulic load appears to be under-recognized and may be particularly important in other hypoxemic lung diseases.

**Pulmonary artery wave reflection as a component of RV load.** Several studies have shown that the pulsatile load is increased in chronic PH, as suggested by the increased characteristic impedance and enhanced wave reflection (131,132). This has generally been attributed to decreased pulmonary artery compliance and complex changes in reflection sites. This abnormal pulsatile load may have detrimental effects on ventricular-vascular coupling by increasing the pulsatile part of ventricular power and thus unfavorably loading the still-ejecting right ventricle. The role of pulmonary arterial input impedance has been under-recognized in the past, and there are compelling reasons why this measure should now be evaluated.

**Cardiac hypertrophy and failure.** Cardiomyocyte hypertrophy occurs in response to an increased load, such as that associated with hypertension and other forms of pressure overload, or to compensate for loss of myocardial tissue after MI. This response has been considered to be adaptive to increased load, because hypertrophy normalizes the increase in wall stress induced by mechanical overload. However, in humans increased cardiac mass is a strong independent risk factor for morbidity and mortality, and prolongation of this hypertrophic response in animals inevitably leads, on the one hand, to contractile dysfunction and heart failure through poorly understood mechanisms. On the other hand, normal postnatal growth of the heart or exercise-induced cardiac growth also occurs through hypertrophy of individual cardiac muscle cells (133). These forms of so-called “physiologic” cardiac hypertrophy are not associated with contractile dysfunction and are morphologically and molecularly distinct from stress-induced hypertrophy.

The distinctions between physiologic hypertrophy and that associated with decompensation in response to excessive hemodynamic stressors and increased neurohormonal stimulation, commonly known as “pathologic” hypertrophy, are many. On the one hand, “pathologic” hypertrophy is characterized by large increases in myocyte size and ventricular thickness that is accompanied by increases in interstitial fibrosis and the induction of the fetal cardiac gene program. “Physiologic” hypertrophy, on the other hand, is characterized by smaller increases in myocyte size and ventricular thickness, no increase in interstitial fibrosis, and no induction of the fetal cardiac gene program. In addition, “physiologic” hypertrophy is reversible, whereas “pathologic” hypertrophy in animals might not be reversible, perhaps as the result of irreversible damage to the heart, such as loss of cardiomyocytes by necrosis and apoptosis.

Almost all the pathways studied involving cardiac hypertrophy and failure have been studies in the left ventricle, with a relative paucity of information validated or confirmed in the right ventricle. This leaves few answers regarding the relative importance of many of these pathways in RV failure. A critical aspect of future study will require comparisons in human RV samples.

**Heart failure and oxidative stress.** Increased ROS generation is a major feature of the transition from hypertrophy to heart failure. In a pro-oxidative environment, the formation of peroxynitrite from superoxide and NO can occur. Peroxynitrite in turn promotes NOS3 uncoupling, such that its synthase activity is redirected from NO production to the generation of superoxide ($O_2^-$). This uncoupling of NOS3 converts the enzyme from an important prosurvival, antihypertrophic, and proangiogenic (via NO) molecule to one that promotes cardiac dysfunction and destruction, including maladaptive hypertrophy, extracellular matrix remodeling, and probably myocyte cell death, although such a direct connection has not been reported. The target for peroxynitrite modification may be the Zn-thiolate cluster of NOS3 itself or the essential cofactor tetrahydrobiopterin (BH4). It has recently been shown that NOS3 uncoupling occurs in chronic pressure overload of the left ventricle, and that oral BH4 supplementation restored NO bioavailability, suppressed NO synthase-derived ROS, and prevented both cardiac dysfunction and maladaptive matrix remodeling (134,135). This may provide a rationale for exploring a similar strategy in right heart failure due to PAH.

**Influence of current and emerging PH therapies on RV function.** With enhanced ability to investigate RV function, there is interest in evaluating the effects of current PAH therapies on RV function. Expression of RV phosphodiesterase-5 (PDE5) is increased in patients with
PAH, and inhibition of this enzyme improves inotropy in animal models. Moreover, magnetic resonance imaging studies have shown that sildenafil acutely promotes RV relaxation. Several other studies have shown improved RV systolic and diastolic function in response to acute and chronic treatment with PG1 analogs, PDE5 inhibitors, and ET receptor antagonists (136). Further studies are needed to translate these observations to clinical PAH.

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