Pulmonary Arterial Hypertension
Epidemiology, Pathobiology, Assessment, and Therapy

(Left) Unexplained PAH: intra-acinar pulmonary artery with marked concentric intimal proliferation of plump cells (Verhoeff-van Gieson-× 400). (Right) Familial PAH: plexiform lesion in an intra-acinar pulmonary artery decorated with anti-smooth muscle actin antibody (SMA). The newly formed microvessels filling the arterial lumen are lined by plump undecorated endothelial cells and by SMA positive (brown) spindle cells (× 350). Reproduced with permission © 2004 Giuseppe G. Pietra, MD

Guest Editors

Nazzareno Galiè, MD
Institute of Cardiology
University of Bologna
Bologna, Italy

Lewis J. Rubin, MD
University of California
San Diego Medical Center
La Jolla, California
Introduction: New Insights Into a Challenging Disease

A Review of the Third World Symposium on Pulmonary Arterial Hypertension

Nazzareno Galie, MD,* Lewis J. Rubin, MD†

Bologna, Italy; and La Jolla, California

The Third World Symposium on Pulmonary Arterial Hypertension (PAH) created the opportunity to evaluate multiple new findings into the causes and management of this life-threatening and devastating disease. Led by task force members, the scientific presentations allowed a collective sharing of knowledge and provided a specialized forum by which to establish new benchmarks in the pathogenesis, pathophysiology, diagnosis, treatment, and monitoring of PAH.

Although mysteries still surround the trigger factor(s) initiating the pathological changes seen in PAH, we now understand more of the mechanisms involved. A genetic mutation of bone morphogenetic protein receptor-2, part of the transforming-growth-factor-beta superfamily, appears to have some association with familial and idiopathic PAH. With much still unknown, research has allowed us to consider that genetic elements may play a fundamental role, although this could cover a broad range of factors such as endothelin (ET), prostacyclin (PGI2) synthase, nitric oxide (NO) synthase, the serotonin transporter, and voltage-gated potassium channels.

Superficially, it may appear that the disease process is driven by vasoconstriction, but it now appears that pulmonary vascular proliferation and remodeling are the primary forces of pathogenesis. Endothelial dysfunction is a key element of the manifestation of disease pathophysiology, marked by prolonged elevation of ET coupled with chronic reductions in NO and PGI2. Identification of these processes has allowed the development of specific pharmacologic targets.

Regardless of the etiology, PAH presents as an extremely serious disease state that is difficult to identify early owing to the insidious nature of early-stage symptoms. A heightened awareness of patients at risk, or of early-stage disease manifestation, is necessary to allow for diagnosis before significant pathophysiological changes. The diagnostic process is now more clearly defined, with consensus reached on an algorithm of various investigative tests and procedures to exclude other causes and to ensure that an accurate diagnosis of PAH can be reached.

Advances in medical therapies over the past decade reflect the inroads made into our understanding of PAH. Co-administered with conventional supportive therapy, new drug classes such as endothelin receptor antagonists and prostanoids are now offering viable treatment options for these patients. A focused session on medical therapy during the Third World Symposium on PAH has led to an updated treatment algorithm to guide treating clinicians on the best practice options for PAH patients.

The dilemma remains as to whom best to treat these patients, given that the patient population is spread across a broad range of disciplines, such as respiratory, cardiology, rheumatology, and immunology. The global trend is moving toward a system of designated centers for PAH care, with medical teams including all specialties working in a shared-care approach to patient management. It is no coincidence that the collaborative approach to this World Symposium reflects the cooperation required across specialty groups in the broader medical community, to ensure that best possible care is delivered to this orphan disease state. To this end, we appreciate the opportunity to disseminate the proceedings of this symposium by means of this supplement to the Journal of the American College of Cardiology, and we hope that the readership finds it informative and useful.

Reprint requests and correspondence: Dr. Lewis J. Rubin, UCSD Medical Center, 9300 Campus Point Drive, M/C 7372, La Jolla, California 92037. E-mail: ljrubin@ucsd.edu.

From the *Institute of Cardiology, University of Bologna, Bologna, Italy; and the †University of California San Diego Medical Center, La Jolla, California.

Manuscript received February 13, 2004; accepted March 2, 2004.
Primary Pulmonary Arterial Hypertension
A Look Back
Alfred P. Fishman, MD
Philadelphia, Pennsylvania

For the first half of the twentieth century, the published reports on primary pulmonary hypertension (PPH) were confined to clinical pathological correlations. In the 1950s the physiological aspects began to be reported followed by epidemiologic studies triggered by an epidemic of PPH due to the ingestion of an appetite suppressant, aminorex fumarate. The epidemic prompted a series of meetings of the World Health Organization, which led, in turn, to the creation of the U.S. Registry of Primary Pulmonary Hypertension, standardization of nomenclature, and an etiologic classification of all types of pulmonary hypertension. (J Am Coll Cardiol 2004;43:2S–4S) © 2004 by the American College of Cardiology Foundation

For more than a century before pulmonary arterial pressures could be measured directly in humans, pulmonary arterial sclerosis was widely accepted as the morphologic signature of chronic pulmonary arterial hypertension (PAH) (1,2). In 1891, Ernst von Romberg, a German physician, was puzzled by his inability to discover at autopsy the etiology of the pulmonary vascular lesions, which he designated as “pulmonary vascular sclerosis.” Light began to be shed on the corresponding clinical syndrome in 1901, when Dr. Abel Ayerza, Professor of Medicine at the University of Buenos Aires, Argentina, lectured on the syndrome of chronic cyanosis, dyspnea, and polycythemia, which was associated with sclerosis of the pulmonary artery. This syndrome was subsequently designated as Ayerza’s disease by one of his students, Dr. F.C. Arrillaga.

The first reports of the disease now known as “primary pulmonary hypertension” (PPH) were clinical-pathological correlations (1–7). These were followed by speculations concerning etiology. They began in 1913 with Dr. Arrillaga, who attributed the disease to syphilitic pulmonary endarteritis. This misconception spurred a controversy about the etiologic role of the spirochete, which lasted for two decades when the theory of a syphilitic etiology was laid to rest by Oscar Brenner, a British physician who searched the literature and the Pathology Department of the Massachusetts General Hospital for clues to etiology (8). Based on published accounts of Ayerza’s disease, coupled with his own observations, Dr. Brenner concluded that so-called Ayerza’s disease was neither a clinical nor a pathological entity. Instead, he concluded accurately that the clinical manifestations of so-called Ayerza’s disease were those of heart failure secondary to pulmonary disease, and he interpreted the pathological findings as morphologic evidence of chronic pulmonary disease, moderate pulmonary atherosclerosis, and right ventricular hypertrophy. He saw no reason for the term “Ayerza’s disease.” However, he did fail to see the connection between the pulmonary vascular disease and the right ventricular hypertrophy, picturing each as a separate entity due to “an unknown cause.” Another half-century was to go by before histopathology of the disease was clarified and the relationships between the pulmonary vascular lesions and the right ventricular hypertrophy were clearly understood (9).

Postmortem studies of the pulmonary vasculature in the 1930s could provide little insight into the functional behavior of the pulmonary resistance vessels (i.e., the small muscular pulmonary arteries and arterioles) during life. The functional aspects began to be explored in the 1940s by experiments in cats and humans, which showed that acute hypoxia (10% O₂ in N₂) elicits pulmonary vasoconstriction (10–12). In 1951, Dresdale, Michtom, and Schultz (13) took the opposite tack (i.e., to relieve pulmonary hypertension): in patients with PPH they showed that tolazoline (Priscoline), a pulmonary vasodilator, relieved pulmonary hypertension. However, the observation was not entirely convincing because tolazoline is not only a pulmonary vasodilator but also a systemic vasodilator, raising the possibility that the effect on the pulmonary circulation was secondary to systemic vasodilation.

To eliminate this uncertainty, Harris et al. resorted to the intravenous injection of acetylcholine, which is destroyed during a single passage through the lungs (14,15). They found that acetylcholine was virtually without effect on the pulmonary circulation during normoxia (i.e., while breathing ambient air) but elicited pulmonary vasodilation if pulmonary vascular tone was previously increased by exposing the subject to a hypoxic-inspired air mixture. Soon thereafter, Wood et al. (16) showed that the intravenous injection of acetylcholine also elicited pulmonary vasodilation in patients with pulmonary hypertension secondary to mitral stenosis.

Such was the state of morphological and physiological knowledge relating to PPH when the epidemic of aminorex-induced pulmonary hypertension broke out in the late 1960s (17). Aminorex fumarate (2-amino-5-phenyl-2-oxazoline) is a catechol derivative, which was sold as an...
over-the-counter appetite suppressant to promote weight loss. Its actions include release of norepinephrine at nerve endings and an increase in levels of serotonin in the circulation. The drug was introduced on the Swiss, German, and Austrian markets in November 1965 and was withdrawn in October 1968 because it was held responsible for an epidemic of pulmonary hypertension. In patients who died from aminorex pulmonary hypertension, the pulmonary vascular lesions were identical with those of PPH. The aminorex outbreak presented two major questions about the pathogenesis of PPH. The first, stemming from the fact that only a few individuals who took the drug developed pulmonary hypertension, raised the possibility that genetic predisposition played a role in the pathogenesis of the disease (18). The second question was concerned with uncertainties about initiating and pathogenetic mechanisms.

THE FIRST WORLD HEALTH ORGANIZATION (WHO) MEETING, GENEVA, 1973

Prompted by the outbreak of aminorex-induced pulmonary hypertension, the WHO convened a group of experts in 1973 to assess the state of knowledge about PPH and to standardize clinical and pathological nomenclature (19). In addition to accomplishing these goals, the 1973 meeting called for an international registry that would gather standardized data about the rare disease.

THE NATIONAL REGISTRY, 1981

The international registry did not materialize. However, in 1981, the National Heart, Lung and Blood Institute of the National Institutes of Health created a National Registry of Patients with PPH (20). The Registry consisted of three components: a statistical-epidemiological core, a pathology core, and 32 clinical centers. By the time the Registry closed in 1987, it had accomplished more than anticipated with respect to the elucidation of the clinical, pathophysiologic, and morphologic features of the disease (21). Moreover, the participants, gratified by the success of this collaborative effort, subsequently embarked upon other clinical trials.

THE SECOND WHO MEETING, EVIAN, FRANCE, 1998

The second WHO meeting, on the 25th anniversary of the original meeting in Geneva, went beyond the confines of PPH. Instead, it undertook to compile a classification of all pulmonary hypertensive diseases. The detailed classification appears in Table 1. Here it will suffice to indicate that it sorts all types of pulmonary hypertensive diseases into five categories: 1) PAH 2) pulmonary venous hypertension, 3) pulmonary arterial hypertension, 4) pulmonary veno-occlusive disease, and 5) PAH caused by disorders of the pulmonary vasculature (22–27).

PAH associated with disorders of the respiratory system or hypoxemia, 4) PAH caused by chronic thrombotic or embolic disease, and 5) PAH caused by disorders of the pulmonary vasculature (22–27).

THE THIRD WORLD SYMPOSIUM ON PULMONARY ARTERIAL HYPERTENSION, VENICE, 2003

The third World Symposium on PAH was prompted by a remarkable surge in the understanding of the mechanisms involved in the pathogenesis of pulmonary hypertension that occurred during the few years after the 1998 session. These advances covered a broad span, ranging from molecular biology, developmental biology, and genetics on the one

<table>
<thead>
<tr>
<th>Table 1. The Evian Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Pulmonary arterial hypertension</td>
</tr>
<tr>
<td>1.1 Primary pulmonary hypertension</td>
</tr>
<tr>
<td>(a) Sporadic</td>
</tr>
<tr>
<td>(b) Familial</td>
</tr>
<tr>
<td>1.2 Related to</td>
</tr>
<tr>
<td>(a) Collagen vascular disease</td>
</tr>
<tr>
<td>(b) Congenital systemic-to-pulmonary shunts</td>
</tr>
<tr>
<td>(c) Portal hypertension</td>
</tr>
<tr>
<td>(d) Human immunodeficiency virus infection</td>
</tr>
<tr>
<td>(e) Drugs/toxins</td>
</tr>
<tr>
<td>(1) Anorexigens</td>
</tr>
<tr>
<td>(2) Other</td>
</tr>
<tr>
<td>(f) Persistent pulmonary hypertension of the newborn</td>
</tr>
<tr>
<td>(g) Other</td>
</tr>
<tr>
<td>2. Pulmonary venous hypertension</td>
</tr>
<tr>
<td>2.1 Left-sided atrial or ventricular heart disease</td>
</tr>
<tr>
<td>2.2 Left-sided valvular heart disease</td>
</tr>
<tr>
<td>2.3 Extrinsic compression of central pulmonary veins</td>
</tr>
<tr>
<td>(a) Fibrosing mediastinitis</td>
</tr>
<tr>
<td>(b) Adenopathy/tumors</td>
</tr>
<tr>
<td>2.4 Pulmonary veno-occlusive disease</td>
</tr>
<tr>
<td>2.5 Other</td>
</tr>
<tr>
<td>3. Pulmonary hypertension associated with disorders of the respiratory system or hypoxemia</td>
</tr>
<tr>
<td>3.1 Chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td>3.2 Interstitial lung disease</td>
</tr>
<tr>
<td>3.3 Sleep-disordered breathing</td>
</tr>
<tr>
<td>3.4 Alveolar hypoventilation disorders</td>
</tr>
<tr>
<td>3.5 Chronic exposure to high altitude</td>
</tr>
<tr>
<td>3.6 Neonatal lung disease</td>
</tr>
<tr>
<td>3.7 Alveolar-capillary dysplasia</td>
</tr>
<tr>
<td>3.8 Other</td>
</tr>
<tr>
<td>4. Pulmonary hypertension caused by chronic thrombotic or embolic disease</td>
</tr>
<tr>
<td>4.1 Thromboembolic obstruction of proximal pulmonary arteries</td>
</tr>
<tr>
<td>4.2 Obstruction of distal pulmonary arteries</td>
</tr>
<tr>
<td>(a) Pulmonary embolism (thrombus, tumor, ova or parasites, foreign material)</td>
</tr>
<tr>
<td>(b) In situ thrombosis</td>
</tr>
<tr>
<td>(c) Sickle cell disease</td>
</tr>
<tr>
<td>5. Pulmonary hypertension caused by disorders directly affecting the pulmonary vasculature</td>
</tr>
<tr>
<td>5.1 Inflammatory</td>
</tr>
<tr>
<td>(a) Schistosomiasis</td>
</tr>
<tr>
<td>(b) Sarcoidosis</td>
</tr>
<tr>
<td>(c) Other</td>
</tr>
<tr>
<td>5.2 Pulmonary capillary hemangiomatosis</td>
</tr>
</tbody>
</table>
hand, to clinical trials, natural history, and epidemiology on
the other.

The third World Symposium on PAH also provided an
opportunity to review the effectiveness of the Evian diag-
nostic classification. To this end, 14 experts from the U.S.
and abroad were asked to review critically the Evian diag-
nostic classification with respect to its value for clinical,
research, and epidemiologic purposes. Virtually all partici-
pants agreed that the classification was primarily useful for
clinical and epidemiologic purposes and less so for research.
This pattern of response was not unexpected as research has
become increasingly devoted to the molecular and develop-
mental aspects of the disease, whereas the classification is
focused on diagnosis and treatment.

CONCLUDING COMMENTS

Progress in the understanding of PPH has catalyzed the
classification of all pulmonary hypertensive diseases. The
etiologic basis for the classification enhances the prospects
for a unified approach to diagnosis and therapy.

Reprint requests and correspondence: Dr. Alfred P. Fishman,
Senior Associate Dean, University of Pennsylvania School of
Medicine, 423 Guardian Drive, 1320 Blockley Hall, Philadelphia,
Pennsylvania 19102. E-mail: fishmana@mail.med.upenn.edu.

REFERENCES

2. Larrabee WF, Parker RL, Edwards JE. Pathology of intrapulmo-
ner arteries and arterioles in mitral stenosis. Proc Mayo Clin
3. Mönckeberg JG. [Ueber die genuine Arteriosklerose der Lungen-
4. Arrillaga FC. [Sclérose de l’artère pulmonaire (cardiaques noirs)]. Bull
5. Brachetto-Brian D. [Concepto anatomio-pathologico de los cardiacos
7. Brill IC, Krygier CK. Primary pulmonary vascular sclerosis. Arch
8. Brenner O. Pathology of the vessels of the pulmonary circulation. Arch
9. Pietra GG. The histopathology of primary pulmonary hypertension.
In: Fishman AP, editor. The Pulmonary Circulation: Normal and
Abnormal. Mechanisms, Management and the National Registry.
10. Von Euler US, Liljestrand G. Observations on the pulmonary arterial
The influence of short periods of induced anoxia upon pulmonary
12. Fishman AP. Respiratory gases in the regulation of the pulmonary
13. Dresdale DT, Michtom RF, Schultz M. Recent studies in primary
pulmonary hypertension including pharmacodynamic observations
14. Harris P. Influence of acetylcholine on the pulmonary arterial pressure.
Br Heart J 1957;19:272–86.
15. Fritts HW, Harris P Jr., Claus RH, Odell JE, Cournand A. The effect
of acetylcholine on the human pulmonary circulation under normal
16. Wood P, Besterman EM, Towers MK, McLroy MB. The effect of
acetylcholine on pulmonary vascular resistance and left atrial pressure
17. Gurtner HP. Aminorex pulmonary hypertension. In: Fishman AP,
editor. The Pulmonary Circulation: Normal and Abnormal. Mecha-
nisms, Management and the National Registry. Philadelphia, PA: Uni-
20. Fishman AP. Induction to the National Registry on Primary Pulmo-
nary Hypertension. In: Fishman AP, editor. The Pulmonary Circula-
tion: Normal and Abnormal. Mechanisms, Management and the National
21. Pietra GG. The histopathology of primary pulmonary hypertension.
In: Fishman AP, editor. The Pulmonary Circulation: Normal and
Abnormal. Mechanisms, Management and the National Registry.
22. Wagenvoort CA. Pulmonary veno-occlusive disease. In: Fishman AP,
editor. The Pulmonary Circulation: Normal and Abnormal. Mecha-
nisms, Management and the National Registry. Philadelphia, PA: Uni-
23. Wagenvoort CA. Pathology of Pulmonary Hypertension. New York,
24. Moser KM. Pulmonary vascular obstruction due to embolism and
25. Shaw AFB, Ghareeb AA. The pathogenesis of pulmonary schisto-
mosis in Egypt with special reference to Ayerza’s disease. J Pathol Bact
1938;44:401–12.
26. Naeve RL. Advanced pulmonary vascular changes in schistosomal cor
27. Badesch DB, Tapson VF, McGoon MD, et al. Continuous intrave-
nous epoprostenol for pulmonary hypertension due to the sclero-
Clinical Classification of Pulmonary Hypertension

Gerald Simonneau, MD,* Nazzareno Galie, MD,† Lewis J. Rubin, MD,‡ David Langleben, MD,§ Werner Seeger, MD,∥ Guido Domenighetti, MD,¶ Simon Gibbs, MD,# Didier Lebrec, MD,** Rudolf Speich, MD,†† Maurice Begetti, MD,‡‡ Stuart Rich, MD,§§ Alfred Fishman, MD||

*Institute of Cardiology, University of Bologna, Bologna, Italy; †Institute of Cardiology, University of Bologna, Bologna, Italy; ‡Division of Pulmonary and Critical Care Medicine, University of California, San Diego, California; §Department of Medicine, Sir Mortimer B. Davis Jewish General Hospital, McGill University, Montreal, Canada; ¶Department of Internal Medicine II, Justus-Liebig-University, Giessen, Germany; ‡Department of Intensive Care and Pneumology, Regional Hospital of Locarno, Locarno, Switzerland; #National Heart and Lung Institute, Imperial College of Science, Technology and Medicine, London, United Kingdom; ∥Department of Hepatology, INSERM U481, Beaumoin Hospital, Clichy, France; ††Department of Internal Medicine, University Hospital of Zurich, Zurich, Switzerland; ¶¶Pediaic Cardiology Unit, Children's University Hospital of Geneva, Geneva, Switzerland; §§Center for Pulmonary Heart Disease, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois; ||University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania.

In 1998, during the Second World Symposium on Pulmonary Hypertension (PH) held in Evian, France, a clinical classification of PH was proposed. The aim of the Evian classification was to individualize different categories sharing similarities in pathophysiological mechanisms, clinical presentation, and therapeutic options. The Evian classification is now well accepted and widely used in clinical practice, especially in specialized centers. In addition, this classification has been used by the U.S. Food and Drug Administration and the European Agency for Drug Evaluation for the labeling of newly approved medications in PH. In 2003, during the Third World Symposium on Pulmonary Arterial Hypertension held in Venice, Italy, it was decided to maintain the general architecture and philosophy of the Evian classification. However, some modifications have been proposed, mainly to abandon the term "primary pulmonary hypertension" and to replace it with "idiopathic pulmonary hypertension"; to reclassify pulmonary veno-occlusive disease and pulmonary capillary hemangiomatosis; to update risk factors and associated conditions for pulmonary arterial hypertension and to propose guidelines in order to improve the classification of congenital systemic-to-pulmonary shunts. (J Am Coll Cardiol 2004;43:5S–12S) © 2004 by the American College of Cardiology Foundation

Pulmonary hypertension (PH) was previously classified into two categories: primary pulmonary hypertension (PPH) or secondary pulmonary hypertension, depending on the absence or the presence of identifiable causes or risk factors. The diagnosis of PPH was one of exclusion after ruling out all causes of PH (1,2).

In 1998, during the Second World Symposium on Pulmonary Hypertension held in Evian, France, a clinical classification of PH was proposed (3–5). The aim of the "Evian classification" was to individualize different categories sharing similarities in pathophysiological mechanisms, clinical presentation, and therapeutic options. Such a clinical classification is essential in communicating about individual patients, in standardizing diagnosis and treatment, in conducting trials with homogeneous groups of patients, and in analyzing novel pathobiological abnormalities in well-characterized patient populations. Obviously, a clinical classification does not preclude other classifications such as a pathological classification based on histological findings, or a functional classification based on the severity of symp-

From the †Department of Pulmonary and Critical Medicine, University of Paris Sud, Paris, France; ‡Institute of Cardiology, University of Bologna, Bologna, Italy; §Division of Pulmonary and Critical Care Medicine, University of California, San Diego, California; ‡Department of Medicine, Sir Mortimer B. Davis Jewish General Hospital, McGill University, Montreal, Canada; ¶Department of Internal Medicine II, Justus-Liebig-University, Giessen, Germany; ‡Department of Intensive Care and Pneumology, Regional Hospital of Locarno, Locarno, Switzerland; #National Heart and Lung Institute, Imperial College of Science, Technology and Medicine, London, United Kingdom; ∥Department of Hepatology, INSERM U481, Beaumoin Hospital, Clichy, France; ††Department of Internal Medicine, University Hospital of Zurich, Zurich, Switzerland; ¶¶Pediaic Cardiology Unit, Children's University Hospital of Geneva, Geneva, Switzerland; §§Center for Pulmonary Heart Disease, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois; ||University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania.

Manuscript received January 28, 2004; revised manuscript received February 13, 2004, accepted February 23, 2004.

toms. The 2003 Third World Symposium on Pulmonary Arterial Hypertension (PAH) held in Venice, Italy, provided the opportunity to assess the impact and the usefulness of the Evian classification and to propose some modifications.

EVIAN CLASSIFICATION

The Evian classification (3,4) consisted of five categories (Table 1) in which PH diseases were grouped according to specific therapeutic interventions directed at dealing with the cause of: 1) PAH, 2) pulmonary venous hypertension, 3) PH associated with disorders of the respiratory system or hypoxemia, 4) PH caused by thrombotic or embolic diseases, and 5) PH caused by diseases affecting the pulmonary vasculature. Within each category are subsets that reflect diverse causes and sites of injury.

Pulmonary arterial hypertension. The first category, termed PAH, included a first subgroup without identifiable cause, or so-called PPH. It incorporated both the familial and sporadic forms of the disease. The second subgroup included a number of conditions or diseases of known causes that have in common the localization of lesions to the small pulmonary muscular arterioles. Among these are drug-related PH, porto-pulmonary hypertension, HIV-related PH, collagen vascular diseases, congenital systemic-to-pulmonary shunts, and persistent PH of the newborn.

Although the mechanisms responsible for remodeling of pulmonary arterioles in these conditions are unknown, they share similar morphological findings, clinical presentation, and clinical responsiveness to treatment with the continuous infusion of epoprostenol (particularly PPH and PAH associated with the scleroderma spectrum of diseases) (6,7).
Pulmonary venous hypertension. This category consisted predominantly of left-sided valvular or myocardial diseases requiring therapies directed at improving myocardial performance or relieving valvular mechanical defects rather than pulmonary vasodilator therapy. Indeed, epoprostenol therapy in patients with pulmonary venous hypertension can be harmful (8). This category also included extrinsic compression of the pulmonary veins (9) and pulmonary veno-occlusive disease (PVOD), which clinically mimics PPH (10).

PH associated with disorders of the respiratory system or hypoxemia. Within this category, the predominant cause is inadequate oxygenation of arterial blood as a result of either lung disease, impaired control of breathing, or residence at high altitude. In this category, the increase in mean pulmonary artery pressure is generally modest (<35 mm Hg) (11). As a rule, survival depends on the severity of the pulmonary disease rather than on pulmonary hemodynamics. Long-term oxygen therapy (16 or 24 h/day) improves survival in patients with chronic obstructive lung disease (12,13). In native residents who develop PH at high altitude, relocation to sea level rapidly improves PH and its associated symptoms.

PH caused by thrombotic or embolic diseases. This category included either chronic thromboembolic PH due to proximal organized clot in major pulmonary arteries, which can benefit from pulmonary endarterectomy (14,15), or more peripheral emboli or thrombi that are indistinguishable from thrombotic lesions observed in PPH and can be treated with chronic pulmonary vasodilator therapy (16). In all cases, life-long anticoagulation is indicated.

PH caused by diseases affecting the pulmonary vasculature. This category involved PH stemming from inflammatory processes or mechanical obstruction (e.g., schistosomiasis, sarcoidosis). Pulmonary capillary hemangiomatosis (17) was also included in this group, although it usually presents clinically, as with PVOD (18).

**ASSESSMENT OF THE EVIAN CLASSIFICATION**

The 2003 World Symposium on PH provided the opportunity to evaluate the impact and usefulness of the Evian classification and to propose modifications. A questionnaire was sent to all the experts (n = 56) who attended the Venice meeting. The first question was: “Do you think the Evian classification is now well accepted and widely used in clinical practice in place of the previous classification?” Among respondents (n = 30), a total of 88% considered the Evian classification to be well accepted and widely used in clinical practice, especially in centers with the largest clinical experience. In contrast, nonexpert physicians apparently still use the old classification (primary vs. secondary).

The second question was: “Do you think the Evian classification is useful for drug evaluation and registration, clinical practice, basic science?” Respectively, 88%, 96%, and 66% of experts considered the Evian classification useful for

---

**Table 1. The Evian Clinical Classification**

<table>
<thead>
<tr>
<th>1. Pulmonary arterial hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 Primary pulmonary hypertension</td>
</tr>
<tr>
<td>(a) Sporadic</td>
</tr>
<tr>
<td>(b) Familial</td>
</tr>
<tr>
<td>1.2 Related to</td>
</tr>
<tr>
<td>(a) Collagen vascular disease</td>
</tr>
<tr>
<td>(b) Congenital systemic-to-pulmonary shunts</td>
</tr>
<tr>
<td>(c) Portal hypertension</td>
</tr>
<tr>
<td>(d) Human immunodeficiency virus infection</td>
</tr>
<tr>
<td>(e) Drugs/toxins</td>
</tr>
<tr>
<td>(f) Other</td>
</tr>
<tr>
<td>(g) Other</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2. Pulmonary venous hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1 Left-sided atrial or ventricular heart disease</td>
</tr>
<tr>
<td>2.2 Left-sided valvular heart disease</td>
</tr>
<tr>
<td>2.3 Extrinsic compression of central pulmonary veins</td>
</tr>
<tr>
<td>(a) Fibrosing mediastinitis</td>
</tr>
<tr>
<td>(b) Adenopathy/tumors</td>
</tr>
<tr>
<td>2.4 Pulmonary veno-occlusive disease</td>
</tr>
<tr>
<td>2.5 Other</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3. Pulmonary hypertension associated with disorders of the respiratory system or hypoxemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1 Chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td>3.2 Interstitial lung disease</td>
</tr>
<tr>
<td>3.3 Sleep-disordered breathing</td>
</tr>
<tr>
<td>3.4 Alveolar hypoventilation disorders</td>
</tr>
<tr>
<td>3.5 Chronic exposure to high altitude</td>
</tr>
<tr>
<td>3.6 Neonatal lung disease</td>
</tr>
<tr>
<td>3.7 Alveolar-capillary dysplasia</td>
</tr>
<tr>
<td>3.8 Other</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>4. Pulmonary hypertension caused by chronic thrombotic or embolic disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1 Thromboembolic obstruction of proximal pulmonary arteries</td>
</tr>
<tr>
<td>4.2 Obstruction of distal pulmonary arteries</td>
</tr>
<tr>
<td>(a) Pulmonary embolism (thrombus, tumor, ova, or parasites, foreign material)</td>
</tr>
<tr>
<td>(b) In situ thrombosis</td>
</tr>
<tr>
<td>(c) Sickle-cell disease</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>5. Pulmonary hypertension caused by disorders directly affecting the pulmonary vasculature</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1 Inflammatory</td>
</tr>
<tr>
<td>(a) Schistosomiasis</td>
</tr>
<tr>
<td>(b) Sarcoidosis</td>
</tr>
<tr>
<td>(c) Other</td>
</tr>
</tbody>
</table>

| 5.2 Pulmonary capillary hemangiomatosis                                                  |
drug evaluation and registration, for clinical practice, and for basic science.

Lastly and probably the best evidence of the impact of the Evian classification is that both the U.S. Food and Drug Administration and the European Agency for Drug Evaluation have recently used this clinical classification for the labeling of newly approved drugs: bosantan (19,20), treprostinil (21), and iloprost (22).

Considering the globally favorable opinion of the Evian classification, the task force on epidemiology and classification decided to maintain the general architecture and philosophy of this clinical classification. However, to improve and to update the Evian classification according to the recent advances in our understanding of PH, it was proposed that some important issues be addressed, including: 1) the need to include a genetic classification, 2) discontinuing use of the term “primary pulmonary hypertension,” 3) the reclassification of PVOD and pulmonary capillary hemangiomatosis (PCH), 4) the update on new risk factors for PAH, and 5) reassessment of the classification of congenital systemic-to-pulmonary shunts.

DO WE NEED A GENETIC CLASSIFICATION OF PH?

In light of the recent advances in our understanding of the genetic basis of PPH, it has been suggested that a genetic classification of PH be considered. Before addressing this question further it may be worthwhile to outline briefly what is known and unknown regarding the genetics of severe PH. Mutations in the gene encoding the bone morphogenetic protein receptor type II (BMPR2), localized to chromosome 2q33, have been suggested to underlie approximately 50% of cases of familial PPH (23). Although many of the other 50% of families show some evidence of linkage to the BMPR2 locus, specific mutations have not been identified in the coding region, or the promoter region (R. Trembath, personal communication, June 2003). Moreover, mutations in BMPR2 have been identified in up to 26% of sporadic cases of PPH (24). Although some of these cases may arise de novo by mutation, the majority represent familial transmission of mutant BMPR2, with low penetrance of the gene for the disease (25). However, the frequency of mutation has not yet been reproduced in larger studies, and so far fewer than 70 BMPR2 mutations have been reported. In addition, there is some evidence for a second locus mapping to 2q31, although this locus has been mapped using a phenotype that includes an abnormal pulmonary vascular response to exercise, rather than manifest PPH.

So far, mutations in BMPR2 gene seem to be quite specific for so-called PPH; however, mutations in BMPR2 have also been identified in rare cases of PAH associated with appetite-suppressant drugs (26) and one patient with PVOD (27). Thus far, a search for BMPR2 mutations in other forms of PH has been negative (28).

Genetic studies have demonstrated that mutations in BMPR2 are not sufficient per se to cause clinical disease. Hence, the chance of a disease gene carrier developing clinical PPH is as low as 20%. This observation highlights the critical role of other genetic/environmental factors in conferring susceptibility to PH (29).

In summary, because our knowledge of the role of genes in various forms of PH remains at an early stage it is probably premature to recommend a classification of PH based on genetic defects. Further studies are needed to identify other genes, modifiers, and regulatory genes of PH and to determine whether PAH patients with BMPR2 mutations differ from PAH patients without identified mutations with respect to response to treatment, age of onset, severity, and natural course of the disease.

TO ABANDON THE TERM “PRIMARY PULMONARY HYPERTENSION”

Primary pulmonary hypertension means unexplained or idiopathic PH.

Initially described by Romberg (30) as “sclerosis of pulmonary arteries” more than a century ago this disease has been the subject of great interest and has successively undergone several name changes. The term “primary pulmonary hypertension” was coined by Dresdale et al. (31) more than 50 years ago, to characterize a condition in which hypertensive vasculopathy existed exclusively in the pulmonary vasculature without a demonstrable cause.

In the last 20 years, it has become recognized that several conditions or diseases, including the intake of appetite-suppressant medications, connective tissue disease, portal hypertension, or HIV infection, may be associated with pulmonary vascular disease, and that they share similar pathologic and clinical features with PH. These conditions were commonly grouped as “secondary pulmonary hypertension” in contrast with primary forms. As a result, the term “secondary pulmonary hypertension” comprised very heterogeneous forms of diseases including other intrinsic pulmonary vascular diseases that resemble PPH as well as disorders that either affect the pulmonary venous circulation or conditions that affect the pulmonary circulation by altering respiratory structure or function.

Thus, the term “secondary pulmonary hypertension” in the Evian classification was abandoned because it was found confusing and without value for diagnosis and treatment. In contrast, the term “primary pulmonary hypertension” was retained because of its common use and familiarity, and because it was emblematic of 50 years of intense scientific and clinical research. However, the main problem with the term “primary” is that it requires use of the modifier “secondary” to distinguish this condition from others. Thus, during the Venice meeting, it was proposed to abandon “primary pulmonary hypertension” and to replace it with “idiopathic pulmonary arterial hypertension.” The first category in the modified Evian classification termed “pulmonary arterial hypertension” now consist of three main
TO RECLASSIFY PVOD AND PCH

Both PVOD and PCH are uncommon conditions, but they are increasingly recognized as causes for PH. In the Evian classification, these two entities were included in separate groups, both distinct from the PAH category: PVOD was included in the pulmonary venous hypertension category, which consists predominantly of left-sided valvular or myocardial diseases; PCH was included in the last and heterogeneous group of PH caused by diseases directly affecting the pulmonary vasculature.

As discussed in the pathology report by Pietra et al. (32) in this supplement, PVOD and PCH are similar in some respects, particularly in relation to the changes in the pulmonary parenchyma (i.e., pulmonary hemosiderosis, interstitial edema, and lymphatic dilation) and to pulmonary arterial intimal fibrosis and medial hypertrophy (18, 33, 34). Similarities in the pathological features and clinical presentation, along with the possible occurrence of pulmonary edema during epoprostenol therapy (35, 36), suggest that these disorders may overlap. Accordingly, it seems logical to include PVOD and PCH within the same group, most appropriately within the category of PAH. Indeed, PVOD and PCH, as well as PAH, show similar histological changes in the small pulmonary arteries, including intimal fibrosis, medial hypertrophy, and plexiform lesions. Moreover, the clinical presentation of PVOD and PCH is generally similar to that of PPH.

Finally, the risk factors or conditions associated with PAH and PVOD/PCH are similar and include the scleroderma spectrum of the disease (37), HIV infection (38, 39), and the use of anorexigenes (F. Capron, personal communication, June 2003). Of particular interest are reports of a familial occurrence in both PVOD (40) and PCH (41) as well as in PAH. Lastly, BMPR2 mutation, the gene associated with familial and IPAH, has been documented in a patient with PVOD (27). These findings suggest that PVOD, PCH, and PAH may represent components of a spectrum of a single disease. Thus, in the new classification, the PAH category comprises another subgroup termed “PAH associated with significant venous or capillary involvement.” This subgroup probably requires similar management to the other PAH subgroups. However, the prognosis seems worse, with a more rapid downhill course. In addition, vasodilators and especially epoprostenol have to be used with great caution because of the high risk of pulmonary edema. As a result, as soon as recognized, these patients should be placed on the list for lung transplantation.

UPDATED RISK FACTORS AND ASSOCIATED CONDITIONS FOR PULMONARY ARTERIAL HYPERTENSION

A risk factor for PAH is 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). The term “associated conditions” is used when it is not possible to determine whether a predisposing factor was present before PH onset. Because the absolute risk of known risk factors for PAH is generally low, individual susceptibility or genetic predisposition is likely to play an important role. During the Evian meeting, different risk factors and associated conditions were categorized according to the strength of their association with PH and their probable causal role. “Definite” indicates an association based on several concordant observations including a major controlled study or an unequivocal epidemic. “Very likely” indicates several concordant observations (including large case series and studies) that are not attributable to identified bases. “Possible” indicates an association based on case series, registries, or expert opinions. “Unlikely” indicates risk factors that were suspected but for which controlled studies failed to demonstrate any association. According to the strength of the evidence, Table 2 summarizes, risk factors and associated conditions that were identified during the Evian meeting.

RECENT EPIDEMIOLOGIC STUDIES

Ever since the Evian meeting, two prospective epidemiologic studies have been performed in the United States.

The SNAP (Surveillance of North American Pulmonary Hypertension) study was a voluntary collaborative survey conducted on 559 patients with PH over a 14-month period (42). This study confirmed the causal role of fenfluramine derivatives in the development of PAH. It showed a clear association between the use of fenfluramine and the diagnosis of PPH but not secondary PH. The adjusted odds ratio (OR) for the use of fenfluramine for more than six months was 7.5. Another interesting observation in the SNAP study was the unexpectedly high reported rate of anorexigen use in secondary PH (11.4%). This finding suggested that the use of anorexigenes increased the likelihood of developing PH in patients with other conditions that cause secondary PH.

The Sophia (Surveillance Of Pulmonary Hypertension In America) study enrolled 13 tertiary-care PH centers within the U.S. and included 1,335 patients with newly diagnosed PH between January 1998 and June 2001 (43). This study demonstrated that the use of fenfluramine during the past five years was preferentially associated with PPH rather than chronic thromboembolic PH (OR, 2.7; 95% confidence interval [CI]: 1.5 to 4.8). Interestingly, this study also showed an unanticipated association between PPH and...


Table 2. Risk Factors and Associated Conditions for PAH Identified During the Evian Meeting (1998) and Classified According to the Strength of Evidence

<table>
<thead>
<tr>
<th>A. Drugs and Toxins</th>
<th>B. Demographic and Medical Conditions</th>
<th>C. Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Definite</td>
<td>1. Gender</td>
<td>1. Definite</td>
</tr>
<tr>
<td>● Aminorex</td>
<td>2. Possible</td>
<td>● HIV infection</td>
</tr>
<tr>
<td>● Fenfluramine</td>
<td>● Pregnancy</td>
<td>2. Very likely</td>
</tr>
<tr>
<td>● Dexfenfluramine</td>
<td>● Systemic hypertension</td>
<td>● Portal hypertension/liver disease</td>
</tr>
<tr>
<td>● Toxic rapeseed oil</td>
<td>3. Unlikely</td>
<td>● Collagen vascular diseases</td>
</tr>
<tr>
<td>2. Very likely</td>
<td>4. Unlikely</td>
<td>● Congenital systemic-pulmonary-cardiac shunts</td>
</tr>
<tr>
<td>● Amphetamines</td>
<td></td>
<td>3. Possible</td>
</tr>
<tr>
<td>● L-tryptophan</td>
<td></td>
<td>● Thyroid disorders</td>
</tr>
<tr>
<td>3. Possible</td>
<td></td>
<td></td>
</tr>
<tr>
<td>● Meta-amphetamines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>● Cocaine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>● Chemotherapeutic agents</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

both “St. John’s wort” and over-the-counter antiobesity agents that contain phenylpropanolamine.

CASE SERIES AND CASE REPORTS

Ever since the Evian meeting, several case series or case reports have been published that provide some evidence of novel “possible” risk factors for PAH.

Hematologic conditions. Recently, a high prevalence (11.5%) of asplenia secondary to surgical splenectomy has been reported in a series of 61 patients with unexplained PAH, suggesting that patients with splenectomy may be at increased risk for developing PAH (44). At the time of diagnosis, PAH was generally severe, and the interval between splenectomy and diagnosis ranged from 4 to 32 years. Histological examination of the lungs in three patients showed pulmonary vascular changes similar to those of IPAH. However, these patients also had many thrombotic lesions in small pulmonary arteries. The underlying pathogenetic mechanisms are unclear; it was hypothesized that because of the loss of the filter function of the spleen, abnormal erythrocytes remained longer in the circulation and might have triggered platelet activation.

Certain hemoglobinopathies represent other possible risk factors for PAH. Pulmonary hypertension is a well-recognized complication of sickle-cell disease. It is a severe complication that significantly reduces the survival rate of these patients as compared with those without PH. It represents the cause of death in 3% of patients with sickle-cell disease. Classically, in situ thrombosis of elastic and small pulmonary arteries was considered to be the predominant finding at autopsy. Recently, a clinical-pathologic study of 20 patients reported pulmonary vascular abnormalities consistent with those of PAH, including plexiform lesions, in 60% of patients (45). Increased shear stress from deformed erythrocytes passing through the pulmonary microvasculature has been proposed as the underlying mechanism of vascular injury. In addition, the bioavailability of nitric oxide is reported to be decreased in these patients (46,47).

Other hemoglobin abnormalities may be associated with PAH, especially beta-thalassemia (48). In some patients, histologic examination at postmortem has found the lesions of IPAH and/or thrombotic pulmonary arteriopathy. The mechanism of PAH in patients with hemoglobinopathy is unclear, but a possible role has been suggested for liver disease, splenectomy, and thrombosis.

The possible association of PAH with chronic myeloproliferative disorders has been reported by several case reports (49,50) and in one cohort of six patients (51). A recent report from the Mayo Clinic dealt with 26 patients seen in that institution between 1987 and 2000 (52). The chronic myeloproliferative disorders included polycythemia vera, essential thrombocytosis, and myelofibrosis with myeloid metaplasia accompanying chronic myeloid leukemia or the myelodysplastic syndrome. In all patients, PH was moderate or severe at diagnosis. In these patients, the main causes of PH, particularly chronic thromboembolism, were excluded on clinical grounds and ventilation-perfusion lung scan. Unfortunately, autopsies were not performed. The etiology of PAH in these patients is probably multifactorial, including splenectomy, portal hypertension, chemotherapy-induced PVOD, and infiltration of the pulmonary parenchyma by hematopoietic cells and extramedullary hemopoiesis.

Rare genetic or metabolic diseases. Unexplained PAH has been reported in patients with certain rare genetic or metabolic diseases. These observations suggest new pathobiologic mechanisms for the pulmonary hypertension (e.g., an alternative role for a known mutated gene, genetic defects in chromosomal regions adjacent to a mutated gene, or a consequence of a new metabolic pathway).

Pulmonary arterial hypertension has been associated with type Ia glycogen storage disease (Von Gierke disease) in fewer than 10 patients since the initial description (53). It is a rare autosomal recessive disorder caused by a deficiency of glucose-6-phosphatase (54). Pulmonary histology is typical of PAH, and the clinical course is that of rapidly developing right heart failure. It has been suggested that in these patients PAH could
be due to an abnormal production of serotonin (55); in some patients, a surgical porto-caval shunt might represent an additional risk factor. The gene responsible for type 1a glycogen storage disease has been cloned on the long arm of chromosome 17 in position 17q21. Further studies should be performed to investigate a possible gene linked to PH in the same chromosomal region.

Gaucher disease is another rare autosomal recessive disorder characterized by a deficiency of lysosomal beta-glycosidase, which results in the accumulation of glucocerebroside in reticuloendothelial cells. The typical manifestations of this lipid storage disorder include hepatosplenomegaly and bone marrow infiltration with dysfunctional monocytes. Several cases of unexplained PAH have been reported in association with Gaucher disease (56). In these patients, liver disease, splenectomy, capillary plugging by Gaucher cells, and enzyme replacement therapy could play a role in the development of PH. Interestingly, a polymorphism in exon 13 of BMPR2 has been found in a patient with Gaucher disease and unexplained PAH (57).

Hereditary hemorrhagic telangiectasia (Osler-Weber-Rendu disease) is a rare autosomal-dominant disorder characterized by the presence of multiple arteriovenous malformations particularly in the pulmonary hepatic and cerebral circulations. Mutations in two genes encoding transforming growth factor-beta (TGF-β) receptor superfamily, namely endoglin and activin-receptor-like kinase-1 (ALK1), which are located on chromosomes 9 and 12, respectively, underlie this disorder. Recently, individual cases (58,59) and one case series of 10 patients (60) with hereditary hemorrhagic telangiectasia associated with PH were reported. These patients were clinically and histologically indistinguishable from PPH. In these patients, mutations in ALK1 (60), or more rarely in endoglin (61), were identified, suggesting that these mutations can give rise to diverse effects, including the vascular dilation characteristic of hereditary hemorrhagic telangiectasia and the occlusion of small pulmonary arteries typical of PPH.

**CLASSIFICATION OF CONGENITAL SYSTEMIC-TO-PULMONARY SHUNTS**

In 1897, Viktor Eisenmenger first described a patient with ventricular septal defect and severe pulmonary vascular disease (62). The term “Eisenmenger syndrome” was coined by Paul Wood, and it is now commonly used to include all systemic-to-pulmonary arterial shunts leading to PH and resulting in a right-to-left or bidirectional shunt (63).

Pulmonary vascular histopathologic changes that accompany congenital heart disease are usually indistinguishable from those of IPAH; the lesions include medial hypertrophy, intimal proliferation fibrosis, and, in more severe PH, plexiform lesions and necrotizing arteritis (64). The pulmonary vascular involvement from congenital heart disease usually follows a period in which pulmonary resistance is low and pulmonary blood flow is high. In these patients, it

| Table 3. Revised Clinical Classification of Pulmonary Hypertension (Venice 2003) |
|---------------------------------|---------------------------------|
| 1. Pulmonary arterial hypertension (PAH) |
| 1.1. Idiopathic (IPAH) |
| 1.2. Familial (FPAH) |
| 1.3. Associated with (APAH): |
| 1.3.1. Collagen vascular disease |
| 1.3.2. Congenital systemic-to-pulmonary shunts** |
| 1.3.3. Portal hypertension |
| 1.3.4. HIV infection |
| 1.3.5. Drugs and toxins |
| 1.3.6. Other (thyroid disorders, glycogen storage disease, Gaucher disease, hereditary hemorrhagic telangiectasia, hemoglobinopathies, myeloproliferative disorders, splenectomy) |
| 1.4. Associated with significant venous or capillary involvement |
| 1.4.1. Pulmonary veno-occlusive disease (PVOD) |
| 1.4.2. Pulmonary capillary hemangiomatosis (PCH) |
| 1.5. Persistent pulmonary hypertension of the newborn |
| 2. Pulmonary hypertension with left heart disease |
| 2.1. Left-sided atrial or ventricular heart disease |
| 2.2. Left-sided valvular heart disease |
| 3. Pulmonary hypertension associated with lung diseases and/or hypoxemia |
| 3.1. Chronic obstructive pulmonary disease |
| 3.2. Interstitial lung disease |
| 3.3. Sleep-disordered breathing |
| 3.4. Alveolar hypoventilation disorders |
| 3.5. Chronic exposure to high altitude |
| 3.6. Developmental abnormalities |
| 4. Pulmonary hypertension due to chronic thrombotic and/or embolic disease |
| 4.1. Thromboembolic obstruction of proximal pulmonary arteries |
| 4.2. Thromboembolic obstruction of distal pulmonary arteries |
| 4.3. Non-thrombotic pulmonary embolism (tumor, parasites, foreign material) |
| 5. Miscellaneous |
| Sarcoïdosis, histiocytosis X, lymphangiomatosis, compression of pulmonary vessels (adenopathy, tumor, fibrosing mediastinitis) |

**Guidelines for classification of congenital systemic-to-pulmonary shunts**

<table>
<thead>
<tr>
<th>1. Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple</td>
</tr>
<tr>
<td>Atrial septal defect (ASD)</td>
</tr>
<tr>
<td>Ventricular septal defect (VSD)</td>
</tr>
<tr>
<td>Patent ductus arteriosus</td>
</tr>
<tr>
<td>Total or partial unobstructed anomalous pulmonary venous return</td>
</tr>
<tr>
<td>Combined</td>
</tr>
<tr>
<td>Describe combination and define prevalent defect if any</td>
</tr>
<tr>
<td>Complex</td>
</tr>
<tr>
<td>Truncus arteriosus</td>
</tr>
<tr>
<td>Single ventricle with unobstructed pulmonary blood flow</td>
</tr>
<tr>
<td>Atrioventricular septal defects</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2. Dimensions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small (ASD ≤2.0 cm and VSD ≤1.0 cm)</td>
</tr>
<tr>
<td>Large (ASD &gt;2.0 cm and VSD &gt;1.0 cm)</td>
</tr>
</tbody>
</table>

| 3. Associated extracardiac abnormalities |

<table>
<thead>
<tr>
<th>4. Correction status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected (spontaneously or surgically)</td>
</tr>
<tr>
<td>Noncorrected</td>
</tr>
<tr>
<td>Partially corrected (age)</td>
</tr>
</tbody>
</table>

*Main modifications to the previous Evian clinical classification are set in **bold** in table body. These include: idiopathic pulmonary hypertension instead of primary hypertension; some newly identified possible risk factors and associated conditions have been added in the APAH subgroup (glycogen storage disease, Gaucher disease, hereditary hemorrhagic telangiectasia, hemoglobinopathies, myeloproliferative disorders, splenectomy); another subgroup has been added in the PAH category: PAH associated with significant venous or capillary involvement (PVOD and PCH); the last group now termed “miscellaneous” includes some conditions associated with pulmonary hypertension of various and multiple etiologies (histiocytosis X, lymphangiomatosis, compression of pulmonary vessels by adenopathy, tumor, fibrosing mediastinitis).
is suspected that shear stress caused by high flow damages endothelial cells and produces pulmonary hypertensive disease. However, in some children, the mechanism of PH is less clear because similar lesions have been found in patients who have never manifested a large left-to-right shunt, suggesting that PH in these individuals may be idiopathic rather than caused by a high pulmonary blood flow secondary to congenital heart disease. Support for this hypothesis comes from reported cases of severe PH in children with small atrial septal defects whose mothers had IPAH (65).

In general, the likelihood of developing Eisenmenger syndrome depends not only on the location but also on the size of the defect and the magnitude of the shunt. Among the simple cardiac defects, ventricular septal defects appear to be the more frequent abnormalities, followed by atrial septal defects and patent ductus arteriosus (66). Development of PH appears to be related to the size of the defects; for example, the natural history of patients with ventricular septal defects shows that 3% of patients who have small or moderate-size defects (≤1.5 cm in diameter) and that about 50% of the patients with large defects (>1.5 cm in diameter) will develop Eisenmenger syndrome.

Among the different forms of congenital heart diseases, great differences exists with respect to the time of onset of the lesions of PH. Thus, patients with a patent ductus arteriosus or a ventricular septal defect who develop Eisenmenger syndrome have an earlier onset of PH than do patients with atrial septal defects. Other more complex abnormalities, such as atrioventricular septal defects or truncus arteriosus, often develop PAH early in life. Lastly, in some patients, severe PAH can be detected after correction of the heart defect. In many of these cases, it is not clear whether the pulmonary vascular disease has progressed despite a successful correction. However, an early correction generally prevents subsequent development of PAH. In summary, among patients with congenital systemic-to-pulmonary shunts, a great heterogeneity can be observed in terms of location and size of the shunt, the presence of complex cardiac abnormalities, and the status regarding surgical correction. These differences could explain some important variability among these patients with regard to response to vasodilator therapy and the evolution of the disease.

The revised clinical classification as proposed at the Venice conference in 2003 is shown in Table 3. This classification has preserved the structure and spirit of the Evian classification. However, some changes were introduced to reflect recent advances in the understanding and management of PH. In addition, the last group, now termed “miscellaneous,” includes some rare conditions associated with PH of various and multiple etiologies: sarcoidosis (67,68), histiocytosis X (69,70), lymphangiomatosis (71), compression of pulmonary vessels by adenopathy, tumor, or fibrosing mediastinitis. These modifications aim at making this clinical classification more comprehensive, easier to follow, and widespread as a tool.

### REFERENCES

Pulmonary arterial hypertension (PAH) is a multifactorial pathobiology. Vasoconstriction, remodeling of the pulmonary vessel wall, and thrombosis contribute to increased pulmonary vascular resistance in PAH. The process of pulmonary vascular remodeling involves all layers of the vessel wall and is complicated by cellular heterogeneity within each compartment of the pulmonary arterial wall. Indeed, each cell type (endothelial, smooth muscle, and fibroblast), as well as inflammatory cells and platelets, may play a significant role in PAH. Pulmonary vasoconstriction is believed to be an early component of the pulmonary hypertensive process. Excessive vasoconstriction has been related to abnormal function or expression of potassium channels and to endothelial dysfunction. Endothelial dysfunction leads to chronically impaired production of vasodilators such as nitric oxide and prostacyclin along with overexpression of vasoconstrictors such as endothelin (ET)-1. Many of these abnormalities not only elevate vascular tone and promote vascular remodeling but also represent logical pharmacological targets. Recent genetic and pathophysiologic studies have emphasized the relevance of several mediators in this condition, including prostacyclin, nitric oxide, ET-1, angiopeptin-1, serotonin, cytokines, chemokines, and members of the transforming-growth-factor-beta superfamily. Disordered proteolysis of the extracellular matrix is also evident in PAH. Future studies are required to find which if any of these abnormalities initiates PAH and which ones are best targeted to cure the disease. (J Am Coll Cardiol 2004;43:13S–24S)© 2004 by the American College of Cardiology Foundation ISSN 0735-1097/04/$30.00

From the *Service de Pneumologie et Réanimation Respiratoire, Centre des Maladies Vasculaires Pulmonaires, UPRES EA2705, Hôpital Antoine-Béclère, Université Paris-Sud, Clamart, France; †Respiratory Medicine Unit, Department of Medicine, University of Cambridge School of Clinical Medicine, Addenbrooke’s Hospital, Cambridge, United Kingdom; ‡Vascular Biology Group and Pulmonary Hypertension Program, Department of Medicine (Cardiology), University of Alberta, Edmonton, Alberta, Canada; §Development Lung Biology Research, University of Colorado Health Sciences Center, Denver, Colorado; ¶Division of Biomedical and Life Sciences, Institute of Biomedical and Life Sciences, Glasgow University, Glasgow, United Kingdom; #Department of Cardiology, University of Vienna, Vienna, Austria; ††Center for Lung Research, Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee; “Department of Medicine, Veterans Affairs Medical Center, Minneapolis, Minnesota; ‡‡Department of Internal Medicine, Justus-Liebig University, Giessen, Germany; §§Pulmonary Hypertension Center, University of Colorado Health Sciences Center, Denver, Colorado; and §§Department of Pediatrics, Stanford University School of Medicine, Stanford, California.

Manuscript received January 7, 2004; accepted February 3, 2004.

The pulmonary circulation is a low-pressure, high-flow system with a great capacity for recruitment of normally unperfused vessels. As a consequence, the walls of pulmonary arteries are thin, in keeping with their low transmural pressure. Pulmonary arterial hypertension (PAH) is a disease of the small pulmonary arteries, characterized by vascular narrowing leading to a progressive increase in pulmonary vascular resistance. The consequence of this increased right ventricle afterload is the failure of the afterload-intolerant right ventricle.

Vasoconstriction, remodeling of the pulmonary vessel wall, and thrombosis contribute to the increased pulmonary vascular resistance in PAH (1). However, it is now recognized that pulmonary arterial obstruction by vascular proliferation and remodeling is the hallmark of PAH pathogenesis (2). The process of pulmonary vascular remodeling involves all layers of the vessel wall and is complicated by the finding that cellular heterogeneity exists within the compartment of the pulmonary arterial wall (2).

CELLULAR CHANGES

Smooth muscle cells and fibroblasts. Each cell type (endothelial, smooth muscle, and fibroblast) in the pulmonary vascular wall plays a specific role in the response to injury (2). A feature common to all forms of PAH remodeling is the distal extension of smooth muscle into small peripheral, normally nonmuscular, pulmonary arteries within the respiratory acinus. The cellular processes underlying muscularization of this distal part of the pulmonary arterial tree are incompletely understood. In addition, a hallmark of severe pulmonary hypertension is the formation of a layer of myofibroblasts and extracellular matrix between the endothelium and the internal elastic lamina, termed the neointima. In some model systems, particularly in hypoxia models, the adventitial fibroblast appears to be the first cell activated to proliferate and to synthesize matrix proteins in response to the pulmonary hypertensive stimulus (3). The mechanisms that enable the adventitial fibroblast to migrate into the media (and ultimately the intima) are currently unclear, but there is good evidence to suggest that upregu-
Cells comprising plexiform lesions are endothelial channels supported by a stroma containing matrix proteins and myofibroblasts. Endothelial cells express markers of angiogenesis, such as vascular endothelial growth factor (VEGF) and its receptors (5). In addition, cells comprising plexiform lesions of idiopathic PAH are monoclonal in origin (6).

Therefore, although the lesions themselves are probably hemodynamically irrelevant, they may represent more than simply the result of severe elevation of intravascular pressures.

It has been suggested that the endothelial proliferation seen in these lesions may be a marker of a fundamental endothelial abnormality in idiopathic PAH, possibly playing a key role in the pathogenesis of the condition. Intriguingly, defects in growth suppressive genes have been reported in plexiform lesions of patients with idiopathic PAH, including transforming growth factor-beta (TGF-β) and the apoptosis-related gene, Bax (7). Thus, in approximately 30% of plexiform lesions there is a somatic frameshift mutation in the transforming growth factor-beta type-2 receptor (TGF-βR2) gene encoding a premature stop codon. Furthermore, in 90% of plexiform lesions the TGF-βR2 protein is not expressed, in contrast to the abundant expression in endothelial cells outside these lesions.

Thus, it has been proposed that somatic mutations in growth regulatory genes allow clonal expansion of endothelial cells, that contribute to the formation of plexiform lesions and vascular obliteration (7). Human herpesvirus-8 infection may also contribute to the growth of monoclonal endothelial cells in plexiform lesions from patients with idiopathic PAH (8). These findings suggest that triggers, including vasculotropic viruses, can encourage the growth of endothelial cells by dysregulating cell growth or growth-factor signaling.

**Inflammatory cells.** Inflammatory mechanisms appear to play a significant role in some types of pulmonary hypertension including monocrotaline-induced cases in rats and PAH of various origins in humans including connective tissue diseases and human immunodeficiency virus infection (9). Interestingly, some patients with severe PAH associated with systemic lupus erythematosus have improved with immunosuppressive therapy, emphasizing the relevance of inflammation in this subset of patients (9). Patients with idiopathic PAH also have some immunological disturbances speaking in favor of a possible role for inflammation in the pathophysiology of this disease. Indeed, a subset of PAH patients have circulating autoantibodies including antinuclear antibodies, as well as elevated circulating levels of proinflammatory cytokines IL-1 and IL-6. Lung histology also revealed inflammatory infiltrates (macrophages and lymphocytes) in the range of plexiform lesions in severe PAH as well as an increased expression of chemokines RANTES and fractalkine (10). Further analysis of the role of inflammatory mechanisms is necessary to understand whether this component of the disease is relevant to its pathophysiology.

**Platelets and thrombosis.** Thrombotic lesions and platelet dysfunction are potentially important processes in PAH (11). In situ pulmonary artery thrombosis may be initiated or aggravated by abnormalities in the clotting cascade, the endothelial cells, or the platelets. Biologic evidence shows that intravascular coagulation is a continuous process in PAH patients, characterized by elevated plasma levels of fibrinopeptide A- and D-dimers. In addition, procoagulant...
activity and fibrinolytic function of the pulmonary endothelium are altered in PAH. This dysfunction is reflected by the demonstration of elevated plasma levels of von Willebrand factor and plasminogen activator inhibitor type-1. At the present time, it is widely accepted that the shear stress itself or injury of the lung vessels generates a thrombogenic surface with subsequent thrombotic lesions. Thus, it appears this prothrombotic diathesis is shared by many forms of pulmonary hypertension, and is not unique to PAH.

Moreover, an increasing body of evidence also suggests that enhanced interactions between platelets and the pulmonary artery wall may contribute to the functional and structural alterations of pulmonary vessels. Vascular abnormalities in PAH may lead to release by platelets of various procoagulant, vasoactive, and mitogenic mediators. Indeed, in addition to its role in coagulation, the platelet stores and releases important contributors to pulmonary vasoconstriction and remodeling such as thromboxane A2, platelet-activating factor, serotonin (5-hydroxytryptamine [5-HT]), platelet-derived growth factor (PDGF), TGF-β, and VEGF. In most cases, however, it remains unclear whether thrombosis and platelet dysfunction are causes or consequences of the disease (11).

**MOLECULAR MECHANISMS**

Pulmonary vasoconstriction is believed to be an early component of the pulmonary hypertensive process. Excessive vasoconstriction has been related to abnormal function or expression of potassium channels, as well as to endothelial dysfunction (1,2). Endothelial dysfunction leads to chronically impaired production of vasodilators such as nitric oxide (NO) and prostacyclin along with prolonged overexpression of vasoconstrictors such as endothelin (ET)-1, which not only affect vascular tone, but also promote vascular remodeling and, therefore, represent logical pharmacological targets (Fig. 1). It appears that most stimuli that acutely enhance vasoconstriction ultimately also cause cell proliferation (e.g., K+ channel inhibition, ET-1).

**Prostacyclin, vasoactive intestinal peptide, and NO.** Prostacyclin (prostaglandin I2) is an important endogenous pulmonary vasodilator acting through activation of the cyclic adenosine monophosphate (cAMP)-dependent pathways. Prostacyclin also inhibits the proliferation of vascular smooth muscle cells and decreases platelet aggregation. Prostacyclin synthesis is decreased in endothelial cells from PAH patients. Analysis of urinary metabolites of prostacyclin showed a decrease in the amount of excreted...
the anatomic location of cells. For instance, ET A mediate was demonstrated, which presumably re...pulmonary artery smooth muscle cells from idiopathic PAH. A...ported by the demonstration of reduced NO synthase expression in pulmonary endothelial cells from PAH patients. A novel therapeutic strategy in PAH aims at increasing NO-dependent, cyclic guanosine monophosphate-mediated pulmonary vasodilation by inhibition of the breakdown of cyclic guanosine monophosphate by phosphodiesterase type-5. In a small group of PAH patients, sildenafil has been shown to be safe and effective on a chronic basis.

Vasoactive intestinal peptide (VIP), a neuropeptide primarily functioning as a neurotransmitter, acts as a potent systemic and pulmonary vasodilator. It also inhibits the proliferation of vascular smooth muscle cells and decreases platelet aggregation; VIP acts through two receptor subtypes (VPAC-1 and -2), which are coupled to adenylate cyclase and expressed in the lung vasculature. Stimulation of VPAC receptors leads to the activation of the cAMP and cyclic guanosine monophosphate (cGMP) systems. Low serum concentrations and decreased VIP immunoreactivity were shown in pulmonary arteries from patients with idiopathic PAH. In addition, higher expression of VIP receptors and elevated specific receptor binding activity in pulmonary artery smooth muscle cells from idiopathic PAH was demonstrated, which presumably reflects VIP deficiency. Acute and chronic responses to inhaled VIP have been recently demonstrated in a small number of PAH patients.

**ET-1.** Through its action on the endothelin receptor A (ET A) in pulmonary artery smooth muscle cells, ET-1 leads to a rapid increase in intracellular calcium and sustained activation of protein kinase C. Early activation of the p42/p44 isoforms of mitogen-activated protein kinase and induction of the early growth response genes c-fos and c-jun are also observed. The mitogenic action of ET-1 on pulmonary artery smooth muscle cells occurs through the ET A or endothelin receptor B (ET B) subtype, depending on the anatomic location of cells. For instance, ET A mediate mitogenesis in cells derived from the main pulmonary artery, whereas in cells from resistance arteries both receptor subtypes may contribute. There is strong evidence that endothelium-derived ET-1 is a major player in the vasodilator/vasoconstrictor imbalance characteristic of PAH. Levels of lung and circulating ET-1 are increased in animals and patients with pulmonary hypertension of various etiologies. These observations indicate that ET-1 is likely to contribute to the vasoactive component of PAH, as well as to the abnormal pulmonary vascular remodeling characteristic of the condition. Results of chronic ET receptor antagonist therapy support the relevance of this pathway in PAH.

**Potassium channels.** Lessons relevant to PAH can be learned from understanding the mechanism of hypoxic pulmonary vasoconstriction, although PAH also involves cell proliferation and abnormalities of apoptosis. Hypoxic pulmonary vasoconstriction is elicited when hypoxia inhibits one or more voltage-gated potassium channels (Kv) in the pulmonary artery smooth muscle cells of resistance pulmonary arteries (Fig. 2). The resulting membrane depolarization increases the opening of voltage-gated calcium channels, raising cytosolic calcium and initiating constriction. The Kv1.5 is downregulated in pulmonary artery smooth muscle cells in humans with PAH and, both Kv1.5 and Kv2.1 are downregulated in rats with chronic hypoxia-induced pulmonary hypertension.

Furthermore, deoxyribonucleic acid microarray studies have shown downregulation Kv channel genes in PAH lungs. The selective loss of these Kv channels leads to pulmonary artery smooth muscle cell depolarization, an increase in the intracellular calcium, and both vasoconstriction and cell proliferation. It is not clear whether these Kv channel abnormalities are genetically determined or acquired. However, it is clear that the appetite suppressants dexfenfluramine and aminorex directly inhibit Kv1.5 and Kv2.1. Augmenting Kv pathways should cause pulmonary vasodilation and promote regression of pulmonary remodeling. Drugs including dichloroacetate and sildenafil may enhance the expression and function of these potassium channels. Most of the hemodynamic effects of NO are mediated by cGMP, which causes vasodilation by activating protein kinase G, which phosphorylates and activates BKCA channels, as one of several mechanisms by which it lowers cytosolic calcium.

**Serotonin.** In PAH, circulating serotonin levels are elevated, whereas the level in platelets, the major repository of serotonin (5-hydroxytryptamine [5-HT]), is low.
5-Hydroxytryptamine is produced by the gastrointestinal tract enterochromaffin cells and pulmonary neuroepithelial bodies and stored in platelets. A role for 5-HT has been suggested in PAH (23,24). First, a correlation between high plasma 5-HT levels and PAH was observed in a patient with congenital thrombocytopathy characterized by a defect in the platelet 5-HT storage capacities. Subsequently, elevated plasma 5-HT levels were demonstrated in a series of PAH patients (23). This could not be corrected by lung transplantation or epoprostenol therapy, indicating that raised plasma 5-HT cannot be the mere consequence of elevated pulmonary pressure (23). In the 1960s, an association between PAH and the anorexigen aminorex was identified. Aminorex induces platelet 5-HT release and inhibits monoamine oxidase, potentially inhibiting its metabolism, thus increasing plasma 5-HT levels. More recently, it was shown that exposure to fenfluramine derivatives increased the risk of developing PAH. By interacting with the 5-HT transporter (5-HTT), these anorexigen release 5-HT from platelets and inhibit its reuptake and raise circulating free 5-HT. Additionally, treatment of rats with 5-HT potentiates the effects of hypoxia on pulmonary arterial pressure and remodeling. The mechanism by which 5-HT affects the pulmonary vasculature is still a matter of debate (Fig. 3).

The 5-HTT expression, activity, or both in pulmonary artery smooth muscle cells contribute to the pulmonary vascular remodeling occurring in both clinical and experimental PAH (24); 5-HTT is encoded by a single gene on chromosome 17q11.2, and a variant in the upstream promoter region of the 5-HTT gene has been described. This polymorphism with long (L) and short (S) forms affects 5-HTT expression and function with the L-allele inducing a greater rate of 5-HTT gene transcription than the S-allele. The L-allelic variant was found to be present in homozygous forms in 65% of idiopathic PAH patients but only in 27% of controls (25). Moreover the 5-HTT gene polymorphism contributes to interindividual differences in hypoxia-induced 5-HTT expression and potentially affects susceptibility to hypoxic pulmonary hypertension (26). Mice overexpressing the 5-HTT gene exhibit spontaneous pulmonary hypertension in absence of hypoxia (and exaggerated pulmonary hypertension after hypoxic exposure) (27). Finally, recent studies have shown that selective serotonin reuptake inhibitors protect against hypoxic pulmonary hypertension in mice (28).

In human large pulmonary arteries, the 5-HT1 receptor mediates 5-HT-induced contraction. Further investigation identified the 5-HT1B as that mediating contraction in human small muscular pulmonary arteries (29). In addition,
there is an increase in the expression of the 5-HT\textsubscript{1B} receptor in PAH. Contractile responses to 5-HT in the rat pulmonary circulation are mediated by the 5-HT\textsubscript{2A} receptor in control rats, but in chronic hypoxic pulmonary hypertensive rats the response is increased, and this is mediated by the 5-HT\textsubscript{1B} receptor. Molecular studies confirmed that messenger ribonucleic acid (mRNA) for the 5-HT\textsubscript{1B} receptor is increased in these vessels. Converging evidence that the 5-HT\textsubscript{1B} receptor may be involved in the development of hypoxia-induced PAH comes from studies using the 5-HT\textsubscript{1B}/1D antagonist and studies in the 5-HT\textsubscript{1B} receptor knockout mouse (30). Development of right ventricular hypertrophy, and enhanced vasoconstriction to 5-HT\textsubscript{1}-receptor stimulation, is absent in chronic hypoxic pulmonary hypertensive 5-HT\textsubscript{1B} knockout mice compared with their wild-type controls, and pulmonary vascular remodeling is markedly reduced.

A role for other 5-HT receptors such as 5-HT\textsubscript{2B} has been suggested. The 5-HT\textsubscript{2B} receptor is activated by nordexfenfluramine, the active dexfenfluramine metabolite. Interestingly, development of chronic hypoxic pulmonary hypertension is ablated in 5-HT\textsubscript{2B} receptor knockout mice, and the 5-HT\textsubscript{2B} receptor transcript is increased in idiopathic PAH patients (31). An interesting link between the K\textsuperscript{+} channel hypothesis and the role of serotonin is the finding that K\textsuperscript{+} channel inhibitors cause serotonin release and inhibit K\textsuperscript{+} currents in megakaryocytes (32). Moreover, the anorexiants, which inhibit serotonin reuptake and cause serotonin release, are K\textsuperscript{+} channel blockers (22). This led to the hypothesis that chronic depolarization of platelets and pulmonary artery smooth muscle cells could lead to a vasoconstricted, pro-proliferative, serotoninemic phenotype.

**TGF-\beta superfamily.** The TGF-\beta superfamily is composed of multifunctional mediators, including the TGF-\beta isoforms (TGF-\beta1–3), the bone morphogenetic proteins (BMPs), activins, and growth and differentiation factors (33,34). The TGF-\beta superfamily has diverse roles in a wide variety of physiological processes (Fig. 4). Germline mutations in the gene coding for BMP type-II receptor (BMPR2) have been identified in 60% of familial PAH and 10% to 30% of idiopathic PAH (35–37). The absence of BMPR2 mutations in some families and in the majority of sporadic and associated cases suggests that there may be further genes, possibly related to the BMP/TGF-\beta pathway, to be identified. Indeed, mutations in the TGF-\beta receptors, ALK-1 and endoglin, have been identified in PAH patients with a personal or family history of hereditary hemorrhagic telangiectasia (38,39).
The BMPR-II is a constitutively active serine/threonine kinase receptor, signaling via formation of heterocomplexes with one or three type-I receptors (ALK-3/BMPR-1A, ALK-6/BMPR-IB or ALK-2) in response to ligand. The main ligands identified for BMPR-II are BMP2, BMP4, BMP7, GDF5, and GDF6. The BMPR-II phosphorylates a glycine-serine-rich domain on the proximal intracellular portion of the associated type-I receptor. Activation of the type-I receptor kinase domain initiates phosphorylation of cytoplasmic signaling via the Smad family of proteins. The BMPs signal via a specific set of Smad proteins (Smad1, Smad5, and Smad8) termed “Receptor activated” or R-Smads, which complex with the common partner Smad or Co-Smad, Smad4, to allow translocation of this signaling complex to the nucleus where they can regulate gene transcription. However, Smads bind only weakly to DNA and require the presence of transcriptional co-activators or co-repressors.

There are multiple levels at which BMP signaling is regulated, including the presence of endogenous inhibitors of BMP-receptor interactions (chordin, noggin, and BAMBI), the formation of specific type-II/type-I receptor heterocomplexes, the activation of inhibitory Smads (I-Smads, Smad6, and Smad7), and the cell-specific expression of transcription factors. Such diverse levels of regulation may be responsible for the tissue specificity of BMP signaling and may, for example, underlie the lung specificity of PAH. Human pulmonary artery smooth muscle cells and endothelial cells express a wide range of TGF-β superfamily receptors, including BMPR-II and BMPR-IB, and bind 125I-TGF-β and 125I-BMP4.

Furthermore, activation of these receptors by BMPs leads to phosphorylation of Smad1 and induction of mRNAs for Smad6 and Smad7. Although signaling via Smads is well characterized, there is increasing evidence that MAP kinases, including ERK, p38MAPK, and JNK kinases, are activated in specific cell types by TGF-β and BMPs (33). It appears that in some cases the p38 kinase pathway can bypass the Smad pathway and mediate some of the BMPR2 pathway effects on nuclear transcription and apoptosis. Recent evidence suggests that abnormal activation of alternative signaling pathways may be critical to the pathogenesis of PAH (40).

The critical role of the BMP pathway in vascular development is evident from studies in knockout mice. Homozygosity for a null mutation in BMPR2 is lethal during early embryogenesis (41), and mice deficient in Smad5, one of the BMP-restricted Smads, die owing to defects in angiogenesis, with failure to recruit vascular smooth muscle to endothelial structures. The net result of TGF-β signaling on vascular growth and structure is complex. Whether the TGF-β superfamily inhibits or promotes cell proliferation is highly context-specific.

In situ hybridization and immunohistochemical studies have demonstrated that both BMPR-II mRNA and protein are present predominantly on the pulmonary vascular endo-thelium, macrophages, and to a lesser extent on medial smooth muscle cells (42). Lung BMPR-II protein expression is dramatically reduced in patients harboring an underlying BMPR2 mutation predicted to cause truncation of the protein (42). In addition, BMPR-II expression is markedly reduced in PAH cases in which no BMPR2 mutation was identified (41). A small but significant reduction was also observed in cases of secondary pulmonary hypertension. Reduced BMPR-II expression was specific for this receptor because no change was observed in the level of expression of other endothelial markers, including CD31. These findings stress the importance of understanding how other environmental and genetic factors regulate the expression of BMPR-II in lung cells. Thus, further characterization of the regulation of BMPR-II expression is likely to add to our understanding of exogenous factors influencing BMPR-II transcription and may provide important clues as to why the vascular abnormality is restricted to the lung, particularly as BMPR-II is widely expressed in normal adult tissues.

A recently published study has provided further support for the hypothesis that intact BMP signaling is important for the maintenance of the normal pulmonary vasculature (43). In this study the type-I receptor BMPR-IA was downregulated in the lung tissue of a heterogeneous group of patients with pulmonary hypertension. Furthermore, investigators showed a reciprocal relationship between BMPR-IA expression and that of angiopoietin-1, and they demonstrated that angiopoietin-1 downregulates BMPR-IA expression in human pulmonary artery endothelial cells (43).

Additionally, BMP2, -4, and -7 inhibit the proliferation of smooth muscle cells derived from normal pulmonary arteries and from PAH patients with congenital heart diseases, but they fail to suppress proliferation of cells from patients with idiopathic or familial PAH (43). An attractive hypothesis is that a failure of the growth inhibitory effects of BMPs in idiopathic or familial PAH cells could contribute to the vascular obliteration and remodeling that characterize the condition. The failure to suppress growth of idiopathic or familial PAH cells was observed in all cases, whether or not specific BMPR2 mutations were identified, suggesting that defective BMP-mediated signaling may be a common factor in idiopathic or familial PAH. The mechanism by which BMPR2 mutations disrupt BMPR-II signaling has begun to be elucidated (Fig. 5) (40,44,45). Interestingly, a feature common to all mutants is a gain of function involving p38MAPK activation.

In pulmonary artery smooth muscle cells from patients with idiopathic PAH, TGF-β1 causes enhanced cell proliferation in contrast to the growth inhibitory effect observed in normal cells (43). This is not due to alterations in TGF-β1 receptor ratios or downregulation of TGF-β1 type-II receptor (44). Transforming growth factor-beta is also known to increase production of extracellular matrix. In human lung fibroblasts, TGF-β increases elastin expression by stabilization of elastin mRNA, and thus it is possible that increased elastin expression observed in PAH may be due to...
alterations in this pathway. Although studies from other cell types have found TGF-β to induce collagen production, no correlation has been found between procollagen and TGF-β staining in lungs of PAH patients (46). The TGF-β superfamily may regulate the activity of other factors implicated in vascular remodeling. The TGF-β1 induces ET-1 in human pulmonary artery cells probably via activation of protein kinase A (47). Connective tissue growth factor production can also be stimulated by TGF-β in pulmonary fibroblasts (48). Clearly much remains to be learned of the interaction of the TGF-β/BMP pathway with other factors already demonstrated to play important roles in the control of vascular tone and growth.

**Angiogenesis and apoptosis.** Vascular endothelial growth factor is an endothelial-cell-specific-angiogenic mitogen acting via two high-affinity tyrosine kinase receptors (VEGFR-1 and VEGFR-2). Although the physiological role of the abundantly expressed VEGF in the lung is unknown, it has been proposed that VEGF supports pulmonary endothelial cell maintenance and survival. In PAH, the VEGF expression is increased within the pulmonary vasculature, including the plexiform lesions (5,49). Although the isoform VEGF-A has been most extensively studied in the context of pulmonary hypertension and has been proposed to play a protective role, a recent study identified a pathogenic role for VEGF-B.

In contrast to VEGF-A, the VEGF-B appears to exacerbate remodeling as VEGF-B knockout mice (VEGF-B−/−) exposed to chronic hypoxia exhibit significantly less pulmonary vascular remodeling compared with wild-type mice (VEGF-B+/+)(50). Recent animal studies have emphasized the positive effects of VEGF in models of pulmonary hypertension (51). Indeed, cell-based VEGF gene transfer has proved an effective method of preventing the development and progression of pulmonary hypertension in the monocrotaline model (51). Vascular endothelial growth factor would minimize...
progression of the disease by preventing loss of existing vessels or by inducing the development of new blood vessels within the lung (51).

In idiopathic PAH, VEGFR-1 expression is increased, whereas within the plexiform lesions it is VEGFR-2 that is expressed (52). In rats, it has been shown that the combination of chronic blockade of VEGFR-2 and chronic hypoxia could cause pulmonary endothelial cell dysfunction and cell death, allowing the selection of an apoptosis-resistant proliferating endothelial cell phenotype and the subsequent development of severe pulmonary hypertension (53). Because endothelial cell death, cell proliferation, and the development of severe pulmonary hypertension could be blocked by a broad-spectrum caspase inhibitor, it appeared that the selection of an apoptosis-resistant endothelial cell phenotype might be the critical event responsible for pulmonary artery endothelial cell proliferation (53). Therefore, apoptosis of endothelial cells may underlie the propensity to vascular disease (53).

Various other growth factors including PDGF, basic fibroblast growth factor, insulin-like growth factor-1, and epidermal growth factor have also been implicated in the development of remodeling and all have been reported to be increased in the pulmonary hypertensive lung. The mechanism that leads to induction of these growth factors in the pulmonary vasculature is unclear, though reactive oxygen species have been implicated because hydrogen peroxide induces PDGF expression in human pulmonary endothelial cells, as does hypoxia and mechanical stretch and shear stress.

Angiopoietin-1 is an angiogenic factor essential for lung vascular development (43). Produced by smooth-muscle cells and precursor pericytes, angiopoietin-1 stabilizes the development of blood vessels by recruiting muscle cells, through migration and division, to endothelial tubes, creating mature arterial structures. The receptor for angiopoietin-1, TIE2, is present only on vascular endothelium. The ligand-receptor interaction between angiopoietin-1 secreted by smooth-muscle cells and endothelium-specific TIE2 during organ development induces the proliferation of muscle cells around the endothelium vascular network.

After development is completed, angiopoietin-1 is expressed at a minimally detectable level in the human lung. Recent studies attempted to analyze the putative role of angiopoietin-1 in pulmonary hypertension, but they reached entirely antithetical conclusions (43,54,55). The findings by Du and colleagues (43) suggest that all forms of nonfamilial pulmonary hypertension are characterized by upregulation of angiopoietin-1 and phosphorylated TIE2, correlating directly with the severity of the disease. A mechanistic link...
between familial PAH and acquired pulmonary hypertension was supported by the finding that angiopoietin-1 shuts off the expression of BMPR1A, a transmembrane protein required for BMPR2 signaling, in pulmonary arteriolar endothelial cells (43). Interestingly, rodents engineered to express angiopoietin-1 in the lung develop pulmonary hypertension (54). These animals manifest diffuse medial thickening in small pulmonary vessels, resulting from smooth muscle cell hyperplasia.

In addition, angiopoietin-1 stimulates pulmonary arteriolar endothelial cells through a TIE2 pathway to produce and secrete 5-HT (54). These revelations suggest that pulmonary hypertensive vasculopathy occurs through an angiopoietin-1/TIE2/5-HT paracrine pathway and imply that these signaling molecules may be targets for strategies to treat this disease.

By contrast, Zhao et al. (55) have demonstrated that angiopoietin-1 may have a protective role in at least some forms of pulmonary hypertension. In their study, cell-based gene transfer with angiopoietin-1 improved survival and pulmonary hemodynamics in monocrotaline-exposed rats by a mechanism involving the inhibition of apoptosis and protection of the pulmonary microvasculature (55). These two approaches of the role of angiopoietin-1 in PAH reflect distinct views on the mechanisms leading to the disease. On the one hand, the investigators assume that the primary cellular defect contributing to the disease is smooth muscle cell hyperplasia, and that this is mediated by excess angiopoietin-1. On the other hand, it is hypothesized that endothelial apoptosis underlies disease progression, and that this can be prevented by administration of angiopoietin-1. At the present time one cannot conclude whether angiopoietin-1 is the cause or the cure of PAH, but more data are required to evaluate the angiopoietin-1/TIE2 pathway in this condition (56).

**Proteolysis.** Evidence that proteolysis of the extracellular matrix may be important in the pathobiology of pulmonary vascular disease came from observations of degradation of elastin in pulmonary arteries from patients with a congenital heart defect and pulmonary vascular disease (57). These studies were supported by work in a variety of rat models of pulmonary hypertension (hypoxia, monocrotaline) in which heightened activity of elastase in the pulmonary arteries was documented as a very early feature after the injurious stimulus (57). Subsequent studies showed that infusion of elastase inhibitors suppressed the disease process (58,59).

It was then shown that serum factors could induce the production of an endogenous vascular elastase from smooth muscle cells and that the mechanism appeared to involve MAP kinase activity and nuclear partitioning of the transcription factor AML1 (60). This is a transcription factor for neutrophil elastase and a putative transcription factor for endogenous vascular elastase, based on studies using antisense blockade of AML1 to repress elastase activity.

Moreover, repression of nuclear partitioning of AML1 as well as repression of phosphorylation of Erk (a member of the MAP kinase family) was achieved with NO donors, and this also repressed elastase activity (61). Suppression of 5-HT receptors repressed elastase activity and TGF-β in chronic hypoxia. Also, to be further investigated is the relationship between BMPR-II and the induction of elastase activity. Hypothetically, it has been proposed that BMPR-II induces Smad/5 interaction with AML1, thus preventing its interaction with Smad4 and repressing its ability to partner with other transcription factors and to induce elastase activity. Conversely, a mutation in BMPR-II would result in derepression of AML1 and induction of elastase and other AML1-dependent genes.

Evidence from the published data indicates that serine elastases activate MMPs and also repress tissue inhibitors of MMPs. In other injury models, elastase activity has been shown to precede MMP activity and is responsible for its induction. In the monocrotaline model, an elevation in elastase activity is seen on day 2 after injection of the toxin, whereas MMP activity is not increased until day 21. Both MMPs and elastases can degrade most components of the matrix in addition to elastin and collagen. Degradation of collagen leads to ligation of β3 integrins activation of the MAP kinase pathway and transcription of tenascin C. This glycoprotein cooperatively interacts with growth factors such as epidermal growth factor in inducing smooth muscle cell proliferation. Repression of this pathway by elastase inhibitors has been shown in cell and organ culture and in whole animals to induce apoptosis of smooth muscle cells, and regression of severe vascular disease (58,59).

**Conclusions.** It is clear that PAH has a multifactorial pathobiology, and it is unlikely that one factor or gene mutation will explain all forms and cases of PAH (Fig. 6). However, the current understanding of the mechanisms underlying PAH has allowed the rapid development of drugs including prostacyclin, endothelin receptor antagonists, and phosphodiesterase inhibitors. Our improved understanding of additional pathways in this condition will presumably lead to the development of novel therapeutic strategies in the near future, such as ion channel replacement therapy or cell-based therapies, using bone marrow precursor cells.

**Reprint requests and correspondence:** Dr. Marc Humbert, Service de Pneumologie, Hôpital Antoine-Béclère, 157, Rue de la Porte de Trivaux, 92140 Clamart, France. E-mail: humbert@ipsc.u-psud.fr.

**REFERENCES**

4. Davie NJ, Crossno JT, Frid MG, et al. Hypoxia-induced pulmonary artery adventitial remodeling and neovascularization: contribu-


47. Kucich U, Rosenbloom JC, Herrick DJ, et al. Signaling events required for transforming growth factor-β stimulation of connective
Pathologic Assessment of Vasculopathies in Pulmonary Hypertension

Giuseppe G. Pietra, MD,* Frederique Capron, MD,† Susan Stewart, MD,‡ Ornella Leone, MD,§ Marc Humbert, MD,¶ Ivan M. Robbins, MD,# Lynne M. Reid, MD,|| R. M. Tuder, MD**

Philadephia, Pennsylvania; Paris and Clamart, France; Bologna, Italy; Nashville, Tennessee; Boston, Massachusetts; and Baltimore, Maryland

Pulmonary arterial hypertension (PAH) includes various forms of pulmonary hypertension of different etiology but similar clinical presentation and functional derangement. Histopathological vascular changes in all forms of PAH are qualitatively similar but with quantitative differences in the distribution and prevalence of pathological changes in various portions of the pulmonary vascular bed. The documentation of these topographic variations in the response of the pulmonary vasculature to injury may be important to understand the pathogenesis of the various subsets of PAH. To standardize the precise histopathological documentation of the pulmonary vasculopathy in PAH we propose a histopathological classification that includes both the predominant segment of the pulmonary vasculature affected and the possible coexistence of pathological changes in other vascular segments. (J Am Coll Cardiol 2004;43:25S–32S) © 2004 by the American College of Cardiology Foundation

The term pulmonary arterial hypertension (PAH) includes a variety of pulmonary hypertensive diseases with different etiologies but similar clinical presentation and, in many cases, similar response to medical treatment (1). Initially, PAH comprised primary pulmonary hypertension (PPH) and pulmonary hypertension related to left-to-right shunts, collagen-vascular diseases, portal hypertension, human immunodeficiency virus (HIV) infection, ingestion of drugs or dietary products, and persistent fetal circulation (1). At the recent Third World Symposium on Pulmonary Artery Hypertension, pulmonary veno-occlusive disease (PVOD) and pulmonary capillary hemangiomatosis (PCH) have been incorporated into PAH because of the high incidence of arteriopathy in these conditions (2,3), the similarity of risk factors with PAH (HIV, drug ingestion), the familial occurrence (4,5), and the recently discovered mutation of bone morphometric protein receptor-2 (BMPR2) in a case of PVOD (6). It has been hypothesized that PAH, PVOD, and PCH may represent part of the spectrum of the same disease, or differing reactions to similar insults.

The Evian classification (1) is based on etiology, clinical presentation, and functional data, and it assumes that all subsets of PAH have a similar spectrum of pathological lesions. This may not always be the case because morphometric studies have shown differences in the distribution and prevalence of arterial changes among PAH of different etiology (7,8). Also, in certain cases of PAH venous changes coexist with arterial lesions; that is, the pulmonary veins and venules can show intimal and adventitial thickening and even arterialization (9–12). In the proximity of organized pulmonary infarcts, veno-occlusive changes can also be present, possibly due to scarring or other disruptions of vascular relationships.

Thus, documentation of the extent of different types of vascular changes among the various subsets of PAH is essential in understanding how various segments of the pulmonary vascular tree react to injury. To standardize pathological reporting and provide clinicians with a precise description of the nature and extent of vascular lesions present in a single case, we have adopted a descriptive histopathologic system of classification (Table 1) in which both the predominant changes and the coexisting pathologic changes are recorded.

HANDLING OF LUNG TISSUE

Fixation of the lung samples in a state of distension avoids crenation of elastic laminae of muscular pulmonary arteries that may induce a state of spurious medial hypertrophy. Adequate sampling of the lungs (at least five blocks from each lobe) is essential. The histological examination should indicate whether adequate samples of blood vessels are present, the nature and number of diseased blood vessels, the presence, location, and nature of inflammatory cells, as well as any evidence of associated pathology in the airways or lung parenchyma. In addition to hematoxylin-eosin (HE), special histological stains (Movat, Masson, Verhoeff-van Gieson, Perls’ iron) are essential to assess vascular pathology. Also useful are immunohistochemical markers for smooth muscle and endothelium (Factor VIII, CD31, CD34).
PULMONARY ARTERIOPATHY

All forms of pulmonary hypertension have some common pathologic features regardless of their etiology, that is, medial hypertrophy of muscular and elastic arteries, dilation and intimal atheromas of elastic pulmonary arteries and right ventricular hypertrophy. These forms of pulmonary vascular remodeling have limited diagnostic value because they are present in all forms of severity of pulmonary hypertension. Also, pulmonary artery medial and intimal thickening can occur as an isolated nonspecific finding in localized areas of the lung or secondary to airway or interstitial diseases and tumors, with no direct relationship to the presence or not of pulmonary hypertension.

In addition to the aforementioned pathologic changes common to all forms of pulmonary hypertension, PAH is characterized by constrictive and complex arterial lesions involving to varying degrees the pre- and intra-acinar pulmonary arteries (3,12). The main histopathological features of this pulmonary arteriopathy are illustrated in Figure 1 and are briefly defined below.

Constrictive lesions. These lesions include medial hypertrophy, and intimal and adventitial thickening. These changes are believed to result from an imbalance between proliferation and apoptosis of the various cell types forming the vascular walls. Because they are diffuse lesions, they may be hemodynamically important if vasorelaxant properties are lost (i.e., prostacyclin or nitric oxide) or there is excessive

Table 1. Pathological Classification of Vasculopathies of Pulmonary Hypertension

<table>
<thead>
<tr>
<th>1. Pulmonary arteriopathy (pre- and intra-acinar arteries)</th>
<th>Previous WHO terminology†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subsets</td>
<td></td>
</tr>
<tr>
<td>Pulmonary arteriopathy with isolated medial hypertrophy</td>
<td>Pulmonary plexogenic arteriopathy Gr. 1</td>
</tr>
<tr>
<td>Pulmonary arteriopathy with medial hypertrophy and intimal thickening (cellular, fibrotic)</td>
<td></td>
</tr>
<tr>
<td>Concentric laminar</td>
<td></td>
</tr>
<tr>
<td>Eccentric, concentric nonlaminar</td>
<td></td>
</tr>
<tr>
<td>Pulmonary arteriopathy with plexiform and/or dilation lesions or arteritis</td>
<td>Pulmonary plexogenic arteriopathy Gr. 2, 3</td>
</tr>
<tr>
<td>Pulmonary arteriopathy with isolated arteritis</td>
<td>Pulmonary embolic arteriopathy</td>
</tr>
<tr>
<td></td>
<td>Pulmonary plexogenic arteriopathy Gr. 4−6</td>
</tr>
<tr>
<td>1a. As above but with coexisting venous-venular changes (cellular and/or fibrotic intimal thickening, muscularization)</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

The presence of the following changes should be noted:

Adventitial thickening; thrombotic lesions (fresh, organized, recanalized, colander lesion); necrotizing or lympho-monocytic arteritis; elastic artery changes (fibrotic or atheromatous intimal plaques, elastic laminae degeneration); bronchial vessel changes, ferruginous incrustation, calcifications, foreign body emboli, organized infarct perivascular lymphocytic infiltrates.

2. Pulmonary occlusive venopathy (veins of various size and venules) with or without coexisting arteriopathy

Histopathologic features:

Venous changes: intimal thickening/obstruction (cellular, fibrotic); obstructive fibrous luminal septa (recanalization).

Adventitial thickening (fibrotic); muscularization; iron and calcium incrustation with foreign body reaction:

Capillary changes: dilated, congested capillaries; angioma-like lesions.

Interstitial changes: edema; fibrosis; hemosiderosis; lymphocytic infiltrates.

Others: dilated lymphatics; alveoli with hemosiderin-laden macrophages; type II cell hyperplasia.

3. Pulmonary microvasculopathy with or without coexisting arteriopathy and/or venopathy

Histopathologic features:

Microvessel changes: localized capillary proliferations within pulmonary interstitium; obstructive capillary proliferation in veins and venular walls.

Venous-venular intimal fibrosis.

Interstitial changes: edema, fibrosis, hemosiderosis.

Others: dilated lymphatics; alveoli with hemosiderin-laden macrophages; type II cell hyperplasia.

4. Unclassifiable‡

PVOD

PCH

n.a.

*Nonvascular lung pathology needs to be listed as separate diagnosis. †Primary pulmonary hypertension. Report on a WHO meeting, Geneva, October 15–17, 1975. S. Hatano and T. Strasser, eds. §Atypical histopathological features or inadequate sampling of blood vessels.

Gr. = grade; n.a. = not applicable.
production of vasoconstrictors (i.e., endothelin). Medial hypertrophy is an increase in the cross-sectional area of the media of pre- and intra-acinar pulmonary arteries (Figs. 1A and 1B). It is due to both hypertrophy and hyperplasia of smooth muscle fibers and to an increase in connective tissue matrix and elastic fibers in the media of muscular arteries and extension of smooth muscle into nonmuscularized intra-acinar arteries. Medial hypertrophy entails both numerical and phenotypical changes of muscle fibers. Atrophy of the media occurs in arteries with marked intimal thickening and in developing dilation lesions.

Intimal thickening can be of three types: concentric laminar, eccentric, or concentric nonlaminar (Figs. 1C to 1F). Concentric laminar intimal thickening can be to varying degrees either cellular or fibrous (Figs. 1C and 1D). Both ultrastructurally and immunohistochemically, the intimal cells show features of fibroblasts, myofibroblasts, and smooth muscle cells (Fig. 1D). However, these morphologic features do not allow conclusions on their derivation because recent experimental work has shown the potential of endothelial cells and fibroblasts to acquire smooth muscle phenotype (13). Concentric laminar intimal thickening is a characteristic feature of the so-called pulmonaryplexogenic arteriopathy and/or of scleroderma arteriopathy. Eccentric and concentric nonlaminar intimal thickenings are predominantly composed of fibroblasts and connective tissue matrix (Figs. 1E and 1F). The notion that these changes are diagnostic of thromboembolic arteriopathy needs revision because intimal thickening may result from localized proliferation of intimal fibroblasts caused by growth factors released by hemodynamic stresses.

Adventitial thickening is difficult to evaluate because of its ill-defined boundaries in conventional histological sections. In most cases of PAH, the adventitia appears uninvolved, but it is expanded in pulmonary hypertension owing to persistent fetal circulation of the newborn.

**COMPLEX LESIONS**

Plexiform lesions, dilation lesions, and arteritis are classified as complex lesions. They are focal changes important as markers of either the severity or rapid progression of pulmonary hypertension. It has also been suggested that endothelial proliferation in plexiform lesions of PPH may be a marker of a fundamental cellular abnormality, possibly of neoplastic nature (14,15).

The plexiform lesion is a focal proliferation of endothelial channels lined by myofibroblasts, smooth muscle cells, and connective tissue matrix. The lesion is located within pre- and intra-acinar pulmonary arteries and is associated with expansion and partial destruction of the arterial wall with extension of the plexiform lesion into the perivascular connective tissue (Figs. 1G and 1H). Within the plexiform lesion, fibrin thrombi and platelets are frequently present. The plexiform lesion is often located at an arterial branching point, or at the origin of a supernumerary artery, distally to marked obliteratorive intimal thickening of the parent artery. The frequency of the plexiform lesion in PAH remains undetermined and probably varies to a great extent. Based on studies with limited sampling, it is estimated that the plexiform lesion involves from 20% to 60% of the pulmonary arteries. It is extremely rare in PAH related to connective tissue diseases and it does not occur in PAH of persistent fetal circulation.

The localization of plexiform lesions along the arterial tree also varies; that is, in congenital left-to-right shunts they tend to occur in arteries 100 to 200 μm in external diameter, whereas in PPH they tend to occur in arteries <100 μm (3,12). Plexiform lesions may be difficult to distinguish from the colander-like lesion of recanalized thromboemboli (Fig. 1J). They are no longer considered pathognomonic for PPH as they have been found in PAH associated with other diseases and even in chronic thromboembolic pulmonary hypertension (16). Endothelial cells within plexiform lesions express vascular endothelial growth factor (VEGF) and its receptors (17), and the lesions are believed to result from disordered angiogenesis attributable to autonomous monoclonal endothelial proliferation in the case of PPH (14,15). Recently, the association of the vasculotropic human herpesvirus-8 with plexiform lesions of PPH has been demonstrated (18).

The dilation lesion is a thin-walled vein-like vessel (Figs. 1H and 1I) usually located distally to a plexiform lesion. This lesion may be the source of pulmonary hemorrhages and subsequent organization and fibrosis.

Arteritis is rarely primary in PAH; most often it is associated with other complex lesions. The arterial wall may be necrotic with fibrinoid insudation and/or be infiltrated with chronic and acute inflammatory cells (Fig. 1K).

In our histopathological classification, the terms "pulmonary occlusive venopathy" (POV) and "pulmonary microvascularopathy" (PM) replace the old terms of PVOD and PCH, respectively (Table 1).

**Pulmonary occlusive venopathy** (POV) (formerly PVOD) accounts for a relatively small proportion of cases of pulmonary hypertension. The diagnosis of POV and PM (described below) in vivo is difficult and is not considered in most patients until signs of marked pulmonary hypertension have developed. Although, by high resolution computed tomography, patchy centrilobular ground-glass opacities, thickened septal lines, pleural effusion, and mediastinal adenopathy are characteristic for POV and PM (19), the cornerstone of the diagnosis of POV/PM is histopathology. The main pathology of POV consists of extensive and diffuse occlusion of pulmonary venules and veins of various sizes (2,5). The occluding fibrous tissue may be loose and edematous with variable cellularity, or dense, sclerotic, and acellular (Fig. 2A). The intimal thickening of POV involves venules and small veins and rarely extends to the larger veins. The luminal occlusion can be either solid or eccentric with multiple lumina (Fig. 2B), suggestive of recanalization of occlusive thrombi. The media of the venules and veins...
may become thickened with an increase in elastic fibers and smooth muscle (i.e., so-called arterialization). All these morphological features are helpful in distinguishing POV from chronic venous hypertension.

A nonspecific but useful histological feature is the presence of calcium-encrusting elastic fibers in the walls of veins or adjacent alveoli. This feature, when present, renders the veins easily identifiable and is therefore a pertinent histological finding when the diagnosis of PVOD (or, indeed, pulmonary hypertension) has not been raised by the referring clinician. Another feature that is useful in distinguishing between POV and chronic passive venous hypertension is the foreign body giant-cell response to the calcium-encrusted elastic fibers. This feature is also helpful when
POV is being considered as a supplementary diagnosis to another pulmonary pathology (i.e., interstitial lung disease or emphysema), a circumstance not uncommon in lung explant pathology.

In POV, large amounts of hemosiderin are found both within the cytoplasm of alveolar macrophages and type II pneumocytes as well as deposits in the interstitium. The presence of fresh blood and/or hemosiderin may be so prominent that idiopathic hemosiderosis or healed Wegener’s granulomatosis or other vasculitis is suggested. Hemosiderosis can be quite striking, and quantification of occult alveolar hemorrhage in bronchoalveolar lavage (BAL) (19,20) has been successfully used to confirm the clinical diagnosis of POV/PM because BAL hemorrhage is not a usual feature of the other forms of PAH. The capillary vessels are engorged and prominent; they may become so tortuous as to resemble pulmonary capillary hemangiomatosis (21,22). The capillaries are generally easily identifiable within the alveolar septa and do not line both sides of the alveolar walls as seen in PM (PCH).

Pulmonary arteries and arterioles can show remodeling in approximately 50% of POV cases with moderate to severe medial hypertrophy and arterialization. Plexiform lesions and fibrinoid arteritis are not described in POV (2). The pulmonary interstitium shows edema particularly located in the lobular septa, which may progress to interstitial fibrosis. This can be sufficiently extensive to raise the possibility of interstitial lung disease such as usual interstitial pneumonitis. Inflammatory lung disease is further mimicked by the marked lymphocytic interstitial infiltrate that is seen in some cases of POV. Lymphatics within the lung and pleura are dilated in POV (Fig. 2A).

Pulmonary microvasculopathy (formerly PCH) is another rare condition characterized by localized capillary proliferation within the lung in which capillaries invade pulmonary interstitium, vessels, and, less commonly, airways (23). The distribution of PM in the lungs is usually panlobar and patchy, resembling an interstitial process at low magnification and mimicking pulmonary congestion (24). However, closer inspection shows diffuse proliferation of microvessels containing large numbers of erythrocytes (Figs. 2C and 2D). These microvessels can form glomeroid tufts or nodules.
that may project into the lumen of veins and lymphatics and within air spaces. A distinguishing feature, which is best appreciated on reticulin staining, is the presence of microvessels on both sides of the alveolar walls. Havlik et al. (25) require the microvessels to form at least two cell layers within the proliferating lesion for diagnosis. The endothelial cells of the abnormal capillaries are cytologically bland with elongated oval nuclei, diffuse chromatin, and indistinct cytoplasm. Mitoses are not frequently seen despite the apparent proliferative nature of the condition. The abnormal proliferating capillaries extend into bronchovascular bundles; they infiltrate the walls of arterioles, arteries, venules, and veins, invading muscular walls and occluding the lumens. Also, microvascular proliferation has been seen in perineural and intraneural positions, in the pleura, and in lymph nodes. Venous occlusion by proliferating capillaries and related intimal fibrosis is distinct from the nonangiogenic occlusion of veins in POV.

In the areas of capillary proliferation, a striking feature is pulmonary hemosiderosis represented by both fresh hemorrhage and abundant hemosiderin-laden macrophages and type II pneumocytes (Fig. 2D). However, cases without striking hemosiderosis are observed. The infiltrating capillaries can mimic plexiform lesions, but neither these nor dilation lesions have been described in PM. Similar to POV, the pulmonary arteries in PM show marked muscular hypertrophy and intimal thickening, but, unlike POV, lesions resembling thrombosis are not a feature.

The pathogenesis of PM (PCH) is controversial. Some investigators consider PM to be neoplastic (26), and it is interesting that some patients with PM have responded to treatment with interferon-α, which presumably acts by depressing endothelial proliferation (27,28). Conversely, PM may be due to unknown angiogenic stimuli, and the infiltrative aspect of the disease may merely be related to the extension of vascular lesions within a preexisting vascular distribution.

The PCH/PM-like lesions, as described by Havlik et al. (25), are not associated with clinical pulmonary hypertension and do not generally show invasion of small vessels with occlusion. Pulmonary microvasculopathy and PCH/PM-like lesions must be carefully distinguished from severe pulmonary congestion and atelectasis (29).

Previously PM was described as a possible hypertensive vasculopathy, such lesions had been described as prominent, hyperplastic capillaries with pseudoangiomatous features. Increasing familiarity with the entity of PM has drawn attention to overlaps and similarities between this and POV, particularly in relation to the parenchymal changes of hemosiderosis, fibrosis, lymphatic dilatation and edema, and the similar arterial modifications of intimal fibrosis and medial hypertrophy. The distinction between the two entities is clear in those cases where the venous obliteration in PM is due to proliferating capillaries and in POV due to intimal fibrosis with or without recanalization.

Some cases are, however, less clear-cut, and the issue is further confounded by the description of PCH/PM-like lesions that can occur in several clinicopathological settings and that have even been described at autopsy in the absence of evidence of pulmonary hypertension. Clearly, use of the term “PCH/PM-like lesions” must be strict and contextual if confusion is to be avoided. This is an area where there is a likely future role for molecular pathology in distinguishing dilatation and tortuosity of existing capillaries from capillary angiogenesis and in elucidating the relationship to vascular remodeling and growth factors. It is important to note that similar microvasculopathy is usual in severe mitral valve disease, and interpretation of venous and capillary congestion with or without proliferation should be made in the knowledge of any valvular disease.

As with many pathological conditions that are poorly understood, it is tempting to consider lesions as part of a continual spectrum, and this view has been justified in relation to POV, POV with PM-like lesions, and true PM, according to whether the venous or capillary lesions are believed to predominate. This can be a hazardous approach if the nature of the sample is not specified. It is likely that explant or autopsy examination of an entire lung will produce a different emphasis on a putative spectrum than will analysis of biopsy material. One point that is abundantly clear, however, is the critical importance of recognizing both POV and PM as rare causes of unexplained pulmonary hypertension because the treatment with vasodilators is contraindicated and may even be life-threatening (30,31).

Presently, the final distinction between POV and PM requires tissue diagnosis, which is not always feasible. Both the role and the need for lung biopsy in the diagnosis and management of pulmonary hypertension are still under debate.

Pulmonary occlusive venopathy and PM are rather uncommon (fewer than 200 cases have been reported in the published data) but increasingly recognized causes of pulmonary hypertension. They could represent part of the spectrum of vasculopathies of PPH, but they are distinct from the precapillary causes of PAH. The hypertensive angiopathies with exclusive involvement of the precapillary bed share similar morphological lesions of intimal fibrosis, medial hypertrophy, and plexiform lesions, and they generally have a favorable response to drugs such as epoprostenol. Patients with POV and PM may respond to vasodilators with life-threatening edema, and it is therefore of great clinical importance that they be distinguished from precapillary PAH (30,31). It is well recognized that POV in some cases shows extensive abnormality of the pulmonary arterial vascular bed with apparently equal involvement of the venous and arterial components of the circulation. For this situation, the term “pulmonary vascular occlusive disease” has been adopted, but in the current state of pathogenetic knowledge it may itself be confusing (32).

In relation to the second issue, the term pulmonary vascular occlusive disease (or vaso-occlusive) generally denotes extensive involvement of large and small pulmonary arteries
by narrowing or occlusion of the same type of intimal fibrosis as seen in the pulmonary veins, indicating that the tendency to thrombosis was not limited to the veins but also affected the arteries. The availability of explant or autopsy tissue may define the main focus of the vascular pathology on either the venous or arterial aspects of the pulmonary circulation (as compared to biopsy material), but there is no doubt that, even with whole lungs to examine, some cases are difficult to classify. Significant advances in the genetics and pathogenesis of pulmonary hypertension may help to resolve these issues.

Additionally, it is now well described that abnormalities in bone morphometric protein receptor-2 (BMPR2) signaling plays a significant role in the majority of familial and in some sporadic cases of pulmonary hypertension. It is therefore fascinating to note a case (6) of POV (PVOD) apparently caused by an inherited mutation in BMPR2. There was no histopathological assessment of the proband mother’s pulmonary hypertension, but features characteristic of POV were found in the proband herself (6) upon review of the open-lung biopsy. Interestingly, the patient improved clinically with epoprostenol and has remained stable for many years, which is quite uncommon in POV. It is likely that the systematic and uniform description of the nature and extent of widespread arterial lesions in PVO/PM and the coexistence of venous lesions in PAH, as recommended in the new pathological classification, together with studies of BMPR2, BMPR1a, TIE-2, and angiopoietin expression in the various forms of pulmonary vasculopathy, will provide information about the position of predominantly arterial, venous, capillary, or mixed forms on a clinical and histological spectrum (33). These exciting developments will surely enable a better informed analysis of the links between POV and PCH/PM with precapillary pulmonary hypertension. This may also allow further refinement of PCH-like lesions in these settings and also in chronic pulmonary venous hypertension. Similar studies may shed light on capillary angiogenesis versus congestion and dilation. Primary pulmonary hypertension appears now to be an angioproliferative disease, and PCH/PM may represent uncontrolled angiogenesis, either de novo or arising from a reactive/hyperplastic process.

Reprint requests and correspondence: Dr. Giuseppe G. Pietra, Via San Giorgio 23, CH 6976 Castagnola, Switzerland. E-mail: gkpietra@freesurf.ch.

REFERENCES


Mutations in two receptors of the transforming growth factor-beta family have recently been shown to be present in the majority of cases of inherited (familial) pulmonary arterial hypertension (PAH). Study of the biology of these receptors, bone morphogenetic protein receptor type-2 (BMPR2), and activin-like kinase type-1 (ALK-1) will certainly reveal pathogenic mechanisms of disease. Exonic mutations in BMPR2 are found in about 50% of patients with familial PAH, and ALK1 mutations are found in a minority of patients with hereditary hemorrhagic telangiectasia and co-existent PAH. Because familial PAH is highly linked to chromosome 2q33, it is likely that the remaining 50% of familial cases without exonic mutations have either intrinsic BMPR2 abnormalities or alterations in the promoter or regulatory genes. Also, only about 10% of patients with sporadic idiopathic PAH have identifiable BMPR2 mutations. Mutations in BMPR2 confer a 15% to 20% chance of developing PAH in a carrier's lifetime. Thus, there must be gene-gene or gene-environment interactions that either enhance or prevent the development of the vascular disease in persons carrying a mutation, and there must be other patterns of susceptibility based on genetic makeup. To elucidate the genetic basis of PAH further, investigations are needed, including genome scanning for major and minor genes, analysis of genetic profiles of patients for candidate genes likely to modify risk for disease (e.g., serotonin transporter alleles, nitric oxide-synthases), proteomics, transgenic mice, and altered signal transduction. Advances in genetic testing, presymptomatic screening, and biomarkers should permit early detection of disease in those at risk of PAH and allow trials of preventive therapy in carriers. (J Am Coll Cardiol 2004;43:33S–39S) © 2004 by the American College of Cardiology Foundation.
although as a member of the TGF-beta family, ALK1 is likely to share signaling abnormalities with mutated BMPR2 (12,13).

THE BMPR2 MUTATIONS IN FAMILIAL PAH (PPH)

The BMPR2 gene, on chromosome 2q33, has 13 exons. Exons 1–3 encode an extracellular domain, exon 4 encodes the transmembrane domain, exons 5–11 a serine/threonine kinase domain, and exons 12 and 13 a very large intracellular C-terminus of unknown function that appears to be unique to BMPR2. Mutations in familial PPH have been reported in all exons except for 5 and 13 (9,10). Polymorphisms have been found in exons 6, 8, and 12. Each mutation is unique to a family and co-segregates with disease. The amino acid substitutions resulting from point mutations are in either highly conserved or functionally critical domains of the receptor, and thus are predicted to alter receptor function (14–16). The TGF-beta family of receptors is highly conserved throughout nature. Other missense mutations predict truncation of coding of the transcript. The location of the mutation in families with BMPR2-related PPH does not seem to alter the gender ratio, age of onset, or severity of disease. Locations of many of the known mutations in BMPR2 are shown in Figure 2. A mutation in BMPR2 was recently reported in a family with pulmonary hypertension due to veno-occlusive disease, suggesting that the signaling abnormality is not confined to the precapillary arterioles in all cases (17).

THE BMPR2 MUTATIONS IN “SPORADIC” PAH

About 25% of sporadic cases of PPH were initially thought to have BMPR2 mutations (18), although more recent unpublished analyses of sporadic cases by multiple groups have placed this closer to 10%. Semantic confusion arises when an apparently sporadic case of PPH is found to have an exonic BMPR2 mutation. In some cases, this is apparently a new spontaneous germline mutation, which presumably can be inherited by any offspring and carries the same 15% to 20% risk of disease. In other cases, the mutation has been found to be inherited, and thus is really a family case, albeit the first diagnosis in the family. The rate of mutations in the general population is unknown, but must be exceedingly low because of their absence in control populations. Of the remaining cases of apparently sporadic PPH, about 90%, a small number might have intronic BMPR2 mutations, ALK1, or other Mendelian causes, but the majority do not appear to have a major genetic basis for disease. It is likely that genetic predispositions exist based on normal variations in genes that may influence the pulmonary circulation (Table 2).

THE BMPR2 MUTATIONS IN OTHER COHORTS

The population carrier frequency for BMPR2 mutations has not been measured. However, an indirect estimate suggests that it is as low as 0.001% and may be as high as 0.01% (19). In data concerning normal control cohorts already published, amounting to about 350 subjects (9,10,14,19), no exonic BMPR2 mutations have been found that would be predicted to alter function of the receptor.

PAH with appetite-suppressant drugs. Mutations of the BMPR2 gene have been reported in PAH associated with fenfluramine derivatives (19). Three BMPR2 mutations were found in 33 unrelated French patients (9%), and a fourth mutation in two sisters. The three single mutations were exon 2 A246C, exon 5 G545A, and exon 11 T1447C, all predicted to reduce function of the receptor. The two sisters had an exon 6 631C >T mutation, resulting in a nonsense mutation (R211X) predicted to produce a truncated protein. The mutation-positive patients had a some-
what shorter duration of fenfluramine exposure before illness than did the mutation-negative patients. The import of this data is compatible with the working hypothesis that gene–gene or gene–environmental interactions are required for the onset of PPH.

**PAH with scleroderma-spectrum disease.** Germline heterogeneous BMPR2 mutations were not found in 24 patients with PAH in the scleroderma spectrum of disease (20). However, one centromere–positive Jewish patient with localized cutaneous CREST had an exon 13 G2948A (R983Q) variant considered likely to be a polymorphism because the same mutation was also found in one of 100 normal Israeli Ashkenazi Jews. This polymorphism has not been found in 350 normal chromosomes. Another report failed to find BMPR2 mutations in 12 patients with PAH and connective tissue diseases (21).

**Human immunodeficiency virus (HIV)-associated PAH, portal hypertension and congenital heart disease.** No BMPR2 mutations were found in 19 French patients with HIV-associated PAH, 11 intravenous drug abuse, and 8 via sexual/blood contact (22). No BMPR2 mutations were found in 11 similar U.S. patients and 25 British patients (J. A. Morse and R. C. Trembath, unpublished data, June 2003). There have been no published reports of BMPR2 mutations in adults and children with PAH and congenital systemic-to-pulmonary shunts and in portopulmonary PAH.

**Immunogenetic studies of PAH.** There are few immunogenetic studies of PPH despite earlier reports of antinuclear antibodies in this disease. Previous immunogenetic studies of PPH have reported antibody/HLA-DR, -DQ correlations in small subsets of patients (23). Anti-fibrillin-1 autoantibodies were the only ones found in high frequency; 70 of 75 adults with PPH (93%), 28 of 33 children with PPH (85%), and 12 of 18 with fenfluramine-associated PAH (67%) (22), but no significant HLA-class II associations were discovered.

A recent study of three U.S. ethnic groups with (scleroderma spectrum of disease), Hispanics, African Americans, and Caucasians, found African Americans more likely to

---

**Figure 1.** The process leading to the discovery of mutations in bone morphogenetic protein receptor type–2 (BMPR2) as the cause of familial primary pulmonary hypertension is depicted. Collection of deoxyribonucleic acid from families with sufficient numbers of affected and unaffected members allowed linkage studies using microsatellite markers that led to identification of a chromosome interval on chromosome 2 at q31–32. Candidate genes known from the Human Genome Project (HGP) in the interval were then identified and tested by deoxyribonucleic acid sequencing. Point mutations in exons of the BMPR2 gene were found that co-segregated with affected individuals known from the family pedigrees.
have diffuse skin involvement and pulmonary hypertension (24). Caucasians had more anticentromere antibodies and African Americans more anti-U1-ribonucleoprotein and anti-Brillarin (U3-RNP) antibodies. Of interest, HLA-DQB1*0301 (DQ7) was also increased in sporadic PPH with tissue plasminogen activator antibodies. Autoantibodies against B23, a nucleolar phosphoprotein, were associated with systemic sclerosis and PAH (25). Also, B23 positivity was related to anti-Brillarin antibodies, anti-RNP antibodies, and decreased lung capacity, but HLA alleles were not assessed. An Italian group found anti-topoisomerase IIα antibodies in SSc with pulmonary hypertension and HLA-B35, but no HLA-class II associations (26). This same group also correlated the menopause with HLA-B35 as a risk factor for isolated pulmonary hypertension in SSc (27). It is hoped that future studies will provide the patient numbers and immunogenetic associations using the required careful ethnic matching of patient cohorts to controls.

**SCREENING OF ASYMPTOMATIC CARRIERS OF IDIOPATHIC PAH (PPH) GENES**

In PPH, symptoms almost never develop until there is advanced obstruction of the vascular bed with extreme pulmonary hypertension and right heart dysfunction (28). It is hoped that earlier diagnosis and treatment of this disease may lead to better outcomes.

One recent approach to early diagnosis is the identification of gene carriers in families with heritable PPH by measurement of pulmonary arterial pressure at rest and during exercise (29). These studies suggest that 95% of family members who possess the risk haplotype or the BMPR2 mutation have an abnormal pulmonary artery systolic pressure (PASP) response to exercise (29,30). Most mutation carriers in the study were asymptomatic and had normal pulmonary vascular resistance and pulmonary artery pressures at rest and normal right heart measurements and function. In most subjects carrying the risk haplotype or the BMPR2 mutation, an abnormal increase of PASP (range, 41 to 80 mm Hg) occurred during exercise at low workloads (50 to 125 W). These pressures equaled or exceeded systolic...
pressures achieved with extreme exercise (240 W) in athletes (31).

Measurement of PASP using Doppler echocardiography during supine bicycle exercise might be useful to reveal genetic susceptibility to the disease. In a preliminary follow-up of 28 PPH-gene carriers, one subject with an abnormal response to exercise manifested PPH within three years. Screening by echocardiography has also resulted in the identification of PPH in several asymptomatic family members (32). Furthermore, only a small portion of asymptomatic abnormal responders will develop overt disease, and predictive biomarkers have not yet been defined.

Pulmonary systolic pressure during exercise may rise to high levels in some normal individuals, potentially leading to a false positive diagnosis. In addition, left ventricular filling dysfunction may lead to reactive pulmonary hypertension and the false diagnosis of PAH. To elucidate these important issues, a prospective, controlled European Union project to evaluate Doppler echocardiography during exercise and hypoxic challenge in PAH families and in control subjects has been started.

GENETIC TESTING AND COUNSELING

The value of a genetic test to estimate an individual’s risk for heritable disease depends on the risks and benefits of such knowledge (33). In some diseases where the penetrance is high and preventive treatment is available (e.g., multiple endocrine neoplasia), the benefits of gene testing far outweigh the risks (34). Conversely, when no effective intervention is available (e.g., Huntington’s disease), the risk of genetic certainty may exceed the benefit of knowing the answer. In all genetic diseases, both pretest and posttest genetic counseling are essential for best care of subjects (35).

Current understanding and the state of clinical testing in familial PPH. The gene that codes for the BMPR2 receptor is large (13 exons); presently, screening for mutations is confined to persons with a known positive family history. Even in this circumstance, the sensitivity of testing for BMPR2 mutations is limited, because at least 50% of families studied to date do not have exonic mutations in BMPR2. Haplotype testing can be performed if a large enough group of affected and unaffected members of a family are available. However, tests for known BMPR2 mutations can be performed at a reasonable cost with high sensitivity and specificity. One problem is that each family has a unique mutation. The penetrance of disease for all known BMPR2 mutations is variable, as low as 15% to 20% in most families, but as high as 80% in some family groups (36). Therefore a deoxyribonucleic acid-based test for known BMPR2 mutations identifies increased risk to develop PPH but not necessarily the disease itself. Genetic testing is simply not ready for broad implementation in idiopathic PAH, but is appropriate in screening programs in families where the mutation is known and counseling is available.

Federally certified clinical laboratories are required by law to assure accuracy of results and avoidance of errors such as contamination of specimens. Genetic testing and counseling must be done solely for the benefit of the person tested. Issues of family planning, family relationships, work environment, self-image, insurability, and social comfort can only be sorted by careful personal counseling by an informed and experienced genetic counselor.

Preliminary study of attitudes and understanding in familial PPH. A first step in measuring and understanding the psychosocial implications of familial PPH was explored before BMPR2 was identified in association with PPH (37). Eighty-two members of a family cohort (75%) agreed to take part, and 62 completed a phone interview. More than 66% of respondents stated that they probably or definitely would have genetic testing if it became available. There was a greater interest in testing if a definite answer could be provided (p < 0.001). The most important reason for wanting testing done was to learn about the risk to their children. Reasons given for not wanting testing included concern about the effects on family, ability to handle the results emotionally, and concern about insurance and insurability (although this factor was less prominent than in other studies). Nearly 20% of the respondents appeared to be confused about the difference between diagnostic testing and the donation of blood for research studies (37).

Numerous investigations have revealed that the expressed interest in predictive genetic testing often exceeds actual uptake. In Huntington’s disease, more than 80% of people at risk expressed interest in testing, but only 10% of these individuals have chosen testing since the gene was identified (38). Factors affecting a person’s decisions about testing vary, and include gender (women are more likely to be tested), perceived risk, desire to decrease uncertainty, availability of effective and acceptable medical interventions, concern about one’s current and future children, and the presence of symptoms of depression (39).

GENETIC MODIFICATION OF THE RISK FOR ACQUIRING PPH

Table 2 lists some known and potential genetic modifiers of the risk for development of PAH. The list is necessarily incomplete, and the relative importance of each or any of these genes is unknown. There may be groupings of polymorphisms that confer risk for PAH, especially if there are environmental stresses (such as fenfluramine or splenectomy). The analysis of associations of genes with the risk for disease is a complex statistical problem. Pulmonary arterial hypertension is a complex genetic disease, and the interaction of underlying major genes (such as BMPR2) and modifying genes coupled with environmental stresses will be the focus of future investigations. In addition to statistical associations, functional studies will be necessary to confirm that an associated gene product actually modifies risk.

The serotonin transporter is the gene and product repre-
senting the best-studied modifier of pulmonary hypertensive states. Cultured pulmonary artery-smooth muscle cells from patients with PPH have an abnormally strong proliferative response to serotonin or serum (40). This abnormal response is due to overexpression of the serotonin transporter (5-HTT), owing in part to a functional polymorphism located on the 5-HTT gene promoter: homozygosity for the (L) allele, the long gene promoter variant associated with a high level of gene transcription, is found in 65% to 75% of patients with PPH as compared to 25% to 30% of controls (41). Recent results showing that 5-HTT gene polymorphism determines the severity of pulmonary hypertension (PH) in hypoxemic patients with chronic obtrusive lung disease support a major role for LL-genotype-driven 5-HTT overexpression in the pathophysiology of various forms of PH. Thus, 5-HTT gene polymorphism may be either an important modifier of the PH phenotype or a factor conveying susceptibility to PH in some individuals (42).

Transgenic models of PAH. Several groups are studying mouse models that carry BMPR2 mutations. The first of these, developed by Beppu et al. (43), is a true null. Mice that are homozygous for this mutant BMPR2 die very early in development during gastrulation. Delot et al. (44) have also developed a mouse that carries an in-frame deletion of exon 2 resulting in loss of the ligand binding portion of the extracellular domain. Mice homozygous for the hypomorphic allele also die in utero, but not until day 12.5. Pathologic analyses of these mice have shown persistence of the truncous arteriosus of the heart, demonstrating the importance of functioning BMPR-II for normal heart development.

Recently, Beppu et al. (43) have reported analyses of mice that carry the null BMPR2 allele in the heterozygous state. Though these mice did not spontaneously develop PH, after three weeks of chronic hypoxia, both pulmonary vascular resistance and right ventricle/left ventricle + septum were greater in the heterozygous mice than in the homozygous wild-type mice. Overexpression of 5-lipoxygenase (5-LO) in the lungs of the BMPR2 null heterozygous mice using a replication-deficient adenovirus led to an increase in PA pressure as compared to homozygous wild-type mice. Thus, 5-LO may modify the susceptibility of BMPR2 null heterozygous mice to the development of PPH.

Genetic manipulation of the laboratory mouse has become a very powerful tool for the study of human disease. Simple transgenics and knockouts can potentially be useful for the “quick” determination as to whether a specific gene, when overexpressed or downregulated, can play a role in the susceptibility to the development of PH. The transgenic and knockout models can be crossed to produce mice carrying mutations in more than one gene to examine the synergistic effects of certain combinations of mutations to determine modifier gene effects. Unfortunately, tissue specificity may play an important role in development of the disease, and these studies will then require animal models for which gene expression in specific tissues can be switched on and off. The transgenic approach to the study of PPH is clearly in its infancy. There are many mouse models yet to be generated, and only time will tell as to their usefulness for the unraveling of the pathogenesis of the disorder.

Summary. Future studies will focus on the search for modifiers, both environmental and genetic, that determine the initiation and perpetuation of the disease in individuals harboring mutations in genes conferring increased risk for this devastating condition. These studies will benefit from recent technological advances that enable tissue and cell-specific profiles of both the deoxyribonucleic acid transcript and the proteomic product together with the generation of targeted animal models of the disease. The search for and subsequent investigation of potential modifier genes will require assessment in patient and family cohorts sufficiently powered to generate statistically robust outcomes. Studies of the functional consequences of such mutations on cellular signaling will be necessary to understand the biology of disease. Recent advances have established the need to develop protocols for presymptomatic screening of at-risk family members as a prelude to the initiation of clinical trials of preventive therapy. Therapy aimed at either prevention or actual reversal of the vascular disease process will be the ultimate goal for the betterment of these patients and their families.

Reprint requests and correspondence: Dr. John H. Newman, Division of Pulmonary and Critical Care Medicine, T 1219 Vanderbilt University Medical Center North, Nashville, Tennessee 37220-2650. E-mail: John.Newman@med.va.gov.

REFERENCES

10. Lane KB, Machado RD, Paicuilo JR, et al. Heterozygous germline mutations in BMPR2, encoding a TGF-beta receptor, cause familial


Diagnosis and Differential Assessment of Pulmonary Arterial Hypertension

Robyn J. Barst, MD,* Michael McGoon, MD;† Adam Torbicki, MD;‡ Olivier Sitbon, MD,§ Michael J. Krowka, MD;† Horst Olschewski, MD;|| Sean Gaine, MD¶

New York, New York; Rochester, Minnesota; Warsaw, Poland; Clamart, France; Giessen, Germany; and Dublin, Ireland

Pulmonary arterial hypertension (PAH) is diagnosed by various investigations that are essential for making the diagnosis, and by additional tests to clarify the category of pulmonary hypertension (PH). A diagnostic algorithm can guide the evaluation of PH, but like all guidelines the algorithm can be modified according to specific clinical circumstances. Most patients are diagnosed as the result of an evaluation of symptoms, whereas others are diagnosed during screening of asymptomatic populations at risk. Right heart catheterization (RHC) must be performed in patients with suspected PH to establish the diagnosis and document pulmonary hemodynamics. Before initiation of medical therapy, assessment of acute vasoreactivity (during catheterization) is necessary to determine the appropriate therapy for an individual patient. An acute response is generally defined as a decrease in mean pulmonary arterial pressure of at least 10 mm Hg with the mean pulmonary arterial pressure decreasing to 40 mm Hg or below, accompanied by a normal or high cardiac output. After PAH is diagnosed, disease severity should be assessed in order to accurately determine riskbenefit profiles for various therapeutic options. Useful tools to predict outcome include functional class, exercise capacity, pulmonary hemodynamics, acute vasoreactivity, right ventricular function, as well as brain natriuretic peptide, endothelin-1, uric acid, and troponin levels. Repeating these tests serially on treatment is useful for monitoring the response to a given therapy. Close follow-up at a center specializing in management of PH is recommended, with careful periodic reassessment and adjustment of therapy. (J Am Coll Cardiol 2004;43:40S–47S) © 2004 by the American College of Cardiology Foundation

The diagnosis of pulmonary hypertension (PH) involves two stages: detection (determining the cause of a patient’s symptoms, or to detect the presence of pulmonary arterial hypertension [PAH] in a high-risk patient) and characterization (determining the specific clinical context of the PH, including causal factors, associated diseases or substrates, hemodynamic perturbations and their localization, and sequelae).

DETECTION

Symptom evaluation. Symptoms that suggest PH are exertional dyspnea, fatigue or weakness, angina, syncope, peripheral edema, and abdominal distension (Fig. 1). Exertional intolerance is quantitated with the World Health Organization (WHO) classification (Table 1).

Screening. Periodic assessment of patients with an underlying predisposition may be warranted to introduce therapy at an early stage, or to initiate more aggressive surveillance to detect progression. Screening for the presence of PAH using Doppler echocardiography is advisable when risk is sufficiently high to justify the expense (i.e., when diagnosis could lead to further evaluation and/or change in management). This currently includes individuals with: 1) a known genetic-mutation–associated PAH or a first-degree relative with idiopathic pulmonary arterial hypertension (IPAH); 2) scleroderma spectrum of disease; 3) patients with congenital heart disease and systemic-to-pulmonary shunts; or 4) portal hypertension undergoing evaluation for orthotopic liver transplantation (1). Other potential PAH substrates do not warrant routine screening (e.g., previous use of appetite suppressant, HIV infection, other connective tissue disorders, obstructive pulmonary disease, or high-altitude residence because of the infrequency and/or low likelihood of altering treatment at a presymptomatic stage).

Incidental discovery. The clinical significance and natural history of asymptomatic or mild PAH is unclear; thus, the implications for further assessment and/or treatment when discovered incidentally, as a result of screening or during evaluation of nonspecific symptoms, remain uncertain. Moreover, the criterion of clinically significant PH when detected under these circumstances by Doppler echocardiography, which in itself is an isolated estimate of right ventricular systolic pressure (RVSP), is not precisely defined. Commonly used definitions of PH are a pulmonary artery systolic pressure (PASP) >35 mm Hg or mean >25 mm Hg at rest or mean >30 mm Hg with exercise. However, PASP >40 mm Hg is present in 6% of otherwise normal individuals older than 50 years and 5% with a body mass index (BMI) >30 kg/m² (2).

In general, any degree of PH should prompt an attempt to define or exclude possible causes, because it may be the first evidence of a modifiable substrate. However, the
severity of PH and the reliability of the measurement should temper the aggressiveness of the evaluation. Confirmation by right heart catheterization (RHC) is warranted before embarking on extensive evaluation for an underlying cause or considerations of prognosis or treatment.

**Detection assessment.** Components of assessment to detect PAH include the physical examination (e.g., left parasternal lift, accentuated pulmonary component of S2, pansystolic murmur of tricuspid regurgitation, diastolic murmur of pulmonary insufficiency, right ventricular S3, jugular vein distension, hepatomegaly, peripheral edema, ascites, cool extremities), chest X-ray, electrocardiogram (ECG), and Doppler echocardiogram. Findings of central pulmonary arterial and/or right ventricular enlargement on chest X-ray suggest the presence of PAH. Additional clues to possible associated diseases should be considered, such as pulmonary venous hypertension—for example, septal lines and pleural effusions (pulmonary venous hypertension due to left heart filling abnormality, pulmonary veno-occlusive disease, pulmonary capillary hemangiomatosis); hyperinflation (chronic obstructive pulmonary disease); or kyphosis (restrictive pulmonary disease). Marked asymmetry of the enlarged central pulmonary arteries may be a clue to chronic thromboembolic disease.

The ECG may provide suggestive or supportive evidence of PAH by demonstrating right ventricular hypertrophy and strain, and right atrial dilation. Right ventricular hypertrophy on ECG is present in 87% and right axis deviation in 70% of patients with IPAH (3). The ECG has inadequate sensitivity (55%) and specificity (70%) to be a screening tool for detecting significant PAH (4).

**Transathoracic Doppler echocardiography (TTE)** estimates PASP and can provide additional information about the cause and consequences of PH. The PASP is equivalent to RVSP in the absence of pulmonary outflow obstruction. The RVSP is approximated by measurement of the systolic regurgitant tricuspid flow velocity v and an estimate of right atrial pressure (RAP) applied in the formula: $RVSP = 4v^2 + RAP$. The RAP is either a standardized value, or an estimated value from characteristics of the inferior vena cava (5) or from jugular venous distension. Tricuspid regurgitant jets can be assessed in 39% to 86% of patients. Careful Doppler examination by experienced sonographers yields audible tricuspid regurgitant signals in 74% of cases (6).

**Abbreviations and Acronyms**

- BMI = body mass index
- CI = cardiac index
- CTEPH = chronic thromboembolic pulmonary hypertension
- ECG = electrocardiogram
- IPAH = idiopathic pulmonary arterial hypertension
- PA = pulmonary artery
- PAH = pulmonary arterial hypertension
- PASP = pulmonary artery systolic pressure
- PH = pulmonary hypertension
- RAP = right atrial pressure
- RHC = right heart catheterization
- RVSP = right ventricular systolic pressure
- TTE = transthoracic echocardiography
- V/Q = ventilation-perfusion

**Figure 1.** Guidelines for evaluating pulmonary hypertension. Abbreviations: BNP = brain natriuretic peptide; CBC = complete blood count; CT = computed tomography; CTD = connective tissue disease; EBCT = electron beam computerized tomography; HIV = human immunodeficiency virus; HRCT = high-resolution computerized tomography; LFTs = liver function tests; PH = pulmonary hypertension; RHC = right heart catheterization; SaO2 = systemic arterial oxygen saturation; TEE = transesophageal echocardiography; VD = vasodilator; V/Q = ventilation/perfusion.
Although obstructive pulmonary disease with hypoxemia may be confirmed by testing, abnormalities occur in other types of PH. Approximately 20% of patients with chronic pulmonary embolism have restrictive parameters (i.e., lung volumes <80% predicted) but may have near normal diffusing capacity for carbon monoxide (DL_{co}). The DL_{co} of 20% of patients with limited systemic sclerosis is below normal; a DL_{co} of <55% of predicted may be associated with future development of PAH.

**Screening overnight oximetry** will exclude significant obstructive sleep apnea/hypopnea.

*Ventilation-perfusion* (*V/Q*) lung scintigraphy is an indispensable component of assessment because chronic thromboembolic pulmonary hypertension (CTEPH) is a potentially curable cause of PH. The *V/Q* scans of patients with chronic pulmonary embolism generally show at least one segmental-sized or larger perfusion defect. Patients with PH who have normal *V/Q* scans are unlikely to have chronic pulmonary embolism, and more likely to have IPAH. In three studies (10–12), *V/Q* scanning showed sensitivity of 90% to 100%, with a specificity of 94% to 100% for distinguishing between IPAH and CTEPH. The *V/Q* scans tend to correlate poorly with the severity of obstruction and to underestimate the degree of severity of large vessel obstruction. Scans consistent with thromboembolism may represent false positives, where the actual underlying pathology is pulmonary artery sarcoma, large vessel pulmonary vasculitis, extrinsic vascular compression, or pulmonary veno-occlusive disease.

*Blood tests* for evaluation of PAH include antinuclear antibody (ANA) titer to screen for connective tissue disease, HIV serology, complete blood count with platelet count, liver function tests, and antiphospholipid antibodies (lupus anticoagulant, anticardiolipin antibodies). Although 40% of patients with IPAH have positive but low ANA titers (≥1:80 dilutions) (13), patients with a substantially elevated ANA and/or suspicious clinical features require further serologic assessment and rheumatology consultation.

*Assessment of exercise capacity* is a key part of the evaluation of PH. The goals of exercise testing vary under different clinical circumstances, and they determine the specific testing modality to employ. These objectives include: searching for alternative or contributory reasons for symptoms (e.g., myocardial ischemia); determining maximal exercise tolerance; characterizing comfortable activity level (functional capacity) of the patient; obtaining predictive data; establishing a baseline measure of exercise capacity and following the response to therapy; assessing the interaction of the circulatory and ventilatory systems; or attempting to discover abnormal pulmonary hemodynamic responses to exercise before clinically evident PH at rest.

**CHARACTERIZATION: ESSENTIAL TESTS**

Certain tests are essential if PH is suspected in order to characterize potential substrates, to determine severity and prognosis accurately, and to select treatment (Fig. 1). The *Doppler echocardiogram* provides essential information in addition to screening, as noted above, including estimation of baseline and follow-up PASP, estimated pulmonary vascular resistance, right ventricular size and function, semiquantitative right atrial size, left ventricular systolic and diastolic function, presence of prognostically relevant pericardial effusion, morphology and function of all cardiac valves, as well as patent foramen ovale, and intracardiac or intrapulmonary shunts (using “bubble” contrast techniques).

**Pulmonary function testing** will exclude or characterize the contribution of underlying airway or parenchymal disease. Although obstructive pulmonary disease with hypoxemia

---

**Table 1. World Health Organization Classification of Functional Status of Patients With Pulmonary Hypertension**

<table>
<thead>
<tr>
<th>Class</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Patients with pulmonary hypertension in whom there is no limitation of usual physical activity; ordinary physical activity does not cause increased dyspnea, fatigue, chest pain, or presyncope.</td>
</tr>
<tr>
<td>II</td>
<td>Patients with pulmonary hypertension who have mild limitation of physical activity. There is no discomfort at rest, but normal physical activity causes increased dyspnea, fatigue, chest pain, or presyncope.</td>
</tr>
<tr>
<td>III</td>
<td>Patients with pulmonary hypertension who have a marked limitation of physical activity. There is no discomfort at rest, but less than ordinary activity causes increased dyspnea, fatigue, chest pain, or presyncope.</td>
</tr>
<tr>
<td>IV</td>
<td>Patients with pulmonary hypertension who are unable to perform any physical activity at rest and who may have signs of right ventricular failure. Dyspnea and/or fatigue may be present at rest and symptoms are increased by almost any physical activity.</td>
</tr>
</tbody>
</table>

The 6MWT, developed for evaluation of congestive heart failure (14), is predictive of survival in IPAH and also correlates inversely with WHO functional status severity, moderately with baseline cardiac output and total pulmonary resistance (but not mean pulmonary arterial pressure), and strongly with peak exercise oxygen consumption (VO₂), peak oxygen pulse, and minute ventilation–carbon dioxide output slope (Ve-VCO₂ slope) in IPAH. Arterial oxygen desaturation >10% during the 6MWT increases mortality risk 2.9 times over a median follow-up of 26 months (15).

Cardiopulmonary exercise testing is discussed in more detail elsewhere in this Supplement. Consistent observations in PAH are reduced peak VO₂, peak work rate, the ratio of VO₂ increase to work rate increase, anaerobic threshold, peak oxygen pulse, and increased Ve-VCO₂ slope. These observations indicate that the mechanism(s) of exercise limitations in PAH include V/Q mismatching, lactic acidosis at a low work rate, arterial hypoxemia, and inability to adequately increase stroke volume and cardiac output (16).

Exercise Doppler echocardiography has been utilized to evaluate RVSP responses with exercise. In healthy men, tricuspid regurgitant velocity increases from an average of 1.72 m/s at baseline to a peak of 2.46 m/s at mid-level exercise and to 2.27 m/s at peak exercise (240 W); in trained athletes the baseline value is 2.25 m/s and increases to 3.41 m/s at peak exercise (17). As with invasive exercise studies, the criteria for an abnormal response are not well established.

Right heart catheterization is required to confirm the diagnosis of PAH. Cardiac output, determined by thermodilution or Fick (with measured oxygen consumption) during RHC is also required to calculate pulmonary vascular resistance. Pulmonary arterial hypertension is defined by a mean pulmonary artery pressure (mPAP) >25 mm Hg at rest or >30 mm Hg with exercise; in PAH, pulmonary capillary wedge pressure or left ventricular end diastolic pressure is ≤15 mm Hg and pulmonary vascular resistance is >3 units. The RHC also characterizes intracardiac shunting, and it establishes pulmonary venous pressure. An elevated pulmonary capillary wedge pressure supports the presence of left heart disease or pulmonary vein obstruction, though a normal pulmonary capillary wedge pressure does not rule out pulmonary veno-occlusive disease.

Hemodynamic measurements have been used to estimate the natural history of IPAH in an individual patient. The probability of survival P(t) one, two, or three years after diagnosis can be estimated as \( P(t) = (H(t))^{10^{(x,y,z)}} \)
where \( H(t) = (0.88 - 0.14t + 0.01t^2) \), \( A(x,y,z) = e^{(0.007325x + 0.0526y - 0.3275z)} \), \( t = \) years, \( x = \) mPAP (mm Hg), \( y = \) mRAP (mm Hg), and \( z = \) cardiac index (CI) (l/min/m²) (18). Other logistic regression equations have been reported to predict survival or death within one year.

Importantly, a vasodilator study should be performed whenever PAH is discovered or confirmed during RHC in patients in whom symptoms and/or disease severity warrant treatment. All patients in whom vasodilator treatment is to be initiated require hemodynamic monitoring for detection of either beneficial or detrimental effects of acute treatment. Uncontrolled studies have suggested that long-term administration of calcium-channel blockers (CCB) prolongs survival in the rare subset of responsive patients compared with unresponsive patients. Unfortunately, no clinical or hemodynamic parameters exist that can predict acute and/or chronic responses to CCB in PAH patients. It is generally accepted that patients who may benefit from long-term CCB can be identified by an acute vasodilator challenge performed during RHC; that is, a sustained benefit from CCB treatment is seen in patients in whom, during acute vasodilator testing, mPAP decreases ≥10 mm Hg to reach a mPAP ≤40 mm Hg with a normal or high cardiac output (19). Owing to the potential risk of severe life-threatening hemodynamic compromise occurring with acute vasodilator challenge with CCB, acute vasodilator testing should be done using a safe, potent, and short-acting vasodilator with limited side effects to identify with accuracy those patients who may benefit from long-term CCB therapy.

The acute pulmonary effect of short-acting vasodilators (e.g., intravenous epoprostenol, inhaled nitric oxide [NO], intravenous enoximone, or inhaled iloprost) predicts the hemodynamic response to long-term CCB. Thus, the efficacy of the short-acting drug has been used to determine whether chronic CCB treatment should be initiated. However, with novel oral and inhaled therapeutic agents combining vasodilatory and antiproliferative properties now available (e.g., endothelin receptor antagonists, prostacyclin analogues, and phosphodiesterase type 5 inhibitors), the usefulness of invasive testing for pulmonary vasoreactivity in selecting optimal treatment is now less clear. Although it is reasonable to believe that patients who respond to intravenous epoprostenol, inhaled NO, intravenous adenosine, or inhaled iloprost will respond to such oral or inhaled therapies, no study has evaluated the acute and chronic responses to these drugs in vasoreactive patients.

The abrupt development of pulmonary edema during acute vasodilator testing suggests the presence of pulmonary veno-occlusive disease or pulmonary capillary hemangiomatosis and is a contraindication to chronic vasodilator treatment.

**CHARACTERIZATION: CONTINGENT TESTS**

Certain tests are contingent on the presentation and results of essential testing (Fig. 1). They may not be necessary in all patients. Transesophageal echocardiography (TEE) provides important data that may alter treatment in up to 25% of patients. Useful in the detection of intracardiac shunts, especially atrial septal defects, TEE can also detect central pulmonary emboli including chronic thromboemboli causing PAH, with a reported sensitivity of 80% (20), and up to 96%, with a specificity of 88%, in patients with documented severe central acute or chronic thromboembolism (21).

Additional imaging may be required if the V/Q scan is
suggestive of chronic pulmonary embolism. Chest computerized tomography (CT) provides supportive noninvasive evidence, but if negative it should not obviate the use of pulmonary angiography. A mosaic pattern of lung attenuation in a noncontrast CT scan raises the possibility of chronic thromboembolism. Contrast-enhanced spiral (or helical) CT or electron-beam CT (EBCT) can visualize central chronic pulmonary thromboemboli. The CT features of chronic thromboembolic disease are complete occlusion of pulmonary arteries, eccentric filling defects consistent with thrombi, recanalization, and stenoses or webs.

The sensitivity of spiral CT for detecting central pulmonary embolism is >85% to 90%. Though sensitivity for detecting distal emboli is lower, detection rates of up to 97% for distal emboli (compared with high probability V/Q scans or angiography) have been reported (20). Specificity is also 90%, with occasional misclassification of IPAH as being CTEPH (20).

The EBCT signs of pulmonary vein obstruction (e.g., pulmonary veno-occlusive disease) are smooth thickening of interlobular septa, peribronchovascular cuffing, and alveolar ground-glass opacification.

High-resolution CT provides detailed views of the lung parenchyma during evaluation of PH or hypoxia and aids in the diagnosis of pulmonary fibrosis. Diffuse bilateral thickening of the interlobular septae and the presence of small, centriflobular, poorly circumscribed nodular opacities suggest pulmonary capillary hemangiomatosis; diffuse central ground-glass opacification and thickening of interlobular septa suggest pulmonary veno-occlusive disease.

Pulmonary angiography is required to confirm CTEPH and assess operability. Chronic thrombi appear different from acute thrombi and occur in highly variable locations, often incorporated into and retracting the vessel wall. Obstructions can take the form of bands or webs, sometimes with post-stenotic dilation. Irregular intimal surface, rounded or pouch-like termination of segmental branches, luminal narrowing of the central vessel, and odd-shaped

Figure 2. (A) Long-term (7-year) survival in patients with idiopathic pulmonary arterial hypertension (IPAH) based on functional class (III vs. IV) at the time of epoprostenol initiation. \( p = 0.0001 \) by log-rank test (30). (B) Survival in patients with IPAH treated with intravenous epoprostenol according to New York Heart Association (NYHA) functional class. Estimated percentages of survival for patients in NYHA functional class IV at baseline (dashed line) were 76%, 60%, and 47% at one, two, and three years, respectively, as compared with 90%, 76%, and 71% for patients in NYHA functional class III at baseline (solid line) \( (p < 0.001 \) by the Cox-Mantel log-rank test) (32).

Figure 3. (A) Subsequent survival in patients with idiopathic pulmonary arterial hypertension (IPAH) stratified by functional class after 1 year epoprostenol treatment: \( p < 0.001 \) for functional class III vs. functional class IV and for functional class III vs. functional class I and functional class II (30). (B) Survival in patients with IPAH treated with intravenous epoprostenol according to New York Heart Association (NYHA) functional class. After three months of treatment with epoprostenol, survival rates for patients reclassified in NYHA functional class I or II (solid line) were 100%, 93%, and 88% at one, two, and three years, respectively, as compared with 77%, 46%, and 33% for patients persisting in NYHA functional class III or IV (dashed line) \( (p < 0.001 \) by the Cox-Mantel log-rank test) (32).
For patients with known or suspected CTEPH, further evaluation of a potential clotting diathesis is warranted (bleeding time, coagulation Factors VIII, VII, II, and V, von Willebrand factors, Protein C and S). Factor V Leiden mutation (the most common cause of activated protein C resistance) has been implicated as high risk for idiopathic venous thromboembolism, though not specifically in pulmonary embolism or CTEPH. Serum viscosity, serum protein electrophoresis, and Hgb electrophoresis may be helpful under certain circumstances.

Additional blood tests may provide prognostic data. Arterial blood gas or oximetry measurements showing desaturation may signal abnormal gas exchange, right-to-left shunting, ventilation/perfusion mismatching, interstitial fibrosis, other parenchymal lung disease, or hypoventilation. Failure to normalize with high FiO₂ oxygen inhalation supports a component of right-to-left shunting. Arterial blood gas measurement or oximetry during exercise may disclose desaturation requiring supplemental oxygen treatment to improve exercise capacity. Overnight oximetry may disclose disordered sleep with frequent desaturations and may be the first clue to sleep apnea sufficient to cause or contribute to PH. Nocturnal hypoxemia occurs in >75% of IPAH patients independent of the occurrence of apneas or hypopneas (22).

Hyperuricemia occurs with high frequency in patients with PH and correlates with hemodynamic abnormalities (e.g., right atrial pressure) and mortality in IPAH (23).

Brain natriuretic peptide (BNP) is elevated in right ventricular pressure overload and correlates with severity of right ventricular dysfunction and mortality in PAH (24).

If the history and/or screening overnight oximetry is suggestive, polysomnography should be considered to assess a possible contributory role in PH. Up to 20% to 27% of patients with sleep apnea syndromes have PH (25). The degree of waking PH in obstructive sleep apnea is generally mild and reversible by six months of continuous positive airway pressures, i.e., CPAP (26). Open or thorascopic lung biopsy entails substantial risk of morbidity and mortality. Because of the low likelihood of altering the diagnosis, routine biopsy is discouraged. Under certain circumstances, histopathologic findings may provide useful information by excluding or establishing a diagnosis of active vasculitis, granulomatous pulmonary disease, pulmonary veno-occlusive disease, pulmonary capillary hemangiomatosis, interstitial lung disease, or bronchiolitis (27).

**ASSESSMENT OF PAH SEVERITY**

After PAH is diagnosed, in order to assess risk-benefit profiles for various therapeutic options accurately, precise assessment of prognosis as a function of disease severity is required. Measurable descriptors of disease severity have several potential applications: 1) accurate comparison of patient populations, 2) precise characterization of a patient population for the purpose of homogeneous enrollment into clinical protocols, 3) valid comparison of post-treatment severity of disease, 4) valid comparison of post-treatment severity of similar patients on alternative treatment strategies, 5) accurate longevity prediction (or surrogate for survival in clinical studies), and 6) useful early prediction for timing of transplantation. The characteristics of ideal descriptor(s) of disease severity include: 1) easily performed assessment (or surrogate for survival in clinical studies), and 6) useful early prediction for timing of transplantation. The characteristics of ideal descriptor(s) of disease severity include: 1) easily performed assessment procedure, 2) reproducible results temporally and between centers, 3) high correlation with outcome of interest (e.g., survival), 4) low risk, 5) low expense, 6) low discomfort level, and 7) wide availability.

Various demographic and hemodynamic characteristics, as well as exercise capacity, acute pulmonary vasoreactivity, assessment of right ventricular function, neurohormonal levels (e.g., BNP [24], norepinephrine [28]), as well as endothelin-1 (29), uric acid (23), and troponin (31), correlate with survival. Some of these modalities may provide
prognostic information similar to that derived from invasive tests (e.g., cardiac catheterization) and may prove more useful and convenient in assessing treatment efficacy. These tools may also increase predictive accuracy when used in combination. Because many of these variables have been shown to correlate with one another, which parameter(s) will prove to be most useful for decision making and optimizing treatment (e.g., timing of transplantation) will require further study. It is important to remember that these tests evaluated IPAH patients and not patients with PAH related to connective tissue disorders, congenital systemic to pulmonary shunts, HIV infection, or portal hypertension. Thus, these parameters must be applied cautiously to PAH patients in whom comorbid factors might contribute to the overall outcome; for example, patients with PAH related to connective tissue disorders are known to have a worse prognosis than do IPAH patients, whereas patients with PAH related to congenital systemic to pulmonary shunts have a more slowly progressive course than do IPAH patients.

In addition to evaluating various parameters, such as functional class (32) or exercise capacity, at the time of diagnosis prior to initiation of medical therapy, repeating these parameters on treatment is useful in predicting outcome with a given therapy (e.g., epoprostenol). McLaughlin et al. (30) as well as Sitbon et al. (32) demonstrated that the baseline functional class in patients with IPAH treated with epoprostenol (i.e., before epoprostenol is started) is predictive of survival with epoprostenol (Fig. 2). In addition, patients’ functional class on chronic intravenous epoprostenol is also predictive of outcome with continued epoprostenol (Fig. 3) (30,32). Additional survival parameters include: 1) right atrial size and pericardial effusion assessed by echocardiography; 2) exercise endurance assessed by the 6MWT, both at the time of diagnosis as well as on chronic medical therapy (e.g., epoprostenol); and 3) hemodynamics. It appears that although the baseline 6-min walk distance prior to epoprostenol treatment is useful as a parameter in predicting disease severity, once a patient is on epoprostenol, the actual 6-min walk distance on chronic epoprostenol is more predictive of survival than the change in 6-min walk distance with epoprostenol (Fig. 4) (32). By multivariate analysis in IPAH patients treated with epoprostenol, Sitbon et al. (32) reported that the only parameter independently predictive of outcome prior to starting epoprostenol is the presence of right heart failure; after three months of epoprostenol, persistence of functional class III or IV, mPAP <59 mm Hg and a drop in pulmonary vascular resistance <30% relative to baseline are associated with a poor prognosis.

In conclusion, evaluating disease severity as a function of: 1) end-organ consequences (e.g., right heart failure), 2) symptoms and functional limitation, and 3) markers of decreased survival is necessary for decision making to optimize the appropriate aggressiveness of PAH patients’ care.

REFERENCES

End Points and Clinical Trial Designs in Pulmonary Arterial Hypertension
Clinical and Regulatory Perspectives

Marius M. Hoeper, MD,* Ronald J. Oudiz, MD,† Andrew Peacock, MD,‡ Victor F. Tapson, MD,§ Sheila G. Haworth, MD,¶ Adaani E. Frost, MD,‖ Adam Torbicki, MD#

Hannover, Germany; Torrance, California; Glasgow, Scotland; Durham, North Carolina; London, United Kingdom; Houston, Texas; and Warsaw, Poland

To date, randomized controlled clinical trials performed in pulmonary arterial hypertension (PAH) have been relatively short-term studies involving mainly patients with advanced disease. The primary end points in these trials have addressed exercise capacity, usually by using the 6-min walk test. Although this approach is still warranted in future trials assessing new treatments, it is likely that the focus will shift toward trials of longer duration, involving patients with less advanced disease, and that different drugs and drug-combination regimens will be compared. In such trials, it is possible that a composite of markers indicating clinical deterioration (e.g., hospitalization for right heart failure, the requirement for the introduction of an alternative treatment, and predefined indicators of worsening exercise tolerance) may be more useful as primary end points. Quality of life will become a very important issue; however, appropriate quality-of-life questionnaires for PAH have yet to be developed. In addition, hemodynamics will likely remain valuable as secondary end points, but future clinical trials should include hemodynamics obtained both during exercise and at rest. Finally, cardiopulmonary exercise testing, echocardiographic studies, and biochemical parameters, such as brain natriuretic peptide or troponin T, may also prove useful as secondary end points in the future. (J Am Coll Cardiol 2004;43:48S–55S) © 2004 by the American College of Cardiology Foundation

Seven randomized, placebo-controlled trials of medical treatments for pulmonary arterial hypertension (PAH) have been published in recent years (1–7). In all of these trials, exercise-related measurements were used as primary end points, the most common measurement being the 6-min walk test (8). If the goal of a clinical trial is to convince patients, physicians, funding agencies, and regulatory bodies of the value of treatments, which are often expensive, it is worth considering whether other end points should be examined that more adequately describe changes occurring with these treatments. From patients’ perspectives and increasingly from the perspective of regulatory agencies, quality of life (QoL) is likely to be one of the most important measures of success or failure of a particular treatment. However, some clinicians treating PAH may not be satisfied with improvements in QoL alone; many might need to see physiological improvements such as changes in pulmonary hemodynamics, exercise physiology, circulating hormones, or cardiopulmonary morphology. Evidence of increased survival is highly desirable.

The choice of appropriate end points for clinical trials in PAH should also take into account the requirements of regulatory agencies, which usually are the ultimate referees in the approval process of a particular treatment.

In the process of decision making for the implementation of new trials for PAH, it appears that the two main issues that need to be addressed are the trial design and the choice of the appropriate primary and secondary reinforcing end points.

The views presented here are a summary from the Third World Symposium on Pulmonary Hypertension, held in Venice, Italy, in 2003, and from an expert symposium held just prior to this meeting in Gleneagles, Scotland, in 2003, in order to prepare the topic of “Endpoints in PAH” for the Venice meeting. Participants of the task force on medical treatment and end points in Venice and the participants of the Gleneagles meeting are listed in the appendix.

CLINICAL TRIAL DESIGN

After the regulatory approval of oral bosentan for the treatment of New York Heart Association/World Health Organization (NYHA/WHO) functional class III and IV PAH patients, and after additional oral compounds became available for clinical studies in PAH, several key questions have arisen: 1) are placebo-controlled studies still possible to assess these new compounds? 2) are noninferiority studies feasible? 3) are withdrawal studies ethical? and 4) how should combination therapies be addressed? All of these questions are examined below.

Placebo-controlled studies. Currently, four treatments are approved either by American (Food and Drug Administra-
shown to improve survival in a randomized, controlled trial(2,4,6). Patients enrolled in these latter randomized trials include the following: 1) it may be considered unethical to expose patients to three to four months of placebo when symptomatic and clinical deterioration is known to occur in such time; 2) NYHA/WHO functional class II patients appear to be a “minority” in the population observed in clinical practice; and 3) the current priority in clinical practice might be one of comparison of new treatments with approved ones.

Weighing these arguments, the working groups in Scotland and Italy concluded that, given the outlined conditions, placebo-controlled trials for shorter periods of time (up to four months) are still justified from an ethical and scientific point of view.

Noninferiority studies. Noninferiority comparative studies can be an alternative to superiority placebo-controlled studies if the latter are considered inappropriate for ethical and/or practical reasons. In fact, comparative studies designed to demonstrate superiority would be difficult to perform in a rare disease like PAH because the required sample size would be quite large, relative to the disease prevalence. The objective of a noninferiority study is to demonstrate that no statistically significant difference exists between two compounds for a given end point. Therefore, noninferiority studies can only be performed with established primary end points that have already been investigated.

Noninferiority studies also require a setting identical to that of the pivotal study of the comparator (inclusion criteria, exclusion criteria, patient population, study protocol, among others). If the comparator were bosentan, for example, the study design would require that the 6-min walk test be used as the primary end point, as it was in the pivotal study of bosentan (2). Although a superiority study over bosentan would be prohibitive in terms of sample size, in the above scenario, the sample size would also need to be quite high (>500 patients), so that even noninferiority trials bear considerable risks and costs for the sponsors.

Withdrawal studies. Withdrawal studies can be suggested by regulatory agencies for different reasons. For example, when pivotal trials have not shown a clinically relevant benefit, or a more consistent proof of efficacy is required, the FDA may suggest or require that a withdrawal study be performed before granting regulatory approval. In a life-threatening disease such as PAH, a withdrawal study might be considered inappropriate for several reasons. Withdrawal of an effective drug may cause clinical deterioration, which could be irreversible and life-threatening even if the previ-
ous treatment were to be reinstituted. As a consequence, the end point of such a study would be to assess clinical worsening. This could be considered against the basic medical principle, primum non nocere ("First, do no harm"). For these reasons, approval of such a study by institutional review boards and patient acceptance of such a trial would be difficult.

During the Venice symposium, the Endpoints Task Force members concluded that withdrawal studies should not be recommended in PAH patients with functional class III or IV symptoms who are receiving a single active treatment that has proven to be effective in randomized, placebo-controlled trials. However, withdrawal studies may be a useful means to study the efficacy of combination therapies (see the following text).

**Combination therapies.** Given that no currently available PAH treatment offers the potential for cure or long-term stabilization for the majority of PAH patients (other than lung transplantation) (10–12), it is likely that a combination of active treatments will be used increasingly in the near future. Preliminary studies suggest that combinations of prostanoids and phosphodiesterase inhibitors or prostanoids and endothelin receptor antagonists are safe and effective in selected PAH patients (13,14). However, for reasons concerning efficacy, safety, and costs, it is imperative that combination treatments are carefully investigated. As stated above, one useful means to study combination treatments could be withdrawal studies. Alternatively, combinations of two (or more) compounds could be studied in an add-on design, using classical end points. Provided that the sample size is high enough, even more information might be derived from three-arm studies including both compounds alone and in combination.

**PRIMARY AND SECONDARY REINFORCE END POINTS ACCORDING TO REQUIREMENTS OF REGULATORY AGENCIES**

For PAH, it appears that regulatory agencies are currently accepting as "primary" end points only traditional clinical end points such as exercise capacity, QoL, time to clinical worsening and mortality. Usually, physiologic parameters such as hemodynamics are considered "secondary."

In PAH trials, assessment of exercise capacity using the 6-min walk test can still be considered a good choice for a primary end point for several reasons, including the possibility to compare the results with previous trials. However, it is important that the clinical relevance of a given improvement in the distance walked is defined a priori in the protocol of the study to avoid inconclusive discussions at the end of the trial. This might be accomplished by defining clinical relevance according to the treatment effect obtained with already approved treatments, because the term "clinical relevance" can be somewhat arbitrary.

Quality of life as a primary end point has been proposed, but concerns include the lack of validation that has been provided for this parameter in PAH thus far (see the following text).

Time to clinical worsening as a combined end point requires standardization to make the end point more objective and comparable. This parameter has most often been defined in PAH trials as the combination of death, hospitalizations due to worsening of PAH, and escalation of treatments (need for epoprostenol or lung transplantation). The last two events are influenced by the judgment of the attending physician, and as an end point it should be supported by some objective findings, such as predefined thresholds defining deterioration in exercise capacity or hemodynamic parameters.

Hemodynamic parameters have traditionally been considered as "secondary," or reinforcing end points, based on their prognostic value (15), and this concept has been accepted by regulatory agencies. The Endpoints Task Force members agreed that using selected echocardiographic parameters as noninvasive substitutes of hemodynamics should be explored.

The usefulness of biological markers as end points for clinical trials, such as brain natriuretic peptide (BNP), troponin, and endothelin, has yet to be tested and validated in clinical studies, and thus, at present, cannot be proposed to regulatory agencies as primary end points.

**END POINTS IN CLINICAL TRIALS**

According to the Venice task force, an end point may be defined as a measurement used by investigators conducting a clinical trial to determine whether patients benefited from drug administration. The choice of an end point should reflect the desired therapeutic goals. In PAH patients with NYHA/WHO functional class III or IV symptoms, improvement of exercise capacity may be considered a primary therapeutic goal, and thus end points addressing exercise tolerance may be appropriate. In patients with NYHA/WHO functional class I and II symptoms, slowing or stopping progression of disease may be considered the primary goal. Most of the clinical trials in PAH performed thus far have included mainly patients with NYHA/WHO functional class III symptoms; however, it is likely that the focus of clinicians treating PAH will shift to early detection of pulmonary hypertension in patients with less severe disease. Thus, in such a scenario it is critical that end points are chosen that reflect clinical stability, that is, lack of deterioration.

**Clinical end points.** Clinically relevant events such as death, hospitalization for right heart failure, or the requirement for the introduction of alternative ("rescue") treatments have been widely used as secondary, often combined, end points in most of the randomized clinical trials of PAH treatments. In those studies, which included mainly class III and IV patients, the active treatments were able to show a significant reduction of these events after the three- to four-month study periods (2,6,9). However, it is anticipated that observation periods will have to be much longer when...
patients with less advanced illness are being studied or when different active treatments are being compared to each other, or combined. In these circumstances, it is important for safety reasons that clinical end points encompass not only the aforementioned indicators of severe complications but also encompass other variables that define clinical deterioration, such as a predefined decline in 6-min walk distance or in peak oxygen consumption. One suggested combined end point might combine all of the above variables, and be termed failure-free survival.

Exercise studies: 6-min walk test and cardiopulmonary exercise testing (CPET). Assessment of functional capacity in both clinical and research applications is an important tool for evaluating disease severity. The most widely used test to assess exercise capacity in PAH patients has been the 6-min walk test, the main advantage being its ease of administration. The 6-min walk test correlates with several parameters of CPET and provides important prognostic information (10,16). Paciocco et al. (17) showed an 18% reduction in the risk of death per additional 50 m walked in patients with PPH performing the 6-min walk test.

The 6-min walk test has also been successfully used in scleroderma-associated pulmonary hypertension (18) and in the Eisenmenger syndrome (19), but there has been no study of the 6-min walk test for portopulmonary hypertension patients. In addition, the 6-min walk test has not been validated as an end point in PAH patients with less severe disease (i.e., NYHA/WHO functional class I and II symptoms).

Measurement of ventilation and pulmonary gas exchange during exercise testing provides additional information to that derived from standard exercise testing. This modality (CPET) offers an objective evaluation of functional capacity and provides information beyond that of most traditional exercise tests, some of which are independent of patient effort. Although the peak oxygen consumption (Vo2) may be limited by factors other than circulatory impairment, it is very helpful in describing the magnitude of exercise limitations in comparison to normal controls. During submaximal exercise, anaerobic or lactic acid threshold can be obtained as a surrogate for peak Vo2, and may reflect circulatory limitations. Finally, the slope of Ve/Vco2 or the Ve/Vco2 ratio measured at the anaerobic threshold is nearly always elevated in patients with PAH, and reflects the impaired pulmonary circulation that is the hallmark of PAH.

Several studies of CPET in PAH have significantly contributed to our understanding of the symptoms and pathophysiology of patients with PAH who experience dyspnea during exercise (20,21). In addition, Wax et al. (22) showed that in 16 PPH patients undergoing CPET, peak Vo2 and O2 pulse increased significantly after two years of treatment with intravenous epoprostenol. Wensel et al. (23) found that baseline peak Vo2 and the baseline maximum systolic blood pressure during exercise were strong and independent predictors of survival.

The 6-min walk test has been used successfully as primary end point in many clinical studies in PAH (3,4,18,24) and is, to date, the only measure of exercise capacity that has been accepted by the FDA, based on its validation as a marker of a treatment effect. In contrast, presently there is limited experience with the use of CPET in multicenter trials. In one recent multicenter study on beraprost in PAH patients, the 6-min walk test revealed a significant improvement after three and six months of treatment, whereas there were no significant changes in CPET variables (5). These results raised several questions and concerns about the quality of the CPET studies performed at each study center, especially as the 6-min walk test itself was used as a surrogate for CPET, based on its correlation with peak Vo2.

It appears that the results of CPET were largely influenced by the experience of the involved centers, whose variability was probably related to the interpretation of the CPET studies rather than to the performance of the studies. For these reasons, the Endpoints Task Force members believe that CPET cannot currently be recommended as a primary end point in multicenter trials on new treatments for PAH. However, the use of a core CPET laboratory might avoid errors in interpretation made at individual study centers. Finally, because of the substantial physiologic information provided by CPET, this technique might be used in substudies involving centers with experience in CPET in order to generate secondary end points in forthcoming studies.

The NYHA and WHO functional classification. The NYHA functional classification system is widely used as a marker of disease severity in cardiovascular disease (25). One of the simplest and crudest assessments of exercise capacity, the NYHA functional classification has been shown to correlate with disease severity and outcome in a multitude of cardiopulmonary disorders, including PPH (10,15). The WHO functional classification system for PAH (26) is an adaptation of the NYHA system. It is nearly identical to the original NYHA functional classification text, with the references to cardiac-specific symptoms such as the deletion of angina, and the addition of references to PAH-related symptoms. In practice, many clinicians refer to both classification systems collectively as NYHA/WHO functional classification.

A major limitation to the NYHA and WHO functional classification systems is that they rely upon patients to report their own limitations. Patients who over- or under-report their physical limitations can lead the clinician to make incorrect conclusions about their patient’s disease severity. In addition, even despite widely accepted definitions, great variability exists in how physicians assign functional classification. It appears that the vast majority of PAH patients reported in the published reports have been classified as having NYHA/WHO functional class III symptoms. It is also evident that this classification may not be revised by treating clinicians even when medical treatments result in subjective and objective functional improvements. A tighter, perhaps subdivided, categorization should be developed and
validated. In the current version, the NYHA/WHO functional classification may be too crude to be used as a primary end point in clinical trials, though it has been utilized successfully as part of a combined primary end point (6). It is likely that functional classification will remain in use as a secondary, reinforcing end point in future clinical trials.

**Mahler dyspnea index.** The Mahler dyspnea index has been used in chronic lung disease (27) and in pulmonary hypertension (6). Compared to the NYHA and WHO functional classification systems, it provides a scale from 0 to 12 with equal contributions of functional impairment, magnitude of task, and magnitude of effort until dyspnea occurs. Systematic comparisons between the Mahler dyspnea index and 6-min walk test or CPET have not been made.

**Hemodynamics.** Progressive remodeling of the pulmonary arteriolar vessels causes an increase in pulmonary vascular resistance (PVR), which increases right ventricular (RV) afterload. Patients become symptomatic mainly because of the inability of their RV to overcome the increased RV afterload, and thus they are unable to adequately increase pulmonary blood flow (cardiac output) for the O₂ exercise demand. Accordingly, a right heart catheterization with measurements of pulmonary vascular pressures and blood flow quantify both the disease process (PVR) and its main functional consequence (cardiac output [CO] limitation). It is therefore understandable that standard hemodynamic measurements in patients with PAH correlate with clinical state, functional class, exercise capacity, and prognosis (10,11,15). However, there has been disappointment related to the fact that these correlations are loose, and that mean pulmonary artery pressures (PAPs) often fail to reach statistically significant correlation with outcome in PAH treatment trials. As such, standard invasive pulmonary hemodynamic measurements have not been considered as a primary end point in randomized controlled trials of new pharmacological approaches to PAH therapy. In fact, there is a current tendency to omit hemodynamic measurements even as a secondary end point. The End Points Task Force believe, however, that a right heart catheterization with pulmonary hemodynamic measurements should be required both for confirming the initial diagnosis of PAH and for evaluating the progression and response to therapy in PAH patients.

The reasons why pulmonary hemodynamic measurements do not appear to be tightly correlated to clinical state in patients with PAH are twofold. First, in most reported studies, measurements are performed at rest only, when RV stress and related symptoms are minimal. Second, mean pulmonary artery pressure (PAPm) and CO determinations may be insufficient to accurately reflect RV afterload. A single-point measurement of PAPm, pulmonary capillary wedge pressure (PCWP), and CO, and derived PVR calculation may be misleading because the inherent assumptions of linearity and zero crossing of the (PAPm – PCWP)/CO relationship are not met (28). It can indeed be shown that multipoint (PAPm – PCWP)/CO coordinates are described by a linear approximation, but present with a positive extrapolated pressure intercept (29,30). Single-point PVR determinations at variable flow therefore may underestimate or overestimate changes in the pulmonary circulation (28). These errors can be avoided by defining PVR along a multipoint pressure/flow line (28).

Recent studies have shown that improvement in exercise capacity with prostacyclin therapy may not be associated with significant changes in pulmonary hemodynamics at rest, whereas PVR defined by a multipoint (PAPm – PCWP)/CO plot shows a significant decrease with therapy (30). Using such a model, variation in flow can be achieved with exercise (30) or with an infusion of low-dose dobutamine (29).

Taken together, right heart catheterization with pulmonary hemodynamic measurements remains central in the diagnosis of PAH, in the evaluation of severity, response to therapy, and prognosis in patients with PAH. Right heart catheterization should therefore remain an essential part of clinical trials. However, in contrast to previous studies, unless there is evidence of severe right heart dysfunction (recurrent syncope, cardiac index <1.5 l/min/m², right atrial pressure >20 mm Hg), pulmonary hemodynamics should be measured both at rest and during exercise to define PVR by defining at least a two-point pulmonary vascular pressure/flow relationship.

**Quality of life.** At present, the objective of patient management in individuals with PAH is to improve survival and QoL. In an attempt to assess the impact of the condition on patients’ QoLs, generic health status measures, or health-related quality of life indicators such as the SF-36 (31), Nottingham Health Profile (32), Minnesota Living with Heart Failure Questionnaire (33), and EuroQoL (34) have been used in studies of PAH. However, these measures assess impairment and disability rather than QoL, and their generic content is too general to provide valid and sensitive assessments. Furthermore, such instruments fail to assess those issues that are of major concern to the QoL of patients (35).

Unlike impairment and disability, QoL questionnaires summarize the impact of both the disease and its treatment on the patient in a single score. The most widely operation-alized model of QoL assessment postulates that QoL is the extent to which patients are able to meet their needs (36). This model goes beyond an assessment of impairment and disability by inquiring how impaired function affects patients’ ability to satisfy their needs. Major developments have been made in the science of QoL assessment (37); however, these are only now starting to be applied in PAH. In these developments, emphasis is being placed on disease-specific measurements, with the content of instruments derived directly from patients, thereby taking account of the specific and unique impact of PAH (38).

Work is currently in progress to develop two new instruments specific to PAH, derived from qualitative, unstruc-
tured interviews with PAH patients. One instrument is designed to assess impairment and disability (i.e., health status or health-related QoL) with particular relevance to PAH. The second is a "needs"-based QoL measure specific to PAH. The former assesses overall impairment, breathlessness, edema, energy level, mood, and physical functioning. Such disease-specific measures also increase the potential for determining disease-specific utility and, consequently, determining the cost-effectiveness of clinical interventions. Once thoroughly evaluated, QoL assessments may be useful as primary or secondary end points in clinical studies on PAH.

**Echocardiography.** Echocardiography is the most widely available and versatile imaging test. Current data indicate that three groups of echocardiographic variables could be potentially useful as end points in PAH. Interestingly, rather than reflecting instantaneous hemodynamics such as right heart pressures and flows, most of those echocardiographic variables reflect chronic consequences of PAH such as: 1) elevated right atrial pressure as evidenced by the presence and size of pericardial effusions (39,40) and by the right atrial area/volume (39,41); 2) RV dysfunction, measured by the Doppler Tei index of myocardial performance or its surrogates (42); and 3) decreased left ventricular (LV) preload, measured by assessing LV eccentricity index or end diastolic area/volume. According to existing evidence, the improvement in LV diastolic dysfunction especially assessed by decreased LV early diastolic filling velocity seems to best reflect the effects of PAH treatment upon echocardiographic measures (39). Most of the variables listed above are simple and easy to obtain from a single apical four-chamber view in patients with PAH.

Tissue Doppler imaging (43) at echocardiography may further simplify the assessment of cardiac dynamics, by providing an assessment of the Tei index from a single tracing. It may also help to break down the Tei index, allowing for separate assessment of RV systolic and diastolic isovolumic time intervals. Also, simple measurements of the duration and flow velocity pattern of RV ejection seem to correlate with results of treatment (39). Recent experimental data suggest that myocardial acceleration during isovolumic RV contraction is load-independent, and strongly correlates with end-systolic RV elastance, the best available measure of RV contractility (44).

Stress studies using dobutamine infusions or exercise may help to increase the sensitivity of detecting abnormal hemodynamics and of following up the effects of treatment. Unfortunately, there is very limited experience with this type of assessment in patients with PAH (45,46).

Although several imaging-based signs have been shown to correlate with indices of functional capacity and survival and have been found to correlate with successful treatment (39,41,47,48), at present none seem suitable for recommendation as a single or primary end point in PAH. Nevertheless, an echocardiographic substudy of the Bosentan Randomized trial of Endothelin Antagonist Therapy (BREATHE)-1 PAH treatment trial (2) performed in centers with substantial experience with this technique provided very useful information validating clinical benefits seen from treatment by showing parallel improvement in several echocardiographic variables (39). This study may serve as a model for including echocardiographic parameters as secondary end points in future clinical studies.

**Hormonal and blood studies.** Several biological markers have been used to aid in our understanding of the pathophysiology of PAH. Despite reports indicating that some biochemical markers may be indicative of poorer survival, these markers have not been included in clinical trials in PAH.

An ideal marker for PAH would be heart-lung specific, abnormal in PAH, easy to collect, easy to measure, and be reproducible; it should also allow patient monitoring (it changes in one direction if patients deteriorate and in the other direction if patients improve), and it should also predict survival. Among the markers that have previously been studied, BNP, uric acid, and the troponins may be the best candidates.

Brain natriuretic peptide is mostly secreted by overloaded myocytes of the ventricles (49). Nagaya et al. (50,51) have found that initial and follow-up supramedian BNP plasma levels were independent markers of prognosis in PAH. The NT-pro BNP (its biologically inactive alternative) is a more stable marker, which may be easier to study (49). Based on the available data, BNP should be included as a secondary end point in upcoming clinical trials on patients with advanced PAH.

Troponin T is another biochemical marker that has recently been linked to the prognosis of PAH (52). Chronic leakage of troponin T can be detected with high-sensitivity tests in a subset of patients with severe pulmonary hypertension. In contrast to other markers, troponin T is indicative of ongoing damage of RV contractile proteins, which may contribute to progressive RV failure. Preliminary studies by Torbicki et al. (52) have suggested that elevation of troponin T was related to poor survival in PAH. They also noted that troponin leakage may disappear with successful treatment as well as appear with progression of the disease. If these findings can be confirmed by larger studies, troponin T may also be a useful secondary end point in trials involving patients with advanced PAH.

Serum uric acid levels correlate inversely with cardiac index and positively with right atrial pressure and PVR (53–55). Baseline and follow-up analyses of this marker indicate that it is related to survival and that hemodynamic improvements are associated with a decrease in uric acid levels (55). This marker is, however, affected by several variables, including drugs, tissue perfusion, decreased glomerular filtration, and hypoxia. Therefore, intra- and interindividual variability of serum uric acid levels may be too high to render this parameter useful for clinical trials.

**Conclusions.** Although short-term placebo-controlled and randomized trials are still feasible for assessing the efficacy
and safety of a PAH treatment, comparative and combina-
tion studies of longer duration will likely be needed in the
future. In patients with moderate to severe functional
impairment, the 6-min walk test will probably remain
widely used as primary end point for proving efficacy of new
treatments. However, in patients with milder functional
impairment, and for longer trials involving patients with
advanced disease (comparative or combination studies),
failure-free survival as defined above may become the most
meaningful end point. Hemodynamic variables remain very
useful as secondary end points, but in future trials these
measurements should be obtained at rest and during exercise
provided that there are no signs of advanced right heart
failure. In addition, imaging studies (e.g., echocardiography
and possibly magnetic resonance imaging) should be in-
cluded in clinical trials. It is also important that PAH-
specific QoL questionnaires be developed and tested. The
usefulness of biochemical variables such as BNP or troponin
T should be studied by including these parameters as
secondary end points, and a similar approach may be
utilized to establish the role of cardiopulmonary exercise
testing in randomized clinical trials.

Reprint requests and correspondence: Dr. Marius M. Hoeper,
Department of Respiratory Medicine, Hannover Medical School,
30623 Hannover, Germany. E-mail: hoeper.marius@mh-
hannover.de.

REFERENCES

endothelin-receptor antagonist bosentan in patients with pulmonary
hypertension: a randomised placebo-controlled study. Lancet 2001;
358:1119–23.
an oral prostacyclin analogue, in patients with pulmonary arterial
hypertension: a randomized, double-blind, placebo-controlled trial.
infusion of treprostinil, a prostacyclin analogue, in patients with
pulmonary arterial hypertension. A double-blind, randomized,
7. Langleben D, Christman BW, Barst RJ, et al. Effects of the throm-
boxane synthetase inhibitor and receptor antagonist terbutalol in
patients with primary pulmonary hypertension. Am Heart J 2002;143:
E4.
new measure of exercise capacity in patients with chronic heart
intravenous epoprostenol (prostacyclin) with conventional therapy for
primary pulmonary hypertension. The Primary Pulmonary Hyperten-
30623 Hannover, Germany. E-mail: hoeper.marius@mh-
epoprostenol infusion in primary pulmonary hypertension. Prognostic
11. McLaughlin VV, Shillington A, Rich S. Survival in primary pulmo-
nary hypertension: the impact of epoprostenol therapy. Circulation
2002;106:1477–82.
12. Kuhn KP, Byrne DW, Arbogast PW, Doyle TP, Loyd JE, Robbins
IM. Outcome in 91 consecutive patients with pulmonary arterial
hypertension receiving epoprostenol. Am J Respir Crit Care Med
Bosentan treatment in patients with primary pulmonary hypertension
long-term adjunct therapy to inhaled iloprost in severe pulmonary
primary pulmonary hypertension. Results from a national prospective
prognostic significance of six-minute walk test in patients with primary
pulmonary hypertension. Comparison with cardiopulmonary exercise
17. Paciocco G, Martinez FJ, Bossone E, Piulsticker E, Gillespie B,
Rubenfire M. Oxygen desaturation on the six-minute walk test and
mortality in untreated primary pulmonary hypertension. Eur Respir J
2001;17:647–52.
18. Badesch DB, Tappson VF, McGoon MD, et al. Continuous intrave-
nous epoprostenol for pulmonary hypertension due to the scleroderma
patients with the Eisenmenger syndrome. Am J Respir Crit Care Med
20. Sun XG, Hansen JE, Oudiz RJ, Wasserman K. Exercise pathophys-
iology in patients with primary pulmonary hypertension. Circulation
detection of exercise-induced right-to-left shunt in patients with
22. Wax D, Garofano R, Barst RJ. Effects of long-term infusion of
prostacyclin on exercise performance in patients with primary pulmo-
23. Wensel R, Opitz C, Anker SD, et al. Assessment of survival in
patients with primary pulmonary hypertension: importance of cardio-
Expert Opin Investig Drugs 2002;11:991–1002.
the Evaluation and Management of Chronic Heart Failure in the Adult:
Executive Summary. A report of the American College of Cardiology/ 
American Heart Association Task Force on Practice Guidelines
(Committee to Revise the 1995 Guidelines for the Evaluation and
Management of Heart Failure): Developed in collaboration with the
International Society for Heart and Lung Transplantation. Endorsed
by the Heart Failure Society of America. Circulation 2001;104:2996–
3007.
27. Mahler DA, Wells CK. Evaluation of clinical methods for rating
28. McGregor M, Sniderman A. On pulmonary vascular resistance: the
of pulmonary vascular resistance in primary pulmonary hypertension.
pressure-flow relations after prostacyclin in primary pulmonary hyper-
31. Ware JE Jr., Sherbourne CD. The MOS 36-item short-form health
survey (SF-36). I. Conceptual framework and item selection. Med Care
32. Hunt SM, McKenna SP, Ewen J, Williams J, Papp E. The
Nottingham Health Profile: subjective health status and medical
33. Rector TS, Cohn JN. Assessment of patient outcome with the
Minnesota Living with Heart Failure Questionnaire: reliability and
validity during a randomized, double-blind, placebo-controlled trial of
pimobendan. Pimobendan Multicenter Research Group. Am Heart J


APPENDIX

Participants of the Gleneagles meeting May 2 to May 4, 2003, on end points in PAH: Andrew Peacock (Chair), Glasgow; Nazzareno Galie (Co-Chair); Lewis J. Rubin (Co-Chair); Joan A. Barbera, Paul Corris, Richard Coulden, Marion Delcroix, Sean Gaine, Philippe Hervé, Marius M. Hoeper, Marc Humbert, David Langleben, Marco Maggiorini, Steven McKenna, Robert Naeije, Joanna Pepke-Zaba, Horst Olschewski, Ronald J. Oudiz, Maurizio Raisino, Jack Reeves, Gerald Simonneau, Olivier Sitbon, Adam Torbicki, Jean–Luc Vachiery.

Task force on medical treatment, Third World Symposium on PAH, Venice, June 23 to June 25, 2003: Nazzareno Galie (Chair); Lewis J. Rubin (Chair); Werner Seeger (Chair); David B. Badesch, Joan A. Barbera, Robyn R. Barst, Ardeschir Ghofrani, Sheila G. Haworth, Marius M. Hoeper, Marc Humbert, Anne Keogh, Vallerie McLaughlin, Horst Olschewski, Ronald J. Oudiz, Andrew J. Peacock, Gérard Simonneau, Olivier Sitbon, Gianni Tognoni, Adam Torbicki.
Prostanoid Therapy for Pulmonary Arterial Hypertension

David B. Badesch, MD,* Vallerie V. McLaughlin, MD,† Marion Delcroix, MD,‡ Carmine Dario Vizza, MD,§ Horst Olschewski, MD,¶ Olivier Sitbon, MD,¶ Robyn J. Barst, MD#

Denver, Colorado; Ann Arbor, Michigan; Leuven, Belgium; Rome, Italy; Giessen, Germany; Clamart, France; and New York, New York

Prostanoids have played a prominent role in the treatment of pulmonary arterial hypertension (PAH). Several compounds and methods of administration have been studied: chronic intravenously infused epoprostenol, chronic subcutaneously infused treprostinil, inhaled iloprost, and oral beraprost. Chronic intravenous epoprostenol therapy has had a substantial impact on the clinical management of patients with severe PAH. It improves exercise capacity, hemodynamics, and survival in patients with idiopathic pulmonary arterial hypertension (IPAH). It also improves exercise capacity and hemodynamics in patients with PAH occurring in association with scleroderma. The complexity of epoprostenol therapy (chronic indwelling catheters, reconstitution of the drug, operation of the infusion pump, and others) has led to attempts to develop other prostanoids with simpler modes of delivery. Treprostinil, a stable prostacyclin analogue with a half-life of 3 h, has been developed for subcutaneous delivery. It has beneficial effects on exercise and hemodynamics, which depend somewhat on the dose achieved. This, in turn, is determined by the patient's ability to tolerate the drug's side effects, including pain and erythema at the infusion site. Inhaled iloprost therapy may provide selectivity of the hemodynamic effects to the lung vasculature, thus avoiding systemic side effects. In a randomized and controlled trial, iloprost resulted in improvement in a combined end point incorporating the New York Heart Association functional class, 6-min walk test, and deterioration or death. Beraprost is the first orally active prostacyclin analogue. In the first of two randomized controlled trials, beraprost increased exercise capacity in patients with IPAH, with no significant changes in subjects with associated conditions. Hemodynamics did not change significantly, and no difference in survival was detected between the two treatment groups. The second study showed that beraprost-treated patients had less disease progression at six months and confirmed the results of the previous trial. However, this improvement was no longer present at 9 or 12 months. In conclusion, though treatment with prostanoids is complicated by their generally short half-lives and complicated drug delivery systems, they continue to play an important role in the treatment of PAH. (J Am Coll Cardiol 2004;43:56S–61S) © 2004 by the American College of Cardiology Foundation

A metabolite of arachidonic acid, prostacyclin is endogenously produced by vascular endothelium. It is a potent vasodilator in both the pulmonary and systemic circulations, and has antiplatelet aggregatory activity. A relative deficiency of prostacyclin may contribute to the pathogenesis of pulmonary arterial hypertension (PAH). Clinical studies have explored the possibility that chronic therapy with exogenous prostacyclin analogues might be of long-term benefit in patients with moderately severe to severe PAH. To date, the following compounds and methods of administration have been studied: chronic intravenously infused epoprostenol, chronic subcutaneously infused treprostinil, inhaled iloprost, and oral beraprost. This report summarizes the rationale for therapy utilizing each of these prostanoids, and it provides currently available evidence supporting the use of each in the treatment of PAH.

From the *University of Colorado Health Sciences Center, Denver, Colorado; †University of Michigan, Ann Arbor, Michigan; §University Hospital Gasthuisberg, Leuven, Belgium; §University of Roma “La Sapienza,” Rome, Italy; ||University Hospital, Justus-Liebig-University, Giessen, Germany; ¶Hôpital Antoine Béclère, Clamart, France; and #Columbia University College of Physicians and Surgeons, New York, New York.

Manuscript received November 26, 2003; accepted February 3, 2004.

EPOPROSTENOL

Rationale. Christman et al. (1) reported a deficiency of prostacyclin and an excess of thromboxane in patients with PAH. Tuder et al. (2) showed decreased expression of prostacyclin synthase in lungs from patients with severe PAH. Exogenously administered prostanoid analogues might help to overcome the adverse effects of decreased endogenously produced prostacyclin. Epoprostenol has a very short half-life in the bloodstream, requiring constant intravenous (IV) administration.

Treatment. In a multicenter, randomized, controlled trial in 81 patients with severe idiopathic pulmonary arterial hypertension (IPAH, formerly known as primary pulmonary hypertension or PPH), continuously intravenously infused epoprostenol plus conventional therapy (oral vasodilators, anticoagulation, others) was compared to conventional therapy alone. The epoprostenol-treated group demonstrated improved survival and exercise tolerance, increased cardiac output, and decreased pulmonary vascular resistance (3). The beneficial effects of epoprostenol therapy are often sustained. Barst et al. (4) reported long-term
and McLaughlin et al. (6) have described sustained benefit in a small group of patients from centers involved in the earliest clinical use of epoprostenol. Shapiro et al. (5) and McLaughlin et al. (6) have described sustained benefit in larger numbers of patients. McLaughlin et al. (7) more recently reported experience with long-term epoprostenol therapy in 162 consecutive patients with IPAH followed for a mean of 36.3 months (median, 31 months) (Fig. 1). Data followed included functional class, exercise tolerance, and hemodynamics. Observed survival with epoprostenol therapy at one, two, and three years was 87.8%, 76.3%, and 62.8%, respectively, and was significantly greater than the expected survival of 58.9%, 46.3%, and 35.4%, respectively, based on historical data. Baseline predictors of survival included exercise tolerance, functional class, right atrial pressure, and vasodilator response to adenosine. Predictors of survival after the first year of therapy included functional class and improvement in exercise tolerance, cardiac index, and mean pulmonary artery pressure.

Sitbon et al. (8) evaluated the factors associated with long-term survival in patients with PPH/IPAH treated with continuous epoprostenol infusion. A total of 178 patients in New York Heart Association (NYHA) functional class III or IV were treated with epoprostenol. Survival rates at one, two, three, and five years were 85%, 70%, 63%, and 55%, respectively. Baseline variables associated with a poor outcome were a history of right-sided heart failure, NYHA functional class IV, 6-min walk test ≤250 m (median value), right atrial pressure ≥12 mm Hg, and, paradoxically, mean pulmonary artery pressure ≤65 mm Hg. Multivariate analysis, including both baseline variables and those measured after three months on epoprostenol, demonstrated that a history of right-sided heart failure, persistence of NYHA functional class III or IV at three months, and the absence of a decline in total pulmonary resistance of ≥30%, relative to baseline, were associated with poor survival. The investigators concluded that survival of patients with IPAH treated with epoprostenol depends both on severity of disease at baseline and the response to three months of therapy.

A multicenter, randomized, and controlled study of chronic IV epoprostenol in patients with PAH occurring in association with the scleroderma spectrum of disease showed improvement in exercise capacity and hemodynamics at 12 weeks as compared to the control group (9). Trends were seen toward greater improvement in severity of the Raynaud phenomenon and fewer new digital ulcers in the epoprostenol group. A survival difference between groups was not seen in this population over the period of study, but the study was not adequately powered to detect such a difference.

Epoprostenol therapy requires continuous IV infusion. The drug has a very short half-life (<6 min), is unstable at acidic PpH, and cannot be taken orally. It is unstable at room temperature, and is generally kept cold prior to infusion. Patients are usually begun on a low dosage of epoprostenol (1 to 2 ng/kg/min), and gradually titrated upward in increments of 1 to 2 ng/kg/min, based upon side effects and tolerance. Many patients seem to reach a “plateau” dose and may not require continued up-titration from that point. Whereas this dose may be between 20 and 40 ng/kg/min for many patients, the dose range is wide, with considerable interindividual variability.

Common side effects of epoprostenol therapy include flushing, headache, jaw pain with the first bite of food, (which is usually tolerable), diarrhea, nausea, a blotchy erythematous rash, and musculoskeletal aches and pain (predominantly involving the legs and feet). These side effects tend to be dose dependent, and they often respond to a cautious reduction in dose. Abrupt or inadvertent interruption of the epoprostenol infusion should be avoided, as this may lead to a rebound worsening of pulmonary hypertension with symptomatic deterioration and perhaps even death. Complications of chronic IV therapy with epoprostenol include line-related infections (which range from exit site reactions, to tunnel infections and cellulitis, to bacteremia or sepsis), catheter-associated venous thrombosis, thrombocytopenia, and ascites (although this may also be a manifestation of severe disease).

**Abbreviations and Acronyms**

- IPAH = idiopathic pulmonary arterial hypertension
- IV = intravenous
- NO = nitric oxide
- NYHA = New York Heart Association
- PAH = pulmonary arterial hypertension
- PPH = primary pulmonary hypertension

**Figure 1.** McLaughlin et al. (7) reported 162 consecutive patients diagnosed with primary pulmonary hypertension and treated with epoprostenol who were followed for a mean of 36.3 months (median, 31 months). Observed survival (diamonds) with epoprostenol therapy at one, two, and three years was 87.8%, 76.3%, and 62.8%, respectively, and was significantly greater than the expected survival (squares) of 58.9%, 46.3%, and 35.4%, respectively, based on historical data. Baseline predictors of survival included exercise tolerance, functional class, right atrial pressure, and vasodilator response to adenosine. Predictors of survival after the first year of therapy included functional class and improvement in exercise tolerance, cardiac index, and mean pulmonary artery pressure. Reprinted with permission from Circulation (Lippincott Williams & Wilkins).
Chronic IV epoprostenol therapy has had a substantial impact on the treatment of patients with moderately severe to severe PAH. It has been best studied in patients with IPAH and PAH occurring in association with the scleroderma spectrum of disease. Because of the complexity of epoprostenol therapy (chronic indwelling catheters, reconstitution of the drug, operation of the infusion pump, and so forth), and the relative rarity of severe PAH, strong consideration should be given to referral to centers of excellence.

**TREPROSTINIL**

**Rationale.** Although epoprostenol is effective therapy for PAH, the nature of the delivery system has a number of potential complications, which range from severity from local exit site infections easily treated with oral antibiotics to life-threatening sepsis. Because of the short half-life of epoprostenol, interruptions in therapy related to catheter life-threatening sepsis. Because of the short half-life of epoprostenol, interruptions in therapy related to catheter displacement or pump malfunction may be life-threatening. Rare adverse events associated with the delivery system include pneumothorax, deep venous thrombosis, and paradoxical embolus. The efficacy of epoprostenol, coupled with the limitations of the delivery system, has led to the development of prostacyclin analogues with alternative routes of delivery. Treprostinil, a stable prostacyclin analogue with a half-life of 3 h, has been developed for subcutaneous delivery.

**Treatment.** In 14 patients with IPAH, treatment acutely with IV epoprostenol and IV treprostinil had similar hemodynamic effects (10). To test the alternative subcutaneous delivery method, the effects of IV treprostinil and subcutaneous treprostinil were compared in 25 patients with IPAH. Acute hemodynamic effects were similar. An eight-week, placebo-controlled, 2:1 randomized trial of subcutaneous treprostinil was subsequently performed in 26 patients with IPAH. An improvement of 37 ± 17 m in the 6-min walk distance occurred in patients receiving the active therapy (from 373 m to 411 m), compared to a 6 ± 28-m reduction in those receiving placebo (379 m vs. 384 m), a nonstatistically significant trend. There was a favorable, but not statistically significant, trend in hemodynamics, with a 20% reduction in pulmonary vascular resistance index over the eight-week period in the group receiving active treprostinil.

Adverse events including headache, diarrhea, flushing, jaw pain, and foot pain were common in the treprostinil group, similar to what had been previously reported with epoprostenol. Pain (occasionally severe), erythema, and induration at the site of the subcutaneous infusion occurred frequently.

A large international, placebo-controlled, randomized study was conducted assessing the efficacy of chronic subcutaneously delivered treprostinil in patients with IPAH or PAH occurring in association with collagen vascular disease or congenital systemic-to-pulmonary shunts (11). Four hundred-seventy patients enrolled between November 1998 and October 1999 in 24 centers in North America and 16 centers in Europe, Australia, and Israel were randomly assigned to receive either continuous subcutaneously infused treprostinil plus conventional therapy or continuous infusion of placebo plus conventional therapy. Owing to the infusion-site pain and reaction that occurred in the proof-of-concept trial, the dosing strategy called for lower doses at initiation, with a maximal allowable dose at the end of 12 weeks of 22.5 ng/kg/min. The primary end point was exercise capacity as measured by the 6-min walk distance, which improved in the treprostinil group and was unchanged with placebo. The median difference between treatment groups was 16 m (p = 0.006), and the effect on exercise tolerance appeared to be dose related. The patients in the lowest two dosing quartiles experienced little improvement in the 6-min walk distance, whereas patients in the highest quartile in terms of dose (≥13.8 ng/kg/min) demonstrated an improvement of 36 m. Treprostinil therapy was also associated with a significant improvement in mean right atrial pressure, mean pulmonary artery pressure, cardiac index, pulmonary vascular resistance, and mixed venous oxygen saturation. Common side effects included headache, diarrhea, nausea, rash, jaw pain, and infusion-site pain. Of the patients, 85% complained of pain at the infusion site, and 83% had erythema or induration at the infusion site. Although statistically significant, improvement in the 6-min walk distance was relatively modest. The reasons for this may be multifactorial. Entry criteria for the treprostinil trial were much more broad than for either of the epoprostenol trials.

**INHALED ILOPROST**

**Rationale.** Inhaled therapy for pulmonary hypertension may provide selectivity of the hemodynamic effects to the lung vasculature, thus avoiding systemic side effects. Pulmonary selective vasodilation has been described for inhaled nitric oxide (NO), but this agent has several drawbacks. Most importantly, there are no data demonstrating improved survival during therapy with inhaled NO, and this agent possesses less vasodilative potency than do prostanoids in IPAH patients (12,13). In contrast, prostacyclin (epoprostenol) has been shown to improve survival, exercise capacity, and hemodynamics in patients with severe IPAH (3,4,6), and the continuous IV infusion of this drug has been approved for therapy in certain groups of patients in the U.S. and several European countries.

**Treatment.** Iloprost is a prostacyclin analogue and has the same biologic profile as the natural substance with respect to prostaglandin receptor binding and cellular effects (14). Its effects and its side effects are similar to those seen during epoprostenol infusion (15). In contrast, the chemical stability of iloprost is considerably better. Where epoprostenol has to be freshly dissolved, continuously cooled, and protected from light to provide full activity, iloprost is stable at room temperature, at pH 7.4, and normal light. Epoprostenol has a half-life of <6 min, whereas iloprost has a
serum half-life of 20 to 25 min (16). Iloprost has been approved for the treatment of PAH in New Zealand, and was recently approved for the treatment of IPAH in Europe. Although epoprostenol is available in the United States and Europe, iloprost is not approved in the U.S.

Owing to the fact that the intra-acinar pulmonary arteries are tightly surrounded by alveolar surfaces, it is possible to vasodilate these vessels by means of alveolar deposition of a prostanoid. For long-term therapy, repetitive inhalations of iloprost are administered six to nine times daily. Each inhalation requires 10 to 15 min. With alternative devices it is possible to reduce the inhalation time to about 4 min (17) and to avoid any noise by use of ultrasound energy for nebulization.

In patients with severe PAH, inhalation of aerosolized iloprost resulted in a substantial decrease in pulmonary artery pressure and resistance, concomitant with an increase in cardiac output, in the absence of significant systemic artery pressure drop and ventilation–perfusion mismatch (18,19). In severe lung fibrosis, an increase of the pulmonary shunt blood flow may limit the use of IV epoprostenol (19), whereas inhaled iloprost can safely be administered. In uncontrolled studies, inhaled iloprost was effective in decompensated right heart failure (20) and showed favorable long-term hemodynamic improvement (21).

A large randomized, double-blind, placebo-controlled European multicenter study with inhaled iloprost (Aerosolized Iloprost Randomized, AIR [22]) involved a total of 203 patients in NYHA functional class III or class IV with IPAH or PAH occurring in association with underlying disorder (mostly IV iloprost), collagen vascular disease, or nonoperable thromboembolic disease. In both the iloprost and placebo groups, approximately 50% suffered from IPAH, and about 60% were in NYHA functional class III and 40% in NYHA functional class IV. The primary end point of the study, defined as an improvement in NYHA functional class compared with at least 10% improvement in the 6-min walking test, and no deterioration or death (combined clinical end point), was reached by 3.4 × more patients in the iloprost versus the placebo group (16.8% vs. 4.9%; p = 0.007). This effect was achieved with a mean inhaled dose of 0.37 ng/kg/min. In the 6-min walk test, the treatment effect was 36.4 m in favor of iloprost (p < 0.01). Hemodynamics significantly deteriorated in the placebo group, whereas in the iloprost group preinhalation values were unchanged compared to baseline, and postinhaletion values were significantly improved. Importantly, the number of patients remaining on study medication, a measure corresponding to event-free survival, was significantly higher in the iloprost than in the placebo group. Over three months of therapy, there was no indication of tachyphylaxis. In the iloprost group, one patient (1.0%) died during the double-blind study period versus four patients (4.0%) in the placebo group. Overall, the therapy was well tolerated. Cough occurred more frequently in the iloprost compared to the placebo group (38.6% vs. 25.5%) as well as headache (29.7% vs. 19.6%) and flushing (26.7% vs. 8.8%). These adverse events were mild and mostly transient. Syncope occurring in the iloprost group was more often rated as serious than in the placebo group, but was not commonly associated with clinical deterioration.

An open-label multicenter study of the two-year effects of inhaled iloprost in pulmonary hypertension with an initial three-month controlled randomized phase was performed in Germany. Inhaled iloprost treatment was administered for up to two years to 63 patients (40 with IPAH and 23 with PAH occurring in association with underlying disorders). The median daily aerosolized dose was 100 μg (total inhaled dose = 24 μg) divided into six inhalations. At study entry, 66.6% of patients were in NYHA functional class III or class IV, and 33.4% in functional class II. During the two-year study period, five IPAH patients were switched to alternative therapy (mostly IV iloprost) but remained in the study, and 13 patients discontinued the study prematurely (7 with IPAH; 6 with other forms of PAH). After two years, 37 patients received inhaled iloprost treatment (25 with IPAH; 12 with other forms of PAH). During the course of the study, eight patients died: three IPAH and five with other forms of PAH. Two of these patients died before receiving inhaled iloprost treatment. The survival rate according to Kaplan–Meier analysis was 0.850 for all patients and 0.914 (95% confidence interval: 0.81; = 1) for IPAH patients for the two-year study period, including the randomized phase. For IPAH patients, the predicted survival rate according to D’Alonzo et al. (23) was 0.631, which corresponds to = 14.8 deaths. In contrast, only three IPAH patients died. This suggests that survival on inhaled iloprost treatment is substantially higher than expected.

In addition to treatment of IPAH, the pulmonary selectivity of inhaled iloprost provides the opportunity to apply prostanoids to patients who are prone to decrease in systemic arterial pressure, as in portopulmonary hypertension, and in emergency situations. The intrapulmonary selectivity also allows prostanoid application to patients who are prone to intrapulmonary right to left shunt, like hepatopulmonary syndrome and lung fibrosis (19).

Inhaled iloprost may provide an alternative to the use of IV epoprostenol. When the clinical effects of inhaled iloprost and IV epoprostenol are compared, inhaled iloprost has some advantages but also certain drawbacks. Most importantly, the inhalation provides potent pulmonary vasodilation with little systemic side effects and no risk of catheter complications. Additionally, it allows therapy in patients with pre-existent ventilation–perfusion mismatch and in those who are prone to develop such a mismatch during systemic prostanoid application. The most important drawback is the fact that the hemodynamic effect of inhaled iloprost plateaus within 30 to 90 min, and that six to nine inhalations per day are needed to achieve satisfactory clinical results. Inhaled iloprost is currently approved in Europe for functional class III IPAH. Long-term survival data are needed.
BERAPROST

Rationale. Beraprost is the first orally active prostacyclin analogue (24). It is rapidly absorbed during fasting, peak concentration is reached after 30 min, and the elimination half-life is 35 to 40 min after oral administration (25). In the monocrotaline-induced pulmonary hypertension model, beraprost has been shown to have a protective effect on the development of pulmonary hypertension lesions (26). High doses of beraprost appear to have inotropic and chronotropic effects in the isolated guinea pig myocardium (27). Beraprost has also been studied in peripheral vascular diseases such as intermittent claudication (28), Raynaud phenomenon, and digital necrosis in systemic sclerosis (29), with variable results.

Treatment. Beraprost has been used to treat PAH since 1995 in Japan; several small open uncontrolled studies have reported beneficial hemodynamic effects with beraprost in patients with IPAH (24,30). Functional class also improved in the majority of the patients after a mean of two months, and pulmonary vascular resistance was reduced by 26% (24,30). Nagaya et al. (31), in a retrospective open uncontrolled study, reported improved survival in 24 IPAH patients treated with beraprost compared to a similar group of 34 patients on conventional therapy. The three-year survival rate was 76% in the beraprost group and 44% in the conventional-therapy group.

Two randomized, double-blind, placebo-controlled trials of beraprost in PAH have been performed. The first was a 12-week double-blind, randomized, placebo-controlled trial performed in 130 NYHA functional class II and class III patients with PAH of various etiologies (IPAH, and PAH associated with collagen vascular diseases, congenital systemic-to-pulmonary shunts, portal hypertension, or human immunodeficiency virus infection) (32). At a median dose of 80 μg administered orally four times daily, beraprost increased exercise capacity assessed by the 6-min walk test: treatment effect was 25 m in the overall population, and 45 m in the IPAH patients, with no significant changes in the exercise capacity of subjects with the associated conditions. Hemodynamics had no statistically significant changes, and no difference in survival was detected between the two treatment groups. Side effects related to systemic vasoconstriction were frequent, mainly in the initial titration period.

A second trial evaluated the effects of beraprost therapy for PAH in 116 NYHA functional class II and class III patients. This study was 12 months in duration, double-blind, randomized, and placebo-controlled (33). It showed that the beraprost-treated patients had less disease progression at six months and confirmed the results of the previous trial (32): improved 6-min walk distance at three months (+22 m from baseline) and six months (+31 m), as compared to placebo. However, this improvement was no longer present at 9 or 12 months (33). No significant changes occurred in hemodynamics or survival at month 12 versus baseline. It is possible that the beneficial effects of beraprost may attenuate with time. Beraprost is approved for treatment of PAH in Japan, and is currently under evaluation by the European Agency for the Evaluation of Medicinal Products (EMEA).

SUMMARY

Chronic IV epoprostenol therapy has had a substantial impact on the clinical management of patients with severe PAH. The complexity of epoprostenol therapy has led to attempts to develop other prostanooids with simpler modes of delivery. Subcutaneous treprostinil, inhaled iloprost, and oral beraprost have all been studied, and they have various relative advantages and disadvantages. In conclusion, although treatment with prostanooids is complicated by their generally short half-lives and complicated drug-delivery systems, they continue to play an important role in the treatment of PAH. Together with the other major classes of therapeutic agents currently utilized or under investigation for the treatment of PAH (including anticoagulants, supplemental oxygen, calcium channel antagonists, endothelin receptor antagonists, phosphodiesterase inhibitors, and NO or NO donors), prostanooids remain an important therapeutic option.

Reprint requests and correspondence: Dr. David B. Badesch, Divisions of Pulmonary Sciences and Critical Care Medicine, and Cardiology, University of Colorado Health Sciences Center, Box C-272, 4200 East Ninth Avenue, Denver, Colorado 80262. E-mail: David.Badesch@UCHSC.edu.

REFERENCES


Endothelin Receptor Antagonists in Pulmonary Arterial Hypertension

Richard N. Channick, MD,* Olivier Sitbon, MD,† Robyn J. Barst, MD,‡ Alessandra Manes, MD,§ Lewis J. Rubin, MD*  

San Diego, California; Paris, France; New York, New York; and Bologna, Italy

Endothelin receptor antagonism has emerged as an important therapeutic strategy in pulmonary arterial hypertension (PAH). Laboratory and clinical investigations have clearly shown that endothelin (ET)-1 is overexpressed in several forms of pulmonary vascular disease and likely plays a significant pathogenetic role in the development and progression of pulmonary vasculopathy. Oral endothelin receptor antagonists (ERAs) have been shown to improve pulmonary hemodynamics, exercise capacity, functional status, and clinical outcome in several randomized placebo-controlled trials. Bosentan, a dual-receptor antagonist, is approved by the U.S. Food and Drug Administration for class III and IV patients with PAH, based on two phase III trials. In addition to its efficacy as sole therapy, bosentan may have a role as part of a combination of drugs such as a prostanoid or sildenafil. The selective endothelin receptor-A antagonists sitaxsentan and ambrisentan are currently undergoing investigation. (J Am Coll Cardiol 2004;43:62S–67S) © 2004 by the American College of Cardiology Foundation

Since the last World Symposium on Pulmonary Hypertension in Evian, France, in 1998, endothelin receptor antagonism has emerged as a cornerstone of therapy for pulmonary arterial hypertension (PAH). Elucidation of the role of endothelin in the pathogenesis and progression of pulmonary vascular disease, the efficacy of endothelin receptor antagonists (ERAs) in randomized clinical trials, and long-term outcome data have placed this therapy at the forefront of the treatment armamentarium. We review here the current state of knowledge related to ERAs in the context of the treatment algorithm described elsewhere in this supplement.

ENDOTHELIN (ET)-1 AS AN IMPORTANT MEDIATOR IN PAH

The endothelins are a family of 21 amino-acid peptides that play a key role in the regulation of vascular tone. The first member of this family identified was ET-1, a 2492-dalton (Da) peptide with potent vasoconstrictor properties, isolated by Yanagisawa et al. in 1988 (1). Two additional endothelin isopeptides—ET-2 and ET-3—were subsequently discovered. The ET-1 appears to play the most prominent role in vascular control. Knowledge of the mechanisms and molecular aspects of ET-1 is important in understanding the therapeutic value of endothelin receptor antagonism.

PRODUCTION OF ET-1

The majority of ET-1 secreted from cultured endothelial cells occurs from the abluminal side of the cells toward the adjacent vascular smooth muscle cells, which contain specific endothelin receptors (2). Thus, it is important to note that, although circulating ET-1 can be detected in the plasma and may have important clinical correlations with pulmonary vascular disease, these plasma levels may not necessarily reflect the paracrine action of ET-1 on adjacent smooth muscle cells.

ENDOTHELIN RECEPTORS

There are two distinct receptors for the endothelin family of peptides, endothelin receptor A (ETₐ) and endothelin receptor B (ETβ). The endothelin receptors belong to the family of receptors connected to guanine nucleotide–binding (G) proteins (3). The two receptors have unique locations (4) and binding affinities (5) for the endothelin peptides. The ETₐ receptors are expressed on pulmonary vascular smooth muscle cells, whereas ETβ receptors are located on both pulmonary vascular endothelial cells and smooth muscle cells.

When activated, the ETₐ receptor located in pulmonary vascular smooth muscle cells mediates a potent vasoconstrictive response, thought to occur via G-protein–induced phospholipase C activation: 1,4,5-inositol triphosphate formation with the consequent release of Ca²⁺ from intracellular stores (3). There is also evidence that the ETₐ receptor mediates increased intracellular calcium by activating non-selective calcium channels on the surface of the smooth muscle cell (6). The vasoconstriction induced by ETₐ has been shown to persist even after ET-1 is removed from the receptor, likely due to persistently elevated concentrations of intracellular Ca²⁺ (7).

In addition to its powerful vasoconstricting effects, ET-1 is known to be a potent mitogen, with the ability to induce cell proliferation in a number of cell types, including vascular smooth muscle cells (8). It has been shown that the
Abbreviations and Acronyms

CTEPH = chronic thromboembolic pulmonary hypertension  
ERAs = endothelin receptor antagonists  
ET = endothelin  
ET\(_{\alpha}\) = endothelin receptor A  
ET\(_{\beta}\) = endothelin receptor B  
IPAH = idiopathic pulmonary arterial hypertension  
6MWT = 6-min walk test  
PAH = pulmonary arterial hypertension  
WHO = World Health Organization  
NYHA = New York Heart Association

mitogenic actions of ET-1 are mediated by both the ET\(_{\alpha}\) (9) and ET\(_{\beta}\) (10) receptors.

In the normal pulmonary vasculature, ET\(_{\beta}\) receptors are predominantly expressed on endothelial cells (11,12). The ET\(_{\beta}\) receptors on endothelial cells mediate vasodilation via increased production of nitric oxide and prostacyclin (12–14). Nitric oxide and prostacyclin also negatively feed back on ET-1 activity by inhibition of prepredoendothelin-1 transcription. In addition, ET-1 is cleared by ET\(_{\beta}\) receptors.

Data suggest that the ET\(_{\beta}\) receptor does not exclusively mediate pulmonary vasodilation. Under some circumstances it may actually contribute to pulmonary vasoconstriction, through a population of ET\(_{\beta}\) receptors located on vascular smooth muscle cells (15). The vasoconstrictive actions of the ET\(_{\beta}\) receptor may become more pronounced in the pathologic setting of pulmonary hypertension (16), possibly due to upregulation of ET\(_{\beta}\) receptors in states of pulmonary hypertension (17). The functions of both receptors under pathologic conditions may therefore determine whether antagonizing one or both receptors is preferable.

In patients with PAH, several derangements in ET-1 expression and activity have been demonstrated. Patients with idiopathic pulmonary arterial hypertension (IPAH, formerly called primary pulmonary hypertension) have been shown to have higher serum levels of ET-1, which has been shown in human investigations to correlate with high levels of ET\(_{\alpha}\) receptor density and circulating ET-1, which in some instances decrease following surgical correction of the cardiac lesions (21–23).

Chronic thromboembolic pulmonary hypertension (CTEPH) has also been associated with increased activity of the ET-1 system in both animal (24,25) and human (26) pathologic studies. Pulmonary hypertensive changes were attenuated in the presence of combined ET\(_{\alpha}/ET_{\beta}\) receptor blockade in a canine model of CTEPH (24). It is known that many patients with CTEPH have a concomitant small vessel vasculopathy that can limit the hemodynamic improvement following pulmonary thromboendarterectomy, suggesting a pathogenic role for endothelin in this process.

**CLINICAL USE OF ERAs**

Given the prominent role that ET-1 appears to play in several forms of pulmonary hypertension, ERAs have a strong rationale. There is now one approved ERA, oral bosentan, for the treatment of PAH. Other ERAs are currently under investigation.

**Bosentan.** Bosentan is an antagonist of both the ET\(_{\alpha}\) and ET\(_{\beta}\) receptors, with only slightly higher in vitro affinity for the ET\(_{\alpha}\) receptor. Two randomized clinical trials led to U.S. Food and Drug Administration (FDA) approval of bosentan for PAH patients who are functional class III or IV.

The first multicenter randomized placebo-controlled study of chronic oral bosentan was performed by Channick and colleagues in 32 patients with IPAH (n = 27) or with PAH related to scleroderma (n = 5) (27). Recruited patients were all World Health Organization (WHO) functional class III, and there was 2:1 randomization to the bosentan group in relation to placebo. Patients in the bosentan group received the drug at a dose of 62.5 mg twice daily for four weeks followed by 125 mg twice daily. Concurrent therapy with digoxin, diuretics, and calcium channel blockers was permitted; however, patients receiving epoprostenol were excluded. The primary end point was exercise capacity as measured by the 6-min walk test (6MWT) and secondary end points included hemodynamic improvement by right heart catheterization, change in functional class, and time to clinical worsening— all measured at 12 weeks. The intention-to-treat analysis demonstrated statistically significant improvements in the bosentan group compared to placebo in 6MWT, with a mean treatment effect of 76 m and pulmonary hemodynamics (cardiac output, pulmonary vascular resistance, mean pulmonary arterial pressure) (Fig. 1).

A subsequent larger double-blind, placebo-controlled study of bosentan in PAH by Rubin et al. (28) enrolled 213 patients with PAH (n = 150) or PAH related to scleroderma (n = 47) or systemic lupus erythematosus (n = 16). All patients belonged to WHO functional class III or IV. Baseline parameters included mean 6MWT of ~330 m, and mean pulmonary artery pressures of ~55 mm Hg. Patients randomized to the bosentan group received 62.5 mg twice daily for four weeks, then either 125 mg twice daily (n = 74) or 250 mg twice daily (n = 70) for an additional 12 weeks, in comparison to placebo (n = 69). The primary end point was functional status as measured by the 6MWT at 16 weeks. This trial also showed a statistically significant improvement in 6MWT in both bosentan groups in comparison to placebo (Fig. 2). Analysis of secondary measures of efficacy revealed a trend in the bosentan groups toward lower Borg dyspnea indices and
improved functional class. There was also a statistically significant increase in the bosentan groups in time to clinical worsening (Fig. 3), as measured by the composite end point of time to death, lung transplantation, hospitalization, or study dropout because of worsening pulmonary hypertension, need for epoprostenol therapy, or atrial septostomy. In addition, in a subgroup of 85 patients enrolled in an echocardiographic substudy, bosentan improved different echocardiographic and Doppler parameters related to the right ventricular systolic function and the left ventricular early diastolic filling (29).

Data on the long-term efficacy of bosentan are now available. A recent report by Sitbon et al. (30) demonstrated sustained improvement in functional class and pulmonary hemodynamics for at least one year.

Mortality data for 169 patients treated with bosentan as first-line therapy was recently presented by McLaughlin et al. (31). In that report, three-year survival was 86% compared to a predicted survival of 48% for these individuals based on a validated National Institutes of Health (NIH) survival equation. In this cohort, at two years, 70% of patients were still maintained on bosentan alone.

**Safety.** Bosentan is primarily eliminated by hepatic metabolism through the P450 enzyme systems CYP2C9 and CYP3A4. Steady-state levels are usually achieved after three to five days with twice daily dosing. Upon reaching steady-state, the elimination half-life becomes constant. One metabolite of bosentan (Ro 48-5033) is pharmacologically active but is believed to contribute <20% of the clinical response to bosentan. Renal clearance of bosentan appears to be negligible.

Clinical evidence suggests that bosentan administration can precipitate hepatocellular injury, particularly at higher doses. Combined data from existing clinical trials reveal greater than threefold elevations of the aminotransferases in 11% of bosentan patients (n = 658) compared with 2% of patients given placebo (n = 280). This effect was observed both early and late in treatment. The more severe elevations in aminotransferases were observed in the patients receiving 250 mg twice daily or higher. The liver abnormalities were often asymptomatic and all resolved with dose reduction or cessation. In some patients, reintroduction of bosentan did not lead to recurrent hepatic enzyme elevations. Studies in rats have revealed that bosentan-induced liver injury is likely mediated by drug-induced inhibition of the hepatocanalicular bile-salt export pump (32).

Patients on bosentan must undergo monitoring of the alanine aminotransferase and aspartate aminotransferase before drug initiation and monthly thereafter. Patients with significant baseline hepatic dysfunction should not be given bosentan. In patients with hepatic congestion from right heart failure, aggressive diuresis may correct abnormal aminotransferases occurring solely on this basis, and consequently requalify these patients for bosentan.

Bosentan is contraindicated in pregnancy. Animal models reveal that the endothelin peptides appear to play an important role in fetal development. In one study (33) ET-1 was implicated in the closure of the ductus arteriosus at birth. Mice with ET-1 deficiency (34) and those given

---

**Figure 1.** Hemodynamic effects of bosentan versus placebo at 12 weeks. Significant improvements in cardiac index (CI), pulmonary vascular resistance (PVR), and mean pulmonary arterial pressure (mPAP) were all noted. The placebo group worsened over the 12-week period. Adapted from Channick et al. (27)—original work. Control group is shown in blue; bosentan group is shown in green. ‡Significant improvement versus baseline; *Significant decline versus baseline.

**Figure 2.** Six-minute walk distance improvement seen in study of 213 patients demonstrating significant treatment effect of bosentan. (From Rubin et al. [28]).
bosentan (product monograph, Actelion Pharmaceuticals, Allschwill, Switzerland) as fetuses develop severe craniofacial abnormalities. Pregnancy must be excluded before therapy with bosentan and prevented thereafter with reliable contraception. Hormonal forms of contraception may not be reliable in the setting of bosentan therapy, and thus should not be the sole form of contraception in females of childbearing potential.

Other common side effects observed with bosentan include a dose-related decrease in hemoglobin of unknown etiology, headaches, and flushing.

Several drugs have been shown to interact with bosentan through the P450 system. Glyburide and cyclosporine A are contraindicated with concurrent bosentan therapy. Although a small study has shown that humans given bosentan 500 mg twice daily have reduced warfarin effect (35), no influence on warfarin activity has been seen in the clinical trials using the 125-mg and 250-mg twice-daily doses of bosentan.

Role in the context of existing treatments for PAH. Bosentan received approval in 2001/2002 by a number of regulatory agencies, including those in Canada and the U.S. The approved indications are PAH, with functional class III or IV. For WHO functional class III and possibly early class IV patients who are not acutely vasoreactive or who have failed calcium channel blocker therapy, bosentan should be considered the initial treatment of choice, based on compelling short- and long-term data. For patients with significant hemodynamic decline, especially with signs of overt right ventricular failure, or those who progress to WHO functional class IV, epoprostenol remains the initial therapy of choice. These recommendations are outlined elsewhere in this supplement as part of a consensus treatment algorithm.

The addition of bosentan to epoprostenol is a potentially attractive approach, as the two agents work through different and possibly complementary mechanisms. A randomized controlled trial of combined epoprostenol–bosentan found a trend toward greater percent reduction in total pulmonary resistance with the combination versus epoprostenol alone (36).

**SELECTIVE ETA ANTAGONISTS**

Selective antagonists of the ET<sub>A</sub> receptor are currently undergoing investigation, with the rationale that the "favorable" actions of the ET<sub>A</sub> receptor will remain intact and efficacy further improved. Sitaxsentan sodium, a potent endothelin receptor antagonist that has oral bioavailability and a long duration of action (t<sub>1/2</sub>, 5 to 7 h), is approximately 6,500-fold more selective as an antagonist for ET<sub>A</sub> compared with ET<sub>A</sub> receptors. In the first randomized, double-blind, placebo-controlled trial with sitaxsentan in PAH, 178 New York Heart Association (NYHA) functional class II, III, and IV patients with either PPH, PAH related to connective tissue disease, or PAH related to congenital systemic to pulmonary shunts were equally randomized to receive placebo, sitaxsentan 100 mg, or sitaxsentan 300 mg, orally once daily (37). Although the primary efficacy end point of maximum oxygen consumption was not improved by sitaxsentan compared to placebo, the drug did improve exercise capacity (6-min walk distance) (Fig. 4) and functional class after 12 weeks of treatment. These functional benefits occurred with both the 100-mg and the 300-mg doses. Treatment effects in the sitaxsentan groups were 35 m (p < 0.01) for the 100-mg dose and 33 m (p < 0.01) for the 300-mg dose. The NYHA functional class improved in 16 of 55 (29%) patients in the 100-mg group and in 19 of 63 (30%) patients in the 300-mg group. In
contrast, only 9 of 60 (15%) patients in the placebo group had improvement in NYHA functional class. Improvements in pulmonary vascular resistance and cardiac index were also noted in the sitaxsentan group.

As with bosentan, liver function abnormalities occurred (10% in the 300-mg group). It should be noted that, in an earlier pilot study, sitaxsentan was associated with fatal hepatitis when used at higher doses (38). In the larger randomized trial, the most frequently reported clinical adverse events with sitaxsentan treatment (and more frequent than in placebo) were headache, peripheral edema, nausea, nasal congestion, and dizziness, reactions previously noted with ET-receptor antagonists. The most frequently reported laboratory adverse event was increased international normalized ratio or prothrombin time, related to the effect of sitaxsentan on inhibition of CYP2C9 P450 enzyme, the principal hepatic enzyme involved in the metabolism of warfarin. A second phase III trial of sitaxsentan is currently underway.

Another ETA antagonist, ambrisentan, is currently in phase III clinical trials in patients with PAH, and information on relative safety and efficacy will be forthcoming in the near future.

**FUTURE DIRECTIONS IN ERA THERAPY**

Endothelin receptor antagonists for the therapy of PAH appear to have great promise. Questions that remain to be answered include 1) the role of ERAs in early PAH (i.e., WHO class I and II); 2) ERAs as part of combination therapy—for example, with epoprostenol, treprostinil, or sildenafil; 3) the value of selective versus nonselective endothelin receptor antagonism; and 4) the role of ERAs in treating other conditions such as CTEPH or fibrotic lung disease.

**REFERENCES**

Nitric Oxide Pathway and Phosphodiesterase Inhibitors in Pulmonary Arterial Hypertension

Hossein A. Ghofrani, MD,* Joanna Pepke-Zaba, MD,† Joan A. Barbera, MD,‡ Richard Channick, MD,§ Anne M. Keogh, MD,¶ Miguel A. Gomez-Sanchez, MD,¶ Meinhard Kneussl, MD,# Friedrich Grimminger, MD*

Gießen, Germany; Cambridge, United Kingdom; Barcelona and Madrid, Spain; San Diego, California; Darlinghurst, Australia; and Vienna, Austria

Pulmonary hypertension (PH) is a disease of various origins. Nitric oxide—a potent vasodilator—is a key player of pulmonary vasoregulation. Nitric oxide signaling is mainly mediated by the guanylate cyclase/cyclic guanylate monophosphate pathway. The effects of this second messenger system are limited by enzymatic degradation through phosphodiesterases (PDEs). Recently, beneficial effects of the oral PDE-5 inhibitor sildenafil (originally approved for the treatment of erectile dysfunction) were reported for the treatment of PH. We provide a brief overview of the experimental and clinical application of PDE inhibitors in the field of PH. In particular, studies reporting the clinical effectiveness of sildenafil are highlighted. This agent, despite oral application, displays characteristics of a pulmonary selective vasodilator. In addition, evidence shows that sildenafil is operative mainly in the vasculature of well-ventilated areas of the lung. However, to date, controlled randomized trials proving the efficacy of this approach for the treatment of pulmonary arterial hypertension are lacking. The results of such studies have to confirm the current encouraging findings before recommendations regarding the use of PDE-5 inhibitors as a new treatment for PH can be made. (J Am Coll Cardiol 2004;43:68S–72S) © 2004 by the American College of Cardiology Foundation

Nitric oxide (NO) is constitutively produced in the lung by NO synthases. The main cellular sources of lung NO production are the vascular endothelium and the airway epithelia (1,2). Local NO production regulates pulmonary perfusion depending on alveolar ventilation to assure optimized ventilation/perfusion distribution (3,4). Nitric oxide synthase activity is regulated on transcriptional and post-translational redox-based modulation level (5). The common signaling pathway of endogenous vasodilators, such as NO, prostaglandins, and natriuretic peptides, engage cyclic nucleotides (cyclic adenylate monophosphate [cAMP] and cyclic guanylate monophosphate [cGMP]) (Fig. 1). These second messengers are mainly produced by activation of adenylate-cyclase and guanylate-cyclase (GC) (6). Phosphodiesterases (PDEs) represent a superfamily of enzymes, with PDE-1 through PDE-11 being currently known, that inactivate cAMP and cGMP, with different tissue distribution and substrate specificities (6,7). Owing to the stabilization of these second messengers, PDE inhibitors differentially regulate levels of cAMP and/or cGMP, depending on their selectivity profile. Therefore, they might offer as therapeutic tools to augment and prolong prostanoid- and NO-related vascular effects. The efficacy of this approach has been proven in several experimental studies (8,9). Interestingly, the major cGMP-degrading PDE, PDE-5, is abundantly expressed in lung tissue (7). The orally administered selective PDE-5 inhibitor sildenafil has been approved for the treatment of erectile dysfunction (10). Despite the broad use in healthy men and in patients with a variety of underlying diseases, sildenafil displays an excellent safety profile (11).

CURRENT TREATMENTS FOR PULMONARY ARTERIAL HYPERTENSION (PAH)

Continuous infusion of prostacyclin has been shown to be a life-saving therapy in severe primary pulmonary hypertension (PPH) (12) and to improve exercise capacity in collagen vascular disease-associated pulmonary hypertension (PH) (13). There are, however, drawbacks of this therapy, including substantial systemic side effects due to lack of pulmonary selectivity of the prostanoïd, the need of progressive dosage increase, and septic complications of the intravenous line. To preserve the advantageous effects of prostacyclin, and avoid several of these side effects, the concept of aerosolized iloprost for treatment of PAH was developed (14,15). Recently, the results of a double-blind, placebo-controlled multicenter study demonstrated that daily inhaled iloprost significantly improved exercise capacity, New York Heart Association (NYHA) functional classification, dyspnea scoring, and event-free survival over a three-month obser-
vation period in patients with selected forms of PAH and chronic thromboembolic PH (16).

Another promising approach to medical treatment of PAH is the use of the nonselective oral endothelin receptor antagonist bosentan. In a controlled phase III study, bosentan showed beneficial effects on exercise tolerance in patients with PPH and those with PH associated with collagen vascular disease (17). However, liver toxicity was documented in a minor percentage of patients, and long-term experience will have to elucidate the occurrence of this complication during chronic treatment of a substantial number of patients.

The search continues for an “ideal” pulmonary vasodilator that combines pulmonary selectivity with simplicity of administration and reduced side effects. Recently, the

**Abbreviations and Acronyms**

- cAMP = cyclic adenylyl monophosphate
- cGMP = cyclic guanylyl monophosphate
- GC = guanylyl cyclase
- HIV = human immunodeficiency virus
- NO = nitric oxide
- NYHA = New York Heart Association
- PAH = pulmonary arterial hypertension
- PDE = phosphodiesterase
- PH = pulmonary hypertension
- PPH = primary pulmonary hypertension

**Figure 1.** Scheme of nitric oxide (NO) metabolism pathway. In this diagram the nitric oxide (NO) pathway is depicted. In presence of oxygen (O₂) and/or alveolar ventilation, NO synthases (NOS) are activated and produce NO from L-arginine via L-citrulline. The NO activates soluble- and membrane-bound guanylate cyclases, which synthesize cyclic guanylate monophosphate (cGMP), which subsequently activates cGMP-kinase. This enzyme—by activation of K⁺-channels and subsequent Ca²⁺-channel inhibition—evokes a reduction of intracellular Ca²⁺ concentration, finally resulting in vasodilation. The downstream effects of NO are limited by phosphodiesterase (PDE)-induced degradation of cGMP.

**Figure 2.** Comparison of pulmonary vasodilative potency of oral sildenafil, inhaled nitric oxide (NO), and inhaled iloprost. Comparative vasodilator testing was performed in 30 patients with precapillary pulmonary hypertension. In each group, the effect of inhaled NO on pulmonary vascular resistance (PVR) was compared with that of sildenafil, inhaled iloprost, or a combination of both. In the upper left figure, inhaled NO and iloprost were compared with a low dose of sildenafil (12.5 mg), while in the upper right figure a combination of sildenafil and iloprost was tested. The lower left figure summarizes the effects of 50 mg sildenafil compared to NO and iloprost, and the lower right figure shows the data of a combination of high-dose sildenafil with iloprost (adapted from Ghofrani et al., Ann Intern Med 2002;136:515–22).
SILDENAFIL IN EXPERIMENTAL PH

In animal experiments, several PDE inhibitors displayed favorable pulmonary vasodilatory potential (8,18,19). Sildenafil in such a setting proved to be a potent and pulmonary-selective vasodilator (9). Most interestingly, this agent was also able to reduce hypoxia-induced PH in man and in an experimental animal model (20). The effects of sildenafil on chronic remodeling processes in the pulmonary vasculature are not yet well known. It is hoped that future results derived from long-term experimental models of PH will provide insight into these mechanisms.

CLINICAL EXPERIENCE WITH SILDENAFIL FOR THE TREATMENT OF CHRONIC PH

The vasodilatory effects of NO administered by inhalation are restricted to the pulmonary vasculature. Nitric oxide has a very short half-life, is used as a screening agent for lung vasoreactivity (21), and is effective for improving gas exchange in selected patients with the adult respiratory distress syndrome (22). Weaning from chronic NO treatment in patients with the adult respiratory distress syndrome was
found to be facilitated by oral sildenafil (23). In patients with PAH, short-term application of sildenafil during right heart catheterization showed the potential to reduce pulmonary vascular resistance in a dose-dependent manner. Interestingly, the vasodilatory effects were mainly operative in the pulmonary circulation and were significantly stronger than the effects seen with inhaled NO (24). In combination with another pulmonary selective vasodilator, inhaled iloprost, augmentation of the pulmonary vasodilatory effect of each single agent was noted (24,25) (Fig. 2). In patients with deteriorating severe PAH despite ongoing prostanoïd treatment, long-term adjunct oral sildenafil improved exercise capacity and pulmonary hemodynamics (26). The combination of prostanoids and sildenafil could prove to be an appealing concept for future treatment of PH. Numerous reports about the clinical use of sildenafil in PAH as short-term application and long-term treatment in uncontrolled trials have been published (24,27–34).

Interestingly, sildenafil also appears to be effective for treating patients with PH of origin other than PPH. In patients suffering from human immunodeficiency virus (HIV)-related PH, sildenafil was similarly effective in reducing pulmonary vascular resistance as in PPH (35). Moreover, this therapeutic approach has been reported to be effective in pediatric patients (36). In the presence of interstitial lung disease, systemic administration of vasodilators regularly increases the blood flow to low or nonventilated lung areas by interfering with the physiological hypoxic vasoconstrictor mechanism, thereby worsening preexistent ventilation/perfusion mismatch and shunt flow. The decrease in arterial oxygenation and the wasting of the small ventilatory reserve of these patients are the negative consequences of this effect. Most interestingly, oral sildenafil was found to cause pulmonary vasodilation in patients with lung fibrosis and PH, with the overall vasodilatory potency corresponding to that of intravenous prostacyclin. Notably, in contrast to the infused prostanoïd, selectivity for well-ventilated lung areas was demonstrated for sildenafil, resulting in an improvement rather than deterioration of gas exchange (37) (Fig. 3). Also, recent data suggest beneficial long-term effectiveness in patients with nonoperable chronic thromboembolic PH (38). The importance of this finding is the fact that there are few therapeutic options that can be offered to these patients (except lung transplantation).

**Conclusions.** The NO/cGMP axis represents a pivotal signaling pathway for the lung circulation. Phosphodiesterases, as regulators of the second messenger response to endogenous NO, are thus of great therapeutic potential for the treatment of lung circulatory disorders. Among the clinically available PDE inhibitors, sildenafil is a most promising agent for pulmonary vasodilation and long-term antiremodeling in the lung vasculature of PAH patients. Although orally administered, sildenafil does possess features of pulmonary selectivity. It may be favorably combined with other vasodilative and antiproliferative agents. Large trials, including a placebo-controlled phase III trial with sildenafil in patients with PAH, are currently underway.

**REFERENCES**

channel blockers in primary pulmonary hypertension (see comments).


Interventional and Surgical Modalities of Treatment for Pulmonary Arterial Hypertension

Walter Klepetko, MD,* Eckhard Mayer, MD,† Julio Sandoval, MD,‡ Elbert P. Trulock, MD,§ Jean-Luc Vachiery, MD,¶ Philippe Dartevelle, MD,‖ Joanna Pepke-Zaba, MD,# Stuart W. Jamieson, MD,** Irene Lang, MD,†† Paul Corris, MD‡‡ Vienna, Austria; Mainz, Germany; Mexico City, Mexico; St. Louis, Missouri; Brussels, Belgium; Paris, France; Cambridge, United Kingdom; and San Diego, California

Beyond medical therapy, different interventional and surgical approaches exist for treatment of pulmonary arterial hypertension (PAH). Atrial septostomy has been applied in patients with lack of response to medical therapy in the absence of other surgical treatment options. With growing experience, procedure-related death rates have been reduced to 5.4%, and the most suitable patient group has been identified among patients with a mean right atrial pressure between 10 and 20 mm Hg. Pulmonary endarterectomy is the accepted form of treatment for patients with chronic thromboembolic pulmonary hypertension. Establishing the diagnosis and the classification of the type of lesions by pulmonary angiography is crucial for optimal patient selection. Perioperative mortality rates have been reduced to <10% in experienced centers, and the hemodynamic improvement is dramatic and sustained. Lung and heart-lung transplantation remains the procedure of choice for patients unsuitable for other treatment modalities. Timing of the procedure is difficult because waiting times vary between centers and usually are in a high range. Early referral of patients unresponsive to other treatment forms is therefore of importance to avoid transplantation of patients with established significant comorbidity. The survival rate during the first five years after transplantation for PAH is intermediate among the lung diseases, lower than chronic obstructive pulmonary disease but higher than idiopathic pulmonary fibrosis. (J Am Coll Cardiol 2004;43:73S–80S) © 2004 by the American College of Cardiology Foundation

Different interventional and surgical approaches for treatment of pulmonary arterial hypertension (PAH) have been developed in the last two decades. We review here the three major components: atrial septostomy, pulmonary endarterectomy, and lung transplantation.

ATRIAL SEPTOSTOMY

The use of atrial septostomy (AS) in PAH is supported by the fact that deterioration in symptoms and death in PAH are associated with right ventricular failure (RVF). An AS in this setting creates a right-to-left shunt that increases cardiac output and, despite the fall in systemic arterial oxygen saturation, augments systemic oxygen transport (SOT). In addition, the shunt decompresses the heart and ameliorates RVF.

Blade balloon atrial septostomy (BBAS) as a palliative therapy for refractory PAH was first reported in 1983 (1,2).

Graded balloon-dilation AS (BDAS), a variant of BBAS, is the technique most used in recent series (3,4), and it has produced results similar to those of BBAS in terms of symptomatic and hemodynamic benefits but with an apparent reduction in the procedure-related risks. Procedures should be performed only in centers experienced in both interventional cardiology and PAH (5,6).

The precise role of AS in the treatment of PAH remains uncertain because most of the knowledge regarding its use comes from small series or case reports. The potential beneficial effects and risks of AS were addressed in a review derived from an analysis of 64 cases from the published reports (5). This knowledge has now expanded with the report of another 56 cases in the last few years (3,7,8). Severe idiopathic PAH has been the main indication for AS. Other indications have included PAH associated with surgically corrected congenital heart disease, peripheral chronic thromboembolic pulmonary hypertension (CTEPH) (5), and, more recently, PAH associated with systemic sclerosis (8). Most patients who have undergone AS had not responded to conventional treatment (5), had failed long-term prostacyclin therapy (3,9), or were treated with the intention of bridging to lung transplantation (3,9).

In most reports the patients have been considered terminally ill. Accordingly, there is an inherent risk of complications and death during the procedure. In the prior worldwide experience (5), there was an overall procedure-related mortality of 16%, and by univariate analysis, a baseline mean right atrial pressure (mRAP) >20 mm Hg was the variable...
transplantation. Current indications for AS, therefore, in-
throughout the world, especially the limited access to lung
justi-
come was dependent on the immediate hemodynamic
bene-
such as a decompression effect on the heart
mechanisms such as a decompression effect on the heart
improvement in right ventricular failure (i.e., syncope and/or signs of
RVF and/or recurrent syncope, bridging to transplantation
and the absence of other therapeutic options (10).
To address some of the unanswered questions prospectively (timing of procedure, combination with other therapies, and so forth) regarding the precise role of AS, an International Registry on the use of AS in PAH is underway.

**PULMONARY ENDARTERECTOMY**

Chronic thromboembolic pulmonary hypertension is an underdiagnosed consequence of unresolved acute pulmonary embolism with an incidence that is higher than generally appreciated (11). It is characterized by intraluminal thrombus organization and fibrous stenoses or complete obstructions of the pulmonary artery (PA) branches, causing a persistent elevation of PA pressure, pulmonary vascular resistance (PVR), and progressive right heart failure (11,12). The prognosis of patients with thromboembolic pulmonary hypertension is poor, and the survival rate is inversely proportional to the degree of PAH (13). As in patients with acute pulmonary embolism, the diagnosis is often missed or delayed because the main symptoms (e.g., dyspnea) are nonspecific. Additionally, there is a general lack of awareness of this disease and the chance of surgical cure.

Adequate preoperative patient evaluation and selection, surgical technique and experience, meticulous postoperative management, and a multidisciplinary approach are the basic prerequisites for pulmonary endarterectomy (PEA).

**Diagnostic investigations.** When PAH is clinically suspected to be the cause of exertional dyspnea, right heart dysfunction can be detected by transthoracic echocardiography, whereas left heart conditions leading to PAH can be excluded. Pulmonary perfusion scanning allows differentiation between thromboembolic and primary pulmonary perfusion defects (12). High-resolution computed tomography using maximal intensity projections shows the distribution of typical obstructive PA lesions at the main, lobar, and segmental levels and a mosaic pattern of lung attenuation due to regional perfusion differences.

Pulmonary angiography remains the gold standard for the diagnosis and preoperative evaluation of patients with thromboembolic pulmonary hypertension. Biplane plate film or digital subtraction angiography shows the exact localization and the type of PA obstructions (11). Specific experience is required for the interpretation of angiograms of CTEPH patients: irregularities of the vascular wall, intraluminal filling defects, stenoses or occlusion of central, lobar, segmental, and peripheral arteries caused by thrombotic masses or fibrous webs and bands are characteristic angiographic features in CTEPH (Fig. 1).

Pulmonary fiberoptic angioscopy can be used to define operability in selected patients with unclear angiographic findings or disproportionately severe PAH with mild angiographic obstructions. With increasing experience, magnetic resonance imaging using high-field technology and
fast imaging techniques is becoming a helpful noninvasive investigation in CTEPH patients because the lumen as well as the organized tissue in the wall of the PA branches can be precisely depicted in combination with an exact evaluation of right heart function.

Patient selection. Once the diagnosis of CTEPH is established, the decision for surgical therapy is made based on the degree of functional impairment, the severity of PAH, and the surgical accessibility of the thromboembolic lesions (14). A preoperative period of at least three months of adequate anticoagulation is mandatory. Patients considered for surgery are usually severely incapacitated with dyspnea at minor levels of exertion or at rest (New York Heart Association [NYHA] functional class III or class IV). Although the mean preoperative PVR in CTEPH patients is 800 to 1,000 dynes·s·cm⁻², young patients with exertional dyspnea, almost normal PVR at rest, and a significant increase at exertion may also be accepted for surgery, as earlier operation could have the potential to prevent a secondary vasculopathy in the unobstructed pulmonary vascular bed.

The surgical accessibility of the thromboembolic lesions is heavily dependent on the experience of the surgical team. With growing experience, endarterectomy of subsegmental PA branches is possible. The operative risk is increased if a major discrepancy exists between the degree of PAH and the extent of angiographic PA obstructions based on significant microvascular disease.

Principles of operation. Although the operation historically has been described as pulmonary thromboendarterectomy (PTE), it is better termed PEA (11). Fewer than 3,000 PEA operations have been performed worldwide, although it is a potentially curative treatment option for very sick patients with CTEPH. The surgical techniques and evolving modifications have been well described by the San Diego group (14,15). With rare exceptions, thromboembolic pulmonary hypertension is a bilateral disease, and therefore PEA is a bilateral procedure. The operation is not an embolectomy but a true endarterectomy removing the organized and incorporated fibrous obstructive tissue from the PAs. Because visibility in the distal PA branches is essential and bronchial artery collateral flow is significant in CTEPH, extracorporeal circulation and periods of circulatory arrest under deep hypothermia are essential for successful endarterectomy.

Following a proximal intrapericardial PA incision, the correct endarterectomy plane is established and circumferentially followed down to the lobar segmental and sometimes subsegmental branches of each lobe using special suction dissectors. The endarterectomy procedure on one side is usually possible within one 20-min period of circulatory arrest followed by a period of reperfusion and another period of circulatory arrest for the endarterectomy on the contralateral side. After closure of the PA incision, additional cardiac procedures can be performed during the rewarming period, if necessary. As tricuspid valve competence usually returns after successful PEA, tricuspid valve repair is not necessary.

Jamieson et al. (16) have proposed an intraoperative classification of CTEPH: type I (central thrombus present) and type II (thickened intima, fibrous webs and bands) represent the typical condition of surgical patients. Type III occlusions in the segmental and subsegmental branches require adequate surgical experience with dissection within the peripheral pulmonary arteries. Type IV disease represents secondary in situ thrombosis in patients with primary pulmonary hypertension (PPH) and cannot be treated by PEA, whereas lung transplantation is an option for these patients.

Postoperative management. In contrast to the majority of cardiac surgical procedures, the postoperative course is determined primarily by the physiological changes of right heart function and pulmonary perfusion, pulmonary hemodynamics, and gas exchange (17). The postoperative management of patients undergoing PEA can be challenging; in addition, the usual complications associated with cardiac surgery are encountered. The most important complications are persistent PAH due to inadequate endarterectomy or significant secondary vasculopathy and reperfusion edema in the endarterectomized parts of the lung (18). Postoperative care centers around maintaining sufficient right ventricular function and organ perfusion, adequate oxygenation and renal function, and in
preventing early pulmonary artery reocclusion. Extensive circulatory monitoring, including continuous online measurement of cardiac output, mixed venous oxygen saturation, and arterial blood gases, has proved to be helpful.

Following nonaggressive pressure-controlled mechanical ventilation, early extubation on the first or second postoperative day is advocated even in cases with modest reperfusion edema and hypoxia. In rare cases with severe reperfusion edema, oxygenation can be improved by prone positioning, continuous nitric oxide inhalation, or extracorporeal membrane oxygenation. By means of cautious fluid and albumin infusion, the doses of vasoactive drugs administered via left atrial catheter can be limited to a low dose level. Reocclusion prophylaxis is started within 4 to 8 h following surgery using intravenous heparin infusion followed by continuous anticoagulation with warfarin between days 8 and 14.

**Outcome.** Recently, Fedullo et al. (18) have reviewed the world literature on thromboembolic pulmonary hypertension and endarterectomy and found mortality rates between

**Figure 2.** Postoperative and long-term follow-up hemodynamic changes after pulmonary endarterectomy (data from Johannes Gutenberg University, Mainz, Germany, n = 50). CI = cardiac index; mPAP = mean pulmonary artery pressure; PVR = pulmonary vascular resistance.
5% and 24%. As there is a distinct learning curve for the preoperative evaluation, the operation, and the postoperative care, mortality rates are lower in centers with a large volume of procedures.

Although there has not been a controlled study, and such an investigation probably will never be performed, the outcomes of PTE with regard to functional status, quality of life, hemodynamics, right ventricular function, and gas exchange are very favorable. Most patients are in NYHA functional class III or IV before the operation and return to class I or II with a good exercise capacity after surgery (19,20).

In several studies, significant and persistent decreases of PA pressures and PVR after PTE surgery are reported (19) (Fig. 2). Cardiac output is increased and oxygenation can be normalized. Right heart function assessed by echocardiography or MRI is significantly and persistently improved postoperatively (Fig. 3). Tricuspid valve regurgitation is also significantly improved within a few days after surgery; in most patients, tricuspid competence returns to normal.

Long-term survival after endarterectomy with a five-year survival rate of 75% to 80% is very favorable compared to medical treatment or lung transplantation. Therefore, lung transplantation is inappropriate for most patients with this condition.

**LUNG AND HEART-LUNG TRANSPLANTATION**

With the advent of effective drug treatments in the last decade, lung transplantation has become the final option in the management of PAH; at the same time, selection of recipients and timing of the procedure have become more complex.

Whereas prostaglandins and endothelin receptor antagonists can alter the clinical features and course of PPH in many patients, their impact on the underlying pathobiology is uncertain, and they are not curative remedies. The majority of patients already have moderate to severe PAH when the diagnosis is made, and all patients do not respond to treatment. Less than 10% of patients with idiopathic PAH have a significant response to a calcium channel antagonist. Moreover, in two recent series approximately 25% of patients with PPH failed to improve with epoprostenol treatment, and those who remained in NYHA functional class III and IV with epoprostenol treatment had a poor prognosis, with three-year survival rates in the range of 30% to 60% (21,22).

Finally, even among responders, the escalating dose of epoprostenol that is often needed to sustain its beneficial effects may eventually cause intolerable side effects. Thus, ultimately transplantation will be the only alternative for many patients with idiopathic PAH and other forms of PAH.

**Pretransplantation assessment.** The purpose of the pretransplantation assessment is to identify patients whose prognosis will be improved by transplantation and whose cardiopulmonary status or other medical problems will not unduly jeopardize the success of transplantation—that is, to choose the right patient and the right time for transplantation. The major specific goals of the evaluation for transplantation are to confirm the diagnosis, to assess the severity of the disease, and to optimize medical management. The potential role of transplantation (prognosis of the disease vs. that of transplantation) has to be established, and suitable candidates for transplantation have to be selected together with the ideal transplant procedure. The clinical status must be reexamined periodically, and medical management and transplantation strategy adjusted accordingly.

Pathways, which are based on vasoreactivity and on functional impairment, have been standardized to optimize the management paradigm, and transplantation should be reserved until it will confer a survival advantage. However, a long waiting period for transplantation should be expected, and this delay has to be incorporated into the plan. Thus, the pretransplantation evaluation and waiting list registration must anticipate the need for transplantation later, or the opportunity for transplantation may be missed.

Consensus guidelines for selecting patients for transplantation have been promulgated by the major societies (23), and most transplant centers use similar criteria. In addition to a comprehensive assessment of the patient’s cardiopulmonary status, any other medical problems should be fully
characterized to ascertain their potential influence on the outcome of transplantation, and all health maintenance testing that is recommended for the patient’s age and gender should be completed.

**Choice of transplant operation.** Choosing the transplant operation is another facet of the preoperative evaluation. Both heart-lung and lung transplantations have been performed for pulmonary vascular disease, but heart-lung transplantation should be reserved for patients who are not candidates for lung transplantation alone. In general, most forms of PAH except complex congenital heart disease do not require heart-lung transplantation unless there is a significant cardiac problem other than cor pulmonale.

The threshold of unrecoverable right ventricular dysfunction is unknown, if such a boundary even exists. Severe right ventricular dysfunction has been reversible after isolated lung transplantation. However, although afterload is immediately reduced by lung transplantation, right ventricular function does not revert to normal right away, and hemodynamic instability is a common problem in the early postoperative period.

Both single and bilateral lung transplantations have been performed for PPH and some other types of PAH (24–28), and these operations have been combined with repair of cardiovascular anomalies for Eisenmenger syndrome (25,29). Single-lung transplantation creates a tenuous ventilation-perfusion imbalance, and any complication in the allograft is associated with severe hypoxemia. Nevertheless, recipient survival rates have been similar after single and bilateral transplantation for PAH, and if technically feasible, either of these operations is an acceptable choice for most cases of PAH. However, in patients with Eisenmenger syndrome, the option of heart-lung transplantation should be carefully considered. For some defects, especially ventricular septal defect, the survival advantage of heart-lung transplantation was most prominent (29), and the option of heart-lung transplantation should be strongly considered for this subgroup.

**Timing.** The pretransplantation assessment does not end after the initial evaluation and waiting list registration. The waiting time for transplantation depends on the organ allocation system, and allocation systems vary significantly in countries around the world. Regardless of the allocation system, however, there is usually a long duration before transplantation, and during the waiting period both medical management and transplantation strategy must be modified in response to changes in clinical circumstances. Patients with PPH who are treated with epoprostenol should have a follow-up right heart catheterization. Those who have not responded to epoprostenol at their initial reassessment have a guarded prognosis (21,22) and they should proceed toward transplantation unless a contraindication intervenes.

Prognostic indexes that can be derived from repeatable, noninvasive tests are needed to supplement clinical judgment about the timing of transplantation after the patient is on the waiting list. As medical therapy has improved, it has been extended to its limit in many patients before transplantation. However, if refractory right heart failure develops on maximal drug treatment, little can be done to restore cardiopulmonary status, and secondary effects on other organs, especially the liver and kidney, become problematic. In this scenario potential recipients can be transformed into high-risk or unacceptable candidates for transplantation, and the opportunity for transplantation can be lost.

Serial noninvasive tests may be helpful in the decision about timing. The results of the 6-min walk testing and cardiopulmonary exercise testing have had prognostic implications in a few studies (30,31) and the predictive value of these tests deserves further study. Six-minute-walk distance correlated well with peak oxygen uptake in one study of PPH patients, and a 6-min walk distance <332 m portended a poor prognosis with a one-year mortality rate of approximately 40% (30). In a study of cardiopulmonary exercise testing in patients with PPH, both peak oxygen uptake and peak systolic blood pressure were prognostically important. A peak oxygen uptake ≤10.4 ml/kg/min and a peak systolic blood pressure ≤120 mm Hg were associated with one-year mortality rates of approximately 50% and 70%, respectively, and among patients with both of these risk factors, only 23% survived for one year (31). Thus, transplantation would offer a survival benefit to patients with any of these risk factors, and this threshold might be useful in foreseeing the favorable time for transplantation.

**Survival.** The outcome of lung transplantation can be gauged by several end points: survival, physiologic function, quality of life, and cost-effectiveness. Actuarial survival is
well known from the U.S. Scientific Registry (32), the International Society for Heart and Lung Transplantation (ISHLT) registry (33), and reports from individual centers (26,27,34,35). Survival rates from the ISHLT registry are presented for PPH, Eisenmenger syndrome, and other forms of congenital heart disease in Table 1.

Recipients with PPH and Eisenmenger syndrome have had the highest perioperative mortality and the lowest three-month survival rates among the major diagnostic categories of lung transplant recipients in both the U.S. Scientific and the ISHLT registries (32,33). This difference is explained by the complexity of the surgery in severe pulmonary hypertension. Cardiopulmonary bypass is required routinely for the operation in cases of PPH or Eisenmenger syndrome, whereas it is rarely needed for other diagnoses. This increases the risk of hemorrhagic complications and contributes to early graft dysfunction. Furthermore, right ventricular function does not recover immediately, and hemodynamic instability is common in the first few days following transplantation.

In the ISHLT registry, both PPH and Eisenmenger syndrome/congenital heart disease have been associated with a significantly higher risk of death in the first year after lung transplantation than with other diagnoses (33). This increased mortality has been concentrated in the perioperative period; thereafter, the attrition rate for recipients with PPH and Eisenmenger syndrome has paralleled the rates for recipients with other diagnoses because the subsequent complications are not strongly influenced by the pretransplantation diagnosis.

The best transplantation operation of PPH has been debated. No clearly significant difference in survival has been apparent among the procedures (single lung transplantation, bilateral lung transplantation, and heart-lung transplantation) in the ISHLT registry and in some case series (27,33), but some centers have reported a different experience (35–37). In general, lung transplantation for PPH should be performed in specialized centers only, those familiar with the unique problems of this particular procedure and the complexity of the patients.

Reprint requests and correspondence: Dr. Walter Klepetko, University Hospital of Vienna, Department of Cardio-Thoracic Surgery, Waehringer Guertel 18-20, 1090 Vienna, Austria. E-mail: walter.klepetko@meduniwien.ac.at.

REFERENCES

32. 2001 Annual Report of the U.S. Organ Procurement and Transplantation Network and the Scientific Registry of Transplant Recipients: Transplant Data 1991–2000: Department of Health and Human Services, Health Resources and Services Administration, Office of Special Programs, Division of Transplantation, Rockville, MD; United Network for Organ Sharing, Richmond, VA; University Renal Research and Education Association, Ann Arbor, MI.
Comparative Analysis of Clinical Trials and Evidence-Based Treatment Algorithm in Pulmonary Arterial Hypertension

Nazzareno Galie, MD,* Werner Seeger, MD,† Robert Naeije, MD,‡ Gerald Simonneau, MD,§ Lewis J. Rubin, MD||

Bologna, Italy; Giessen, Germany; Brussels, Belgium; Paris, France; and San Diego, California

The numerous controlled clinical trials performed recently in pulmonary arterial hypertension (PAH) can allow us to abandon a clinical-based treatment strategy and adopt an evidence-based therapy. Both uncontrolled and controlled clinical trials with different compounds and procedures are reviewed and compared in order to define the efficacy-to-side-effect ratio of each treatment. A grading system for the level of evidence of treatments based on the number of favorable controlled clinical trials performed with a given compound is adopted; a treatment algorithm based on the evidence derived by clinical trials is proposed. It includes drugs approved by regulatory agencies for the treatment of patients with PAH and/or drugs available on the market for other indications. The algorithm is restricted to patients in New York Heart Association (NYHA) functional class III or IV because they represent the largest population included in controlled clinical trials. In addition, the different treatments have been evaluated mainly in sporadic, idiopathic PAH and in PAH associated with scleroderma or to anorexigen use. Extrapolation of these recommendations to the other PAH subgroups should be done with caution. Oral anticoagulation is proposed for all patients, whereas 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 are responders to acute vasoreactivity testing. Nonresponders to acute vasoreactivity testing, or responders who remain in NYHA functional class III, should be considered candidates for treatment with either an endothelin receptor antagonist or a prostanoid. Continuous intravenous administration of epoprostenol is proposed as rescue treatment in NYHA functional class IV patients. Phosphodiesterase-V inhibitors should be considered in patients who have failed or are not candidates to other therapies. Combination therapy can be attempted in selected cases. Both balloon atrial septostomy and lung transplantation are indicated for refractory patients or where medical treatment is unavailable. (J Am Coll Cardiol 2004;43:81S–88S) © 2004 by the American College of Cardiology Foundation

Treatment of pulmonary arterial hypertension (PAH) has shown a dramatic change from the slow progress in the past decades to the remarkable number of randomized controlled trials (RCTs) accomplished in the past few years.

Initially, up to the 1980s, attempts to reduce pulmonary arterial pressure were performed with various vasodilators. Favorable and sustained results were convincingly shown only by the use of high doses of calcium-channel blockers (CCBs), but only in the minority of patients who responded to acute vasoreactivity testing. Also, oral anticoagulant treatment was considered effective based on retrospective or uncontrolled studies. Finally, in the 1990s, the complex treatment with continuous intravenous (IV) administration of epoprostenol was shown in three non-blinded RCTs to consistently improve symptoms and survival in the more severe cases. In the same period, favorable results of different uncontrolled series of PAH patients who underwent balloon atrial septostomy or lung transplantation were also reported.

Recently, three phase II studies and nine phase III RCT’s with new compounds have been completed in PAH patients and six more are ongoing or planned. In addition, three RCTs testing the combination of new compounds with epoprostenol have been completed or planned. More than 2,500 patients have been or will be involved in this unprecedented effort to find effective treatments and, ultimately, a cure for PAH.

The impressive amount of knowledge derived from these studies can allow us, for the first time in the history of PAH therapy, to shift from a clinical-based to an evidence-based treatment strategy. The purpose of the present report is to compare the RCTs performed in PAH and to propose an evidence-based grading system and a treatment algorithm that incorporate the currently available therapies.

UNCONTROLLED CLINICAL STUDIES IN PAH

Anticoagulants. The evidence for favorable effects of oral anticoagulant treatment in patients with idiopathic pulmonary arterial hypertension (IPAH) or PAH associated with
anorexigens is based on retrospective analysis of single-center studies (1–3). The survival of anticoagulated patients, selected on the basis of clinical judgment, was improved as compared to 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. (1) and from 31% to 47% in the series of Rich et al. (2). The design of these studies was 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 and anorexigen-related PAH patients were included in the studies. In recent RCTs, oral anticoagulants were administered in 51% to 86% of subjects at study entry. Interestingly, the highest prevalence of oral anticoagulant treatment was seen in the trials involving mainly IPAH patients in New York Heart Association (NYHA) functional class III and IV, whereas the lowest prevalence was observed in the trial of patients with scleroderma. It should be emphasized that there is no evidence of any difference in efficacy of oral anticoagulant therapy based on functional class severity.

**Diuretics, digoxin, and oxygen.** The clear symptomatic and clinical benefits of diuretic treatment in right heart failure preclude the need for controlled trials to show efficacy in PAH. In the recent RCTs on new treatments, 49% to 70% of patients were treated with diuretics. However, the lack of trials with specific classes of diuretics in PAH and the individual variability in responses leave the choice of the type and the dose of drug to be used in individual cases to the experience of the physician. Short-term IV administration of digoxin in IPAH produces a modest increase in cardiac output and a significant reduction in circulating norepinephrine; however, 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 18% to 53% of patients upon entering recent RCTs in PAH. No consistent data are currently available on the effects of long-term oxygen treatment in PAH. Although improvement in pulmonary hypertension with low-flow supplemental oxygen has been reported in some PAH patients, this has not been confirmed in controlled trials. In a controlled study of Eisenmenger syndrome, nocturnal oxygen therapy had no effect on hematologic variables, quality of life, or survival (4).

**Calcium channel blockers.** Favorable clinical and prognostic effects of high doses of calcium channel blocker (CCB) drugs in vasoreactive patients with IPAH have been shown in single-center, nonrandomized, noncontrolled studies (2,5–8). In these studies, the control group consisted of nonvasoreactive patients who may have a poorer prognosis as compared to vasoreactive individuals (6). Conversely, the demonstration of a consistent reduction of pulmonary artery pressure by acute pharmacological testing in vasoreactive patients raises ethical questions on the appropriateness of performing a placebo-controlled clinical trial in these patients.

According to the definition of “a positive acute vasoreactive response” proposed in this Supplement (reduction of mean pulmonary arterial pressure ≥10 mm Hg to reach a mean pulmonary arterial pressure ≤40 mm Hg with a increase or unchanged cardiac output with acute pulmonary vasodilator challenge), only about 10% to 15% of IPAH patients will meet these criteria (8). In addition, only about one-half of these will manifest a sustained response to high doses of CCB therapy as defined by a long-term hemodynamic (assessed after at least three months) and functional (NYHA functional class I and II) improvement in the absence of additional treatments (8).

Favorable results of long-term administration of high doses of CCBs have also been shown in children with IPAH (7). In contrast, the effects of high-dose CCBs on associated forms of PAH have not yet been clearly demonstrated (9).

**Balloon atrial septostomy.** The role of balloon atrial septostomy in the treatment of PAH patients is uncertain because its efficacy has been reported only in small series and case reports, totalling approximately 120 published cases (10,11). In most circumstances, this intervention has been performed in severely ill patients as a bridge to lung transplantation, which may explain a procedure mortality rate ranging from 5% to 15%. In addition to symptomatic and hemodynamic improvement, an increase of survival as compared with historical control groups has also been shown (12). At present, balloon atrial septostomy is indicated for advanced NYHA functional class III and class IV patients who are refractory to all available medical treatments; septostomy is used either as a bridge to lung transplantation or as the sole treatment modality when other options are not available (11).

**Lung transplantation.** As with many other surgical procedures performed in severely ill patients, lung transplantation in PAH has been assessed only in prospective uncontrolled series, because formal RCTs are considered unethical in the absence of alternative treatment options (11). The three- and five-year survival after lung transplantation is approximately 55% and 45%, respectively (13). Accordingly, lung transplantation is indicated in PAH patients with advanced NYHA functional class III and IV symptoms that are refractory to available...
medical treatments. The unpredictability of the length of time on the waiting list and of donor organ shortage complicate the decision making regarding the appropriate timing of listing for transplantation.

**CONTROLLED CLINICAL TRIALS IN PAH**

**Synthetic prostacyclin and prostacyclin analogues.** The efficacy of continuous IV administration of epoprostenol (synthetic prostacyclin, Flolan) has been tested in three unblinded, controlled clinical trials in IPAH (14,15) and in PAH associated with the scleroderma spectrum of diseases (16), and the results are summarized in Table 1. Epoprostenol improves symptoms, exercise capacity, and hemodynamics in both clinical conditions, and it is the only treatment to be shown in RCTs to improve patient survival in IPAH.

Four RCTs have been performed with prostacyclin analogues and results are summarized in Table 2. The effects of continuous subcutaneous administration of treprostinil in PAH were studied in the largest worldwide RCT performed in this condition, and improvements in exercise capacity, hemodynamics, and clinical events were shown (Table 2) (17). One additional pilot controlled study was performed with treprostinil in 26 PAH patients and showed trends in the improvement of 6-min walk distance and in the reduction of pulmonary vascular resistance (18).

The orally active prostacyclin analogue beraprost has been evaluated in PAH patients in two RCTs in Europe (19) and in the U.S., respectively (20) (Table 2). In the first study, an increase in exercise capacity was seen in IPAH subjects after three months. In the second randomized trial that lasted 12 months, improvement in exercise capacity was observed at six months but not thereafter. No hemodynamic improvements were observed in the long-term study, and clinical events were reduced only at the six-month evaluation.

Inhaled iloprost has been evaluated in one RCT that enrolled both patients with PAH and chronic thromboembolic pulmonary hypertension (21) (Table 2). The study showed an increase in exercise capacity and improvement in symptoms, pulmonary vascular resistance, and clinical events in IPAH patients only. Continuous IV administration of iloprost has been shown to be effective in a small series of patients with PAH and chronic thromboembolic pulmonary hypertension (22).

**Endothelin-1 receptor antagonists.** Three RCTs with endothelin-1 receptor antagonists (ERAs) have been performed in PAH patients (Table 3). The orally active, dual ERA bosentan has been evaluated in PAH patients in two RCTs that have shown improvement in exercise capacity, functional class, hemodynamics, echocardiographic and Doppler variables, and time to clinical worsening (23–25).

Sitaxsentan, a selective orally active endothelin A receptor (ET_{A}) antagonist has been assessed in PAH patients in one RCT that demonstrated improvement in exercise capacity, hemodynamics, and clinical events (only for the lower dose tested) (26,27). An additional pilot study with this compound in 20 PAH patients has shown similar results (28).

Ambrisentan, a selective, orally active ET_{A} receptor antagonist, has thus far been evaluated only in a pilot blinded, dose-comparison study in 64 PAH patients. Pre-
liminary results show improvements in exercise capacity and hemodynamics that appear similar to the results observed with other ERAs (29).

Additional phase III RCTs with sitaxsentan and ambrisentan are ongoing or are planned; these trials will further explore both the efficacy and side effects profile of selective ETA receptor antagonists and allow comparisons with bosentan.

**Table 3.** Controlled Clinical Trials With Endothelin-1 Receptor Antagonists in Patients With Pulmonary Arterial Hypertension

<table>
<thead>
<tr>
<th>Trial</th>
<th>Bosentan Pilot (Ref. 21)</th>
<th>BREATHE-1 (Ref. 24)</th>
<th>STRIDE-1 (Ref. 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>469</td>
<td>130</td>
<td>116</td>
</tr>
<tr>
<td>Trial type</td>
<td>Controlled</td>
<td>Controlled</td>
<td>Controlled</td>
</tr>
<tr>
<td>Drug</td>
<td>Treprostinil/sc</td>
<td>Beraprost/os</td>
<td>Beraprost/os</td>
</tr>
<tr>
<td>Duration (months)</td>
<td>3</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Primary end points</td>
<td>6-min walk</td>
<td>6-min walk</td>
<td>Disease progression</td>
</tr>
<tr>
<td>NYHA functional class (%)</td>
<td>II</td>
<td>11</td>
<td>49</td>
</tr>
<tr>
<td>Etiology (%)</td>
<td>IPAH</td>
<td>58</td>
<td>48</td>
</tr>
<tr>
<td>Treatment effect</td>
<td>Peak VO2 (% predicted)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>6-min walk change (m)</td>
<td>16+</td>
<td>25</td>
<td>31+‡</td>
</tr>
<tr>
<td>Hemodynamics</td>
<td>Improved</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>Clinical events</td>
<td>Reduced</td>
<td>No change</td>
<td>Reduced‡</td>
</tr>
</tbody>
</table>

*Combined end point of an improvement in NYHA functional class and a >10% improvement in 6-min walk. †Sum of percentage may not be 100% for rounding to the nearest unit; 0.5 is rounded to the upper unit. ‡Statistically significant at six months. Only pulmonary vascular resistance improved in preinhalation period, and a more consistent improvement of other parameters is observed in postinhalation period.

**AIR** = Aerosolised Iloprost Randomized Study; **ALPHABET** = Arterial Pulmonary Hypertension And Beraprost European Trial; **CHD** = congenital heart disease (congenital systemic-to-pulmonary shunts); **CTD** = connective tissue disease; **CTEPH** = chronic thromboembolic pulmonary hypertension; **IPAH** = idiopathic pulmonary arterial hypertension; **inh** = inhaled; **N/A** = not available; **NYHA** = New York Heart Association; **os** = oral; **P-PH** = porto-pulmonary hypertension; **sc** = subcutaneous.
**Phosphodiesterase-V inhibitors.** A number of uncontrolled studies have reported favorable effects of the orally active phosphodiesterase-V inhibitor sildenafil in PAH (30–32). A RCT with a cross-over design has been recently published: sildenafil 25 to 100 mg tid administered in 22 NYHA functional class II and III PAH patients has improved after six weeks' symptoms, the exercise capacity as assessed by the Naughton protocol on the treadmill, and the hemodynamics (33). Results of a pivotal RCT on approximately 300 PAH patients are expected in the second quarter of 2004; the findings of this major trial will define the precise role of sildenafil in the treatment algorithm of PAH.

**Combination therapy.** Combination treatment is an attractive option to address the multiple pathophysiologic mechanisms that are present in PAH. Both the efficacy and the safety of the combination of bosentan and epoprostenol were investigated in a study of 33 patients with severe PAH enrolled in a placebo-controlled, prospective study (BREATHE-2). Improved hemodynamics, exercise capacity, and functional class were observed in both groups. A preliminary report shows that there was a trend for a greater (though nonsignificant) improvement in all hemodynamic parameters in the combination treatment group (34). Additional RCTs that are ongoing or planned will explore the effects of combinations using epoprostenol with sildenafil, sitaxsentan, and ambrisentan, respectively.

In patients with PAH who are deteriorating despite chronic treatment with nonparenteral prostanooids, addition of bosentan (35) or sildenafil (36) to the ongoing treatment resulted in favorable improvements of pulmonary hemodynamics and exercise capacity in uncontrolled studies.

**General comments on controlled clinical trials.** The RCTs in PAH have produced a tremendous increase of both knowledge and therapeutic options in PAH. Although these studies have similar designs, treatment duration, and end points, close analyses of baseline NYHA functional class and etiology profiles (Tables 1 to 3) show substantial differences. Accordingly, comparisons may be misleading; improvement, albeit to different degrees, of the mean exercise capacity as assessed by the 6-min walk distance has been observed in all these studies. Peak VO2 was assessed only in two trials, and the results were not completely consistent with changes in the 6-min walk distance. In evaluation of the clinical relevance of exercise capacity improvements, additional elements should be considered such as baseline functional class, effects on combined clinical events (i.e., hospitalizations, mortality, rescue therapies), and hemodynamic effects (Tables 1 to 3). As mentioned previously, a survival benefit has been demonstrated in only one controlled, unblinded study using epoprostenol in patients with severe IPAH. Because epoprostenol is considered rescue therapy based on these results, the more recent RCTs could not be ethically performed to assess mortality as an end point. Furthermore, severely ill subjects requiring epoprostenol treatment were excluded in the recent RCTs, resulting in an overall low mortality of these study populations.

### Table 4. Grading of Evidence for Efficacy

<table>
<thead>
<tr>
<th>Level of Evidence</th>
<th>Data Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level of Evidence A</td>
<td>Data derived from multiple randomized clinical trials or meta-analyses.</td>
</tr>
<tr>
<td>Level of Evidence B</td>
<td>Data derived from a single randomized clinical trial or from multiple randomized clinical trials with heterogeneous results.</td>
</tr>
<tr>
<td>Level of Evidence C</td>
<td>Data derived from small nonrandomized studies and/or consensus opinion of experts.</td>
</tr>
</tbody>
</table>

**LEVEL OF EVIDENCE FOR EFFICACY**

Various grading systems for the definition of the level of evidence of treatments have been proposed. Table 4 shows the grading system adopted in the current review, which is based on the number of favorable RCTs performed with a given compound. The analysis takes into consideration the studies reported and the RCTs presented in Tables 1 to 3. The grading system was adapted from the European Society of Cardiology recommendation for guidelines (37). The only difference is that we did not include in category B “nonrandomized studies” because all these studies in PAH are rather small; therefore, they are included in category C (Table 4). In category B, we included the wording “multiple randomized clinical trials with heterogeneous results” because this situation may happen (and has happened) and this definition is more comprehensive even if the outcome is that “a single randomized clinical trial” resulted positive.

This approach may present some limitations that should be taken into account:

1. The level of evidence may change over time as a result of additional studies performed.
2. The grading system does not address the sample sizes of the RCTs.

### Table 5. Grading of Evidence for Efficacy and Regulatory Approval Status of Selected Treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of RCTs*</th>
<th>Grading of Evidence</th>
<th>Regulatory Approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral anticoagulants</td>
<td>3†</td>
<td>C</td>
<td>—</td>
</tr>
<tr>
<td>Diuretics</td>
<td>—</td>
<td>C</td>
<td>—</td>
</tr>
<tr>
<td>Digi oxin</td>
<td>—</td>
<td>C</td>
<td>—</td>
</tr>
<tr>
<td>Oxygen</td>
<td>—</td>
<td>C</td>
<td>—</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>5†</td>
<td>C</td>
<td>—</td>
</tr>
<tr>
<td>Epoprostenol</td>
<td>3</td>
<td>A</td>
<td>Europe, U.S., Canada</td>
</tr>
<tr>
<td>Treprostinil</td>
<td>2</td>
<td>B</td>
<td>U.S.</td>
</tr>
<tr>
<td>Iloprost (inhalation)</td>
<td>1</td>
<td>B</td>
<td>Europe, Australia</td>
</tr>
<tr>
<td>Iloprost (IV)</td>
<td>1†</td>
<td>C</td>
<td>New Zealand</td>
</tr>
<tr>
<td>Beraprost</td>
<td>2</td>
<td>B</td>
<td>Japan</td>
</tr>
<tr>
<td>Bosentan</td>
<td>2</td>
<td>A</td>
<td>Europe, U.S., Canada</td>
</tr>
<tr>
<td>Sitaxsentan</td>
<td>1</td>
<td>B</td>
<td>—</td>
</tr>
<tr>
<td>Ambrisentan</td>
<td>1†</td>
<td>C</td>
<td>—</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>1</td>
<td>B</td>
<td>—</td>
</tr>
<tr>
<td>BAS</td>
<td>Multiple†</td>
<td>C</td>
<td>—</td>
</tr>
<tr>
<td>Lung transplantation</td>
<td>Multiple†</td>
<td>C</td>
<td>—</td>
</tr>
</tbody>
</table>

*See appropriate paragraph of the text for references. †Uncontrolled and/or retrospective study. BAS = balloon atrial septostomy; IV = intravenous; RCT = randomized controlled trials.
3. The level of evidence for efficacy should not be confused with the strength of clinical efficacy, which depends on the pharmacodynamic effects of the compound and on patient response. For example, a compound that increased the 6-min walking distance in two controlled clinical trials by 40 m will be rated with a Level of Evidence A (Table 4). If a more potent compound increased the 6-min walking distance by 80 m in only one controlled clinical trial it will be rated Level of Evidence B even though the clinical relevance of the effect is greater.

4. Regulatory agencies may grant approval to a given treatment on the basis of a single RCT with an appropriate sample size and prespecified adequate statistical requirements.

Table 5 shows the grading of evidence for efficacy and regulatory approval status of selected treatments.

**EVIDENCE-BASED TREATMENT ALGORITHM**

A treatment algorithm based on the evidence derived by clinical trials is depicted in Figure 1. The algorithm is restricted to patients in NYHA functional class III or IV because such patients represent the predominant population included in RCTs. For NYHA functional class I or II, very few data are available, and the most appropriate strategy has still to be determined and possibly validated by specific studies. In addition, the different treatments have been evaluated mainly in sporadic idiopathic pulmonary arterial hypertension (IPAH) patients, and in pulmonary arterial hypertension (PAH) associated with scleroderma or to anorexigen use. Extrapolation of these recommendations to the other PAH subgroups should be done with caution.
near-normal hemodynamics) should be confirmed after three to six months of treatment. Nonresponders to acute vasoreactivity testing, or responders who remain in NYHA functional class III, should be considered candidates for treatment with either an ERA or a prostanooid. At present, the only commercially available and approved ERA is the oral active dual-antagonist bosentan. Among prostanooids, treprostinil is administered subcutaneously and has been approved in the U.S.; iloprost, administered by aerosol, has been approved in Europe, whereas beraprost is approved in Japan. Continuous IV administration of epoprostenol may also be used in NYHA functional class III patients who are refractory to ERAs or other prostanooids.

The choice of drug is dependent on a variety of factors, including the approval status, route of administration, side-effect profile, and the physician’s experience. The orally active phosphodiesterase-V inhibitor sildenafil is not approved for the treatment of PAH, and its use should be considered in patients with PAH, who have failed or are not candidates for other available therapy. The role of this drug will be better defined after the evaluation of the pivotal RCT that is currently ongoing.

Continuous IV epoprostenol, approved in the U.S. and Europe, may be considered as first-line therapy for IPAH patients in NYHA functional class IV because of the demonstrated survival benefit in this subset. Although both bosentan and treprostinil are approved for this population, only a small number of NYHA functional class IV patients were included in the clinical trials of these agents. Accordingly, most experts consider these treatments as a second line for severely ill patients. Although no RCTs have been performed with the continuous IV delivery of iloprost this prostanoid analogue, administered intravenously is approved in New Zealand for PAH patients.

Combination therapy (e.g., ERA + prostanooids) may be considered for patients who fail to show improvement or who deteriorate with first-line treatment, even though data on this specific strategy are limited and largely uncontrolled at this point. Appropriate protocols for timing and dosing to limit possible side effects of the combination have still to be implemented.

Finally, both balloon atrial septostomy and lung transplantation are indicated for refractory PAH or where medical treatments are unavailable. These procedures should be performed only in experienced centers.

Reprint requests and correspondence: Dr. Nazzareno Galie, Istituto di Cardiologia, Università di Bologna, via Massarenti, 9, 40138-Bologna, Italy. E-mail: n.galie@bo.nettuno.it.

REFERENCES

Identification of mutations in the bone morphogenetic protein receptor-2 (BMPR2) in the majority of cases of familial pulmonary arterial hypertension (FPAH) has been a major advance in the elucidation of the pathogenic sequence in pulmonary arterial hypertension (PAH) (1,2). However, fewer than 20% of individuals with a BMPR2 mutation develop FPAH, and most individuals who develop PAH do not have an identifiable mutation (3); accordingly, it is likely that other factors, including genes and environmental stimuli, are needed to initiate the pathological sequence that leads to vascular injury and the pulmonary hypertensive state. Both the role of these other factors in initiating the vasculopathic process and the mechanisms through which they interface with genetic abnormalities are unknown (4).

Various cellular pathway abnormalities have been described that may play important roles in the development and progression of PAH (5–9). These include altered synthesis of nitric oxide, prostacyclin and endothelin, impaired potassium channel and growth factor receptor function, altered serotonin transporter regulation, increased oxidant stress, and enhanced matrix production. However, the relative importance of each of these processes is unknown, and the interactions between these various pathways should be explored. Additionally, the intermediate steps involved in the transduction of signals related to BMPR2 are unknown; clarification of these pathways will lead to a more complete understanding of how impaired BMPR2 signaling leads to hypertensive pulmonary vascular disease (10,11).

**THERAPY OF PAH**

Less than a decade ago, the treatment of PAH was based on a limited understanding of the disease pathogenesis and was largely empiric and usually ineffective. The treatment of PAH has advanced dramatically since then, with a number of well-designed clinical trials demonstrating efficacy of several therapies that target specific abnormalities present in PAH (12–15). Furthermore, the complexity of these treatments has devolved from continuous intravenous (IV) delivery to oral and inhaled modes of drug delivery. Despite these successes, the response to therapy of PAH is not universal and is often incomplete. Future studies targeting newly identified alterations in endothelial and smooth muscle cell function, including phosphodiesterase-5 (PDE5) and angiotensin activity, vasoactive intestinal peptide synthesis and activity (16), and the serotonin pathway (9,17) may provide novel treatments.

Drugs currently marketed to treat other conditions may have effects that are beneficial in PAH as well. For example, the hydroxymethylglutaryl-coenzyme-A reductase inhibitors manifest pleiotropic effects that have been suggested to be responsible for a component of their benefit in arteriosclerotic disease (18), and these agents attenuate the pulmonary arteriopathy induced by the administration of monocrotaline to experimental animals (19,20). Formal clinical studies with the statins may, therefore, be appropriate. Similarly, currently available platelet inhibitors (i.e., aspirin) and newer antithrombotic agents may have a role in the treatment of PAH, in light of the beneficial effects (and inherent risks) of anticoagulation with warfarin in idiopathic PAH.

As with other diseases with a complex pathogenesis, targeting a single pathway in PAH is unlikely to be uniformly successful. With the development of several pathway-specific therapies, the opportunity exists for evaluating multidrug therapy in PAH: for example, studies combining an endothelin receptor antagonist with a prostanoïd or a PDE5 inhibitor may lead to either a more aggressive first-line treatment strategy combining several drugs, or to a strategy of layered therapy for disease progression, or both.

**MEASURING OUTCOMES AND MONITORING THE COURSE OF THERAPY**

The development of treatments for PAH has prompted the challenge of how to best assess and monitor the efficacy of long-term therapy. Because it is believed that randomized, placebo-controlled trials using survival as an end point would be unethical to perform in PAH, alternative strategies are required to measure and compare the relative effects
of the available treatments. Similarly, noninvasive markers of disease severity, either biomarkers or physiological tests, are needed that can be widely applied to reliably monitor clinical course. Studies that assess the value of these outcome measures, alone or in combination, will enable physicians to time and select therapy in a more structured fashion.

Conclusions. Although major advances in our understanding of the mechanisms of disease development and in the treatment of PAH have been achieved over the past decade, substantial gaps in our knowledge remain. Bringing together physicians and scientists representing multiple disciplines and expertise, all sharing an interest in PAH, afforded the opportunity to explore areas of mutual interest and collaboration that will, it is hoped, narrow these gaps of knowledge in the future. Ultimately, the success of the Third World Symposium on Pulmonary Arterial Hypertension will be best measured by the progress achieved in understanding and treating PAH over the next few years.

Reprint requests and correspondence: Dr. Lewis J. Rubin, UCSD Medical Center, 9300 Campus Point Drive, M/C 7372, La Jolla, California 92037. E-mail: ljrubin@ucsd.edu.

REFERENCES