

Canadian Cardiovascular Society and Canadian Thoracic Society position statement on pulmonary arterial hypertension

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The Canadian Cardiovascular Society and the Canadian Thoracic Society requested a position statement on pulmonary arterial hypertension from leading Canadian experts. The present document is intended to act as an update for the clinician, to provide a template for the initial evaluation of patients, to enable understanding of current therapeutic paradigms based on approved indications for Canada, to highlight new therapies on the horizon, and to state the positions of the Canadian Cardiovascular Society and the Canadian Thoracic Society on resource management for pulmonary arterial hypertension in Canada.

Key Words: *Canadian health system; Endothelin; Nitric oxide; Prostaglandins; Pulmonary artery; Pulmonary hypertension*

In the past decade, considerable progress has been made in the understanding, diagnosing and treating of pulmonary hypertension. The present document provides a brief overview of that progress. It is intended to act as an update for the clinician, to provide a template for the initial evaluation of patients, to enable understanding of current therapeutic paradigms based on approved indications for Canada, to highlight new therapies on the horizon, and to state the positions of the Canadian Cardiovascular Society and the Canadian Thoracic Society on resource management for pulmonary arterial hypertension (PAH) in Canada.

DEFINITIONS

The normal adult pulmonary vasculature is a low-pressure, high-capacitance bed that can accommodate increases in cardiac output with little or no increase in pressure. Many factors contribute to this low resistance, including the large cross-sectional area of the pulmonary vascular bed and the availability of a low-pressure drainage area (ie, the left atrium) for blood leaving the lung. All disorders that increase pulmonary vascular resistance will increase pulmonary arterial

Énoncé de position de la Société canadienne de cardiologie et de la Société canadienne de thoracologie sur l'hypertension artérielle pulmonaire

La Société canadienne de cardiologie et la Société canadienne de thoracologie ont demandé à un groupe d'experts canadiens de rédiger un énoncé de position sur l'hypertension artérielle pulmonaire. Le présent document se veut une mise à jour pour le clinicien et lui fournit un modèle d'évaluation initiale des patients. Il vise également à faciliter la compréhension des algorithmes thérapeutiques actuels fondés sur les indications approuvées au Canada. Ce document met en évidence les nouveaux traitements à l'horizon et énonce les positions de la Société canadienne de cardiologie et de la Société canadienne de thoracologie sur la gestion des ressources consacrées à l'hypertension artérielle pulmonaire au Canada.

pressure (Ppa). However, when assessing pulmonary hemodynamics, the pulmonary arterial blood flow must also be considered because some types of hyperdynamic circulation can raise Ppa without it representing a true increase in pulmonary vascular resistance. Thus, in defining pulmonary hypertension, the pulmonary vascular resistance should exceed 3 Wood units (ie, greater than 240 dynes \times s \times cm⁻⁵). Conversely, in conditions with only mildly increased pulmonary vascular resistance, pulmonary hypertension may not be evident at rest, and may be revealed only with exercise or pharmacological elevation of cardiac output. Precapillary pulmonary hypertension, which includes PAH, is defined for clinical purposes as a mean end-expiratory Ppa of greater than 25 mmHg at rest, with a mean end-expiratory pulmonary artery wedge pressure (Pwedge) of less than 15 mmHg and a calculated pulmonary vascular resistance of greater than 3 Wood units. It is critical to have a reliable and accurate Pwedge tracing. If a Pwedge cannot be obtained reliably, then the left ventricular end-diastolic pressure should be less than 15 mmHg at end-expiration, to exclude left heart disease, and there should be no indication of left-sided cardiac valvular disease.

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This position statement was jointly commissioned by the Canadian Cardiovascular Society and the Canadian Thoracic Society. The content has been approved by both societies and is being published simultaneously in *The Canadian Journal of Cardiology* and *the Canadian Respiratory Journal*. Correspondence and reprints: Dr David Langleben, Centre for Pulmonary Vascular Disease, Room E-258, Sir Mortimer B Davis Jewish General Hospital, 3755 Cote Ste Catherine, Montreal, Quebec H3T 1E2. Telephone 514-340-7531, fax 514-340-7534, email david.langleben@mcgill.ca

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TABLE 1
The “Venice” classification of pulmonary hypertension

Pulmonary arterial hypertension
<ul style="list-style-type: none"> • Idiopathic • Familial • Associated with: <ul style="list-style-type: none"> Connective tissue disease Congenital systemic-to-pulmonary shunts Portal hypertension HIV infection Drugs and toxins Other • Associated with significant venous or capillary involvement (eg, pulmonary veno-occlusive disease or pulmonary capillary hemangiomatosis)
Pulmonary hypertension with left heart disease
<ul style="list-style-type: none"> • Left-sided atrial or ventricular disease • Left-sided valvular heart disease
Pulmonary hypertension associated with lung diseases and/or hypoxemia
<ul style="list-style-type: none"> • Chronic obstructive pulmonary disease • Interstitial lung disease • Sleep-disordered breathing • Alveolar hypoventilation syndromes • Chronic exposure to high altitude • Developmental abnormalities
Pulmonary hypertension due to chronic thrombotic and/or embolic disease
<ul style="list-style-type: none"> • Thromboembolic obstruction of proximal pulmonary arteries • Thromboembolic obstruction of distal pulmonary arteries • Nonthrombotic pulmonary embolism (eg, tumour, parasites and foreign material)
Miscellaneous
<ul style="list-style-type: none"> • Sarcoidosis, histiocytosis X, lymphangiomatosis or compression of pulmonary vessels

Data from reference 4

ETIOLOGY AND CLASSIFICATION

Numerous classification schemes have been developed since the 1950s, when Dresdale et al (1) first described primary pulmonary hypertension (PPH) and Wood et al (2) performed seminal studies on the effects of vasodilators. Because several etiologies as well as PPH seem to cause similar morphological changes in the precapillary vessels (see below), and these etiologies have many common features of pathogenesis and abnormal mediators, the consensus group of the World Health Organization has included them in the PAH classification (3). This classification was slightly revised in June 2003 at the Third World Symposium on PAH in Venice, Italy, and is now termed the “Venice” classification (4) (Table 1). With the recognition that some patients formerly diagnosed with PPH in fact have an identifiable, specific genetic abnormality, notable changes in the Venice classification are the replacement of ‘PPH’ with ‘idiopathic PAH’, and the inclusion of ‘familial PAH’ as a distinct type of PAH. The classification has proven to be extremely useful in clinical research and studies of pathogenesis. The present document will concentrate on PAH. A functional classification for PAH was also established by the World Health Organization, and is analogous to that of the Canadian Cardiovascular Society for angina and the New York Heart Association for congestive heart failure, with four classes of severity of symptoms (5).

PATHOGENESIS

The earliest hypotheses regarding the development of PPH proposed that vasoconstriction was the initial and principal abnormality and that it led to structural changes in vessels (ie, remodelling), resulting in ‘fixed’ disease (ie, unresponsive to vasodilators). However, recent studies (6) have suggested that the remodelling may be the first and most predominant abnormality. Moreover, at the time of hemodynamic evaluation, by which time most patients have advanced disease, less than 10% of patients with idiopathic PAH have a significant vasodilator response to exogenous dilators such as inhaled nitric oxide or intravenous epoprostenol. PAH is associated with structural alterations in normally thin-walled precapillary pulmonary microvessels of approximately 300 µm to 50 µm in size. The lesions consist of endothelial intimal thickening, smooth muscle proliferation and plexiform lesions (7). The vessel lumen is narrowed or obliterated, leading to increased pulmonary vascular resistance. Plexiform lesions, which are vascular tufts consisting principally of endothelial cells, form mainly downstream from bifurcations (6). These abnormal structures may result from increased cell proliferation or decreased apoptosis. Based on the finding that in some patients with idiopathic PAH, the endothelial ‘expansion’ is monoclonal, this growth may represent a form of localized neoplasia (8). Plexiform lesions may appear late in the course of the disease and are not universally present, but the intimal thickening of ‘plexogenic arteriopathy’ is an early and almost universal finding (6). Recent evidence (9) suggests that endothelial cell apoptosis, likely a result of environmental factors and genetic predisposition, may be an initiating event in PAH, which leads secondarily to endothelial proliferation and, in some cases, to the emergence of apoptosis-resistant endothelial cell populations. Larger conduit arteries develop medial smooth muscle hypertrophy and hyperplasia, further thickening and stiffening the vessel wall (7).

A deficiency in the expression of certain voltage-gated potassium channels, particularly Kv1.5, which results in membrane depolarization and increased intracellular calcium levels in smooth muscle, has been described in patients with idiopathic PAH (10,11). This ionic remodelling may contribute to vasoconstriction and hypertrophy, and is also involved in suppression of apoptosis through its effects on intracellular potassium. Adventitial thickening and inflammation can also be present, and the inflammatory process may be an essential element in the development of the vascular lesions (12). Increased levels of elastase and other proteinases contribute to disorganization of the vascular wall matrix and may promote remodelling (13).

The recognition that idiopathic PAH may be genetically transmitted in families led to the identification of mutations causing dysfunction in the gene for the type-II bone morphogenic protein receptor (BMPR-II) (14,15). These mutations have been found in up to 66% of familial cases and up to 20% of sporadic cases of idiopathic PAH, although estimates vary between centres that are performing the genetic analyses, with some estimates being much lower. Bone morphogenic protein is a member of the transforming growth factor beta superfamily of peptides and is thought to normally suppress cell proliferation or to induce apoptosis (16). Although loss of these negative growth influences on normally quiescent lung vascular cells could result in increased cell proliferation and deficient apoptosis, the actual mechanisms by which BMPR-II

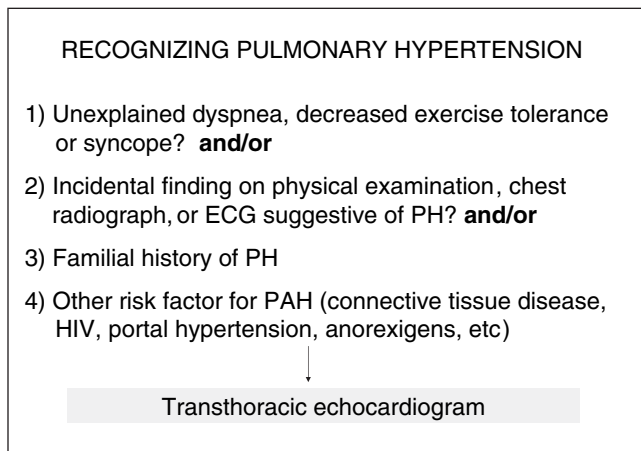


Figure 1) Indications for screening for pulmonary hypertension (PH). The transthoracic echocardiogram is the most useful noninvasive screening tool. ECG Electrocardiogram; PAH Pulmonary arterial hypertension

mutations lead to plexogenic pulmonary arteriopathy remain to be elucidated. As with other diseases, this genetic susceptibility may, in many cases, be inadequate to cause the disease without additional environmental stimuli. Many patients with BMPR-II mutations do not develop PAH (due to incomplete penetrance) and, conversely, animal models of pulmonary hypertension seem to be associated with decreased BMPR-II gene expression in the absence of a genetic abnormality. Microsatellite instabilities of other tumour growth-related genes have been reported for other types of PAH and may contribute to the increased cell numbers that occur (9). Thus, PAH may principally be a disorder of endothelial growth and maintenance of vascular integrity.

MEDIATORS AND MARKERS OF PAH

Abnormal tissue, plasma or urinary levels of several vasoactive mediators have been described in PAH (17). Reduced expression and synthesis of prostacyclin, increased expression and synthesis of thromboxane A₂ and endothelin-1, and increased levels of serotonin and serotonin reuptake receptors have been described (18-22). All of these changes favour vasoconstriction and cellular proliferation. Reduced fibrinolytic activity and a prothrombotic state have also been reported, with increased levels of tissue factor. Identification of these abnormalities has provided a rationale for much of the currently used therapy. In addition, brain natriuretic peptide, troponin and uric acid may be useful, though nonspecific, markers of PAH severity (23-25). Troponin or brain natriuretic peptide measurement may allow the noninvasive monitoring of therapy in PAH using point-of-care testing.

SCREENING AND IDENTIFICATION

Pulmonary hypertension should be considered in the differential diagnosis of unexplained dyspnea (Figure 1). When pulmonary hypertension is in more advanced stages, it may be detected during the physical examination or by electrocardiography (P pulmonale, right axis deviation, right bundle branch block, right ventricular hypertrophy) or chest radiography. However, physical examination is often too insensitive to detect milder forms of the disease. In this regard, two-dimensional and Doppler echocardiography have greatly improved the detection rate. While echocardiography is an extremely useful

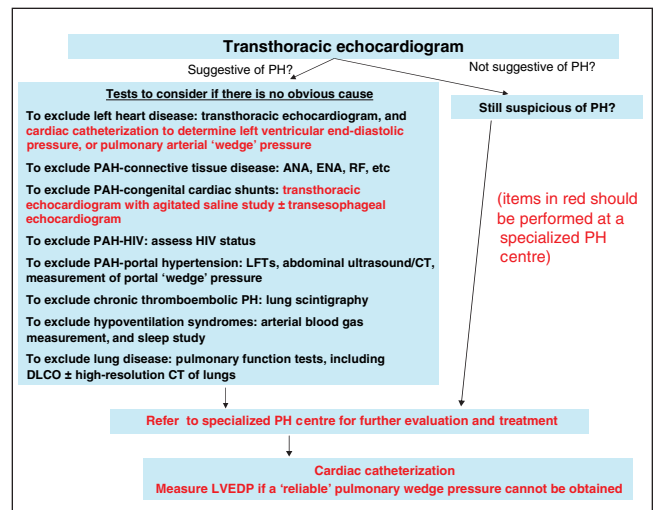


Figure 2) The paradigm for diagnostic evaluation of a suspected case of pulmonary hypertension (PH). Actions in red should be performed at a specialized PH centre. ANA Antinuclear antibody test; CT Computed tomography; DLCO Carbon monoxide diffusing capacity test; ENA Extractable nuclear antigens test; LFTs Liver function tests; LVEDP Left ventricular end-diastolic pressure; PAH Pulmonary arterial hypertension; RF Rheumatoid factor

technique, it provides only an estimate of right ventricular systolic pressure, and is less sensitive and specific when Ppa is closer to normal. This is also a problem when stress echocardiography is used to assess the effects of exercise on Ppa. Pulmonary hypertension is a hemodynamic disorder and cardiac catheterization remains the gold standard for its diagnosis because it provides the ability to directly measure Ppa, calculate pulmonary blood flow, determine the response to acute vasodilators (in appropriate patients), determine disease severity (ie, prognosis) and exclude secondary causes of pulmonary hypertension. Cardiac magnetic resonance imaging is an emerging tool for the evaluation of right ventricular function. It is able to estimate right ventricular mass, volume and ejection fraction. Magnetic resonance imaging studies of the right ventricle do not require injection of gadolinium or other contrast material.

INVESTIGATIONS AT SPECIALIZED HYPERTENSION CLINICS

Beyond the initial screening, all patients suspected of having PAH should be referred to specialized pulmonary hypertension clinics at academic centres that provide a critical mass of expertise and patient volume (Figures 1 and 2). This avoids inadequate or duplicate testing, and fosters research in these relatively rare and complex patients. Given Canadian provincial population sizes, one or two adult care centres per province should suffice. Despite increasing recognition of specific genetic abnormalities in idiopathic PAH, the specific diagnosis remains in the domain of the clinician through the exclusion of a variety of other causes of pulmonary hypertension, and genetic testing is not yet routinely advocated. All patients with pulmonary hypertension require a careful history and physical examination followed by testing to identify specific abnormalities (Figure 2). A basic battery of tests will allow for the correct classification of most patients with pulmonary hypertension. In more complex cases, other testing may be needed. For patients

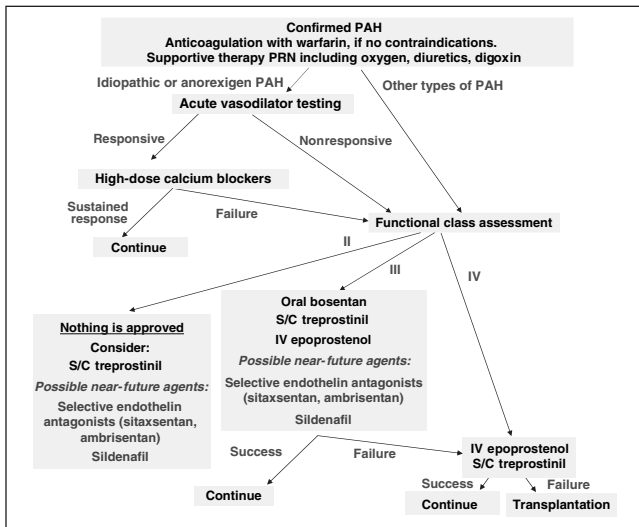


Figure 3) The paradigm for therapy for patients with pulmonary arterial hypertension. As discussed in the text, this therapy should be initiated and followed at the specialized pulmonary hypertension centre. IV Intravenous; PAH Pulmonary arterial hypertension; PRN As necessary; S/C Subcutaneous

with idiopathic PAH or PAH related to anorexigens, vasodilator testing to assess vasoreactivity should be performed at the pulmonary hypertension clinic (Figure 3) to identify an appropriate therapy, as well as for prognostic reasons, using short-acting vasodilators, such as intravenous adenosine, epoprostenol or inhaled nitric oxide (26-28). All are approximately equipotent, but inhaled nitric oxide has the advantages of selectively dilating the pulmonary bed and causing fewer side effects. A successful response to vasodilator testing is defined as a reduction in the mean Ppa by at least 10 mmHg to 40 mmHg, and predicts a response to high-dose calcium channel blocker therapy (26,28). There is no evidence for the use of vasodilator testing in other forms of PAH and other types of pulmonary hypertension, and vasodilator testing in such cases may be hazardous (29).

GENETIC TESTING

There was great excitement in the pulmonary hypertension community regarding the identification of the BMPR-II gene mutations and there was hope that it would rapidly translate into novel therapies and early detection. However, although transmission in families is autosomal-dominant, the penetrance is extremely variable. The phenotype may disappear for several generations, only to reappear with severe manifestations. Thus, many gene carriers never develop clinically overt pulmonary hypertension. Therefore, the use of either routine screening for the gene in at-risk populations or screening for PAH in asymptomatic individuals who carry the gene is uncertain. It is unknown whether treating idiopathic PAH at an early stage will prevent progression, and all of the therapies presently available have risks and side effects. Thus, at the present time, informing gene carriers of their status presents significant psychological and, possibly, financial risk without obvious benefit, and it is, therefore, unethical. Screening pulmonary hypertension populations for BMPR-II and other genetic mutations remains an important and ethical research tool as long as patient anonymity is maintained and patients understand that they will not be informed of their status.

ACCESS TO THERAPY

Medications currently approved for the treatment of PAH substantially improve survival and/or quality of life. However, with few exceptions, they are extremely expensive and well beyond the means of individual citizens. The availability of high-quality health care in Canada is a right and a national priority. It is the authors' position that these medications must be available and financially supported within all provincial formularies across Canada in a timely fashion (ie, within three months of approval by Health Canada). These medications should be prescribed only by the provincially designated pulmonary hypertension centres regardless of the method of administration (oral, subcutaneous, intravenous or other), and these centres must have mechanisms in place for adequate follow-up to ensure that the chosen therapy remains effective and does not have deleterious side effects.

THERAPY

Approved therapies for PAH in Canada consist of two types: prostaglandins and endothelin receptor antagonists (Figure 3). Intravenous epoprostenol (prostacyclin) is approved for functional classes III and IV idiopathic PAH, and PAH related to connective tissue diseases without significant lung fibrosis. The medication must be kept cool and shielded from light. It has an extremely short half-life, necessitating continuous infusion with a portable pump and an indwelling central venous catheter. The major risks include interruption of the infusion, which can be fatal, and sepsis from catheter infections. Many patients experience troubling but nonserious side effects such as jaw pain, diarrhea, flushing and headache. Nevertheless, this is the only approved medication that has been shown to improve survival in PAH and it is an effective bridge to lung transplantation (30-34). Anecdotal evidence suggests that some patients may improve to a degree that they can be removed from transplant lists. A subcutaneously administered prostaglandin, treprostinil, is approved for functional classes III and IV PAH (35). It has a longer half-life and is stable at room temperature. The major side effect is injection site inflammation and pain, which in some patients may be intolerable. The orally active nonselective endothelin antagonist bosentan is approved for functional classes III and IV idiopathic PAH and PAH related to connective tissue disease without significant lung fibrosis (36-38). The evidence for its use in functional class IV is weak. The principal risk associated with its use is hepatotoxicity, which may be severe. Monthly measurement of serum hepatic transaminase and bilirubin levels must be performed long-term. Of the approved agents, epoprostenol seems to provide the greatest degree of benefit. The oral route of bosentan makes it the initial treatment of choice for milder (functional class III) disease.

The Third World Symposium on PAH in Venice provided a paradigm for therapy, which includes all the medications available in Canada (39). It must be re-emphasized that acute vasodilator challenge should be reserved for idiopathic or anorexigen-related PAH, as should high-dose calcium channel blocker therapy (40,41). Calcium channel blockers should be reserved for patients with a true acute vasodilator response (as defined above), and one of nifedipine, amlodipine or diltiazem may be prescribed, the choice depending on the degree of right ventricular dysfunction and the heart rate. This therapy is used by Canadian specialists in an off-label fashion because the indication has never been evaluated or approved by Health Canada.

Similarly, warfarin therapy may provide a survival benefit as suggested in uncontrolled series in idiopathic PAH, and is used in an off-label fashion (40,42). The potential benefit of warfarin in idiopathic PAH has been extrapolated for use in other forms of PAH, as long as there are no contraindications, but there are no studies to support this extrapolation.

Considering that not all patients in a given functional class may be appropriate candidates for the therapies of choice, the experts at pulmonary hypertension centres may find it necessary to prescribe therapies in an off-label fashion (eg, sildenafil for functional class III when unable to take bosentan) or when the data for effectiveness are weak (eg, bosentan in a functional class IV patient in whom prostaglandins cannot be used). Therapies such as these must be reserved for expert use, with extremely close follow-up. Application may have to be made to provincial payers on a case-by-case basis.

SUPPORTIVE THERAPY

Patients with PAH may require oxygen to maintain systemic oxygen saturations at levels over 90%. It is important to emphasize that severe hypoxemia is not a characteristic of idiopathic PAH except in its final stages. If severe hypoxemia is present, other causes (eg, intracardiac shunts or lung diseases) must be considered. In patients with severely reduced cardiac outputs, transcutaneous measurements of oxygen saturation on the fingers may provide falsely low estimates because of sluggish blood flow in the digits, which provides time for increased oxygen extraction. Diuretics may be used to control edema, recognizing that the right ventricular preload should not be compromised. Digoxin may reduce levels of neurohormonal activation and may be considered for use (43).

THERAPIES UNDER EVALUATION

Several therapies are under evaluation in phase III studies, and may soon be available if they prove to be safe and effective. Nitric oxide levels are reduced in PAH, as are levels of its downstream signalling messenger, cyclic GMP (cGMP). Sildenafil inhibits phosphodiesterase type 5, the enzyme that breaks down cGMP and is highly expressed in the lung and genitalia, accounting for the localization of its hemodynamic effects largely to these organs. The resultant increase in cGMP may in turn promote vasodilation and possibly lead to a reduction in smooth muscle proliferation. There are several case series and two small placebo-controlled crossover trials suggesting that sildenafil may be effective in PAH. The preliminary results of a placebo-controlled, blinded trial suggest that sildenafil may be an effective therapy for PAH, although there was an as yet unexplained dissociation between clinical benefit and hemodynamic benefit. Inhaled nitric oxide is effective as an acute vasodilator in PAH (26) but its use in chronic therapy awaits randomly assigned clinical trials and it seems unlikely to achieve widespread use due to the complexities of ambulatory administration of this very short-lived and expensive gas. New forms of endothelin antagonists are also being tested. Two endothelin antagonists, which have more selectivity for the endothelin-A than for the endothelin-B receptor compared with bosentan, are also under evaluation in phase III trials. Sitaxsentan is highly selective for the endothelin-A receptor (endothelin-A to endothelin-B ratio of 6500:1), while ambrisentan is fourfold more selective (77:1) than bosentan. In a recent blinded, placebo-controlled trial, sitaxsentan was found to be both safe and effective in improving functional

capacity in PAH patients with class II and class III symptoms (44). Preliminary data (45) using ambrisentan are also encouraging. Selective endothelin-A blockade may be preferable in PAH because it preserves endothelin-B activity, which is responsible for endothelin-1 clearance and stimulates production of beneficial endogenous prostacyclin and nitric oxide. However, whether selective endothelin blockers will be superior to nonselective blockers remains to be determined; similar to bosentan, selective endothelin blockers manifest hepatotoxicity as a class effect to varying degrees. Preliminary Canadian studies in animals, using cell-based gene therapy, are extremely encouraging and may translate into novel clinical therapies in 2005. Inhaled prostaglandins (eg, iloprost and treprostinil) are also under investigation.

CONCLUSIONS

Tremendous progress has been made in the past decade in understanding the pathogenesis of PAH, establishing its classification and providing effective therapy. This has brought great hope and encouragement to patients and caregivers. This progress must continue. The sobering reality is that most patients with PAH are still at risk of dying within a decade after diagnosis, and new insights into pathogenesis and novel therapies will be required to improve the situation.

FURTHER READING

This position statement provides an expert consensus of the authors, tailored for the Canadian health care system. For further information on PAH, and referenced sources, readers should consult two recent reviews: *Chest* 2004;126(Suppl 1):1S-92S and *J Am Coll Cardiol* 2004;43(12 Suppl S):S1-90. Please note that the Canadian position and conclusions may differ slightly from the comments in these reviews.

CONFLICTS OF INTEREST: Dr Langleben has received support from GlaxoSmithKline, United Therapeutics, Northern Therapeutics, Actelion, Encysive Pharmaceuticals, Myogen and Pfizer. Dr Archer has received funding from Pfizer and ICOS-Lilly, and holds a patent on strategies to enhance potassium channel expression as therapy for primary pulmonary hypertension (PPH). Dr Granton has received support from GlaxoSmithKline, United Therapeutics, Northern Therapeutics, Actelion, Encysive Pharmaceuticals and Pfizer. Dr Hirsch has received funding from Actelion, Encysive Pharmaceuticals, United Therapeutics and Pfizer. Dr Levy has received funding from Actelion, Northern Therapeutics and Encysive Pharmaceuticals. Dr Mehta has received funding from Actelion, GlaxoSmithKline, Northern Therapeutics, Pfizer and Encysive Pharmaceuticals. Dr Michelakis has received funding from Encysive Pharmaceuticals and Pfizer, and holds a patent on strategies to enhance potassium channel expression as therapy for PPH. Dr Stewart is the Founding Scientist and Chief Scientific Officer of Northern Therapeutics.

REFERENCES

1. Dresdale DT, Schultz M, Michtom RJ. Primary pulmonary hypertension. I. Clinical and hemodynamic study. *Am J Med* 1951;11:686-705.
2. Wood P, Besterman EM, Towers MK, McIlroy MB. The effect of acetylcholine on pulmonary vascular resistance and left atrial pressure in mitral stenosis. *Br Heart J* 1957;19:279-86.
3. Fishman AP. Clinical classification of pulmonary hypertension. *Clin Chest Med* 2001;22:385-91.
4. Simonneau G, Galie N, Rubin LJ, et al. Clinical classification of pulmonary hypertension. *J Am Coll Cardiol* 2004;43(Suppl S):5S-12S.

5. Barst RJ, McGoon M, Torbicki A, et al. Diagnosis and differential assessment of pulmonary arterial hypertension. *J Am Coll Cardiol* 2004;43(Suppl S):40S-7S.
6. Cool CD, Stewart JS, Werahera P, et al. Three-dimensional reconstruction of pulmonary arteries in plexiform pulmonary hypertension using cell-specific markers. Evidence for a dynamic and heterogeneous process of pulmonary endothelial cell growth. *Am J Pathol* 1999;155:411-9.
7. Pietra GG, Capron F, Stewart S, et al. Pathologic assessment of vasculopathies in pulmonary hypertension. *J Am Coll Cardiol* 2004;43(Suppl S):25S-32S.
8. Lee SD, Shroyer KR, Markham NE, Cool CD, Voelkel NF, Tuder RM. Monoclonal endothelial cell proliferation is present in primary but not secondary pulmonary hypertension. *J Clin Invest* 1998;101:927-34.
9. Yeager ME, Halley GR, Golpon HA, Voelkel NF, Tuder RM. Microsatellite instability of endothelial cell growth and apoptosis genes within plexiform lesions in primary pulmonary hypertension. *Circ Res* 2001;88:E2-11.
10. Weir EK, Reeve HL, Johnson G, Michelakis ED, Nelson DP, Archer SL. A role for potassium channels in smooth muscle cells and platelets in the etiology of primary pulmonary hypertension. *Chest* 1998;114(Suppl 3):200S-4S.
11. Yuan XJ, Wang J, Juhaszova M, Gaine SP, Rubin LJ. Attenuated K⁺ channel gene transcription in primary pulmonary hypertension. *Lancet* 1998;351:726-7.
12. Voelkel NF, Cool CD, Lee SD, Wright L, Geraci MW, Tuder RM. Primary pulmonary hypertension between inflammation and cancer. *Chest* 1998;114(Suppl 3):225S-30S.
13. Rabinovitch M. Elastase and the pathobiology of unexplained pulmonary hypertension. *Chest* 1998;114(3 Suppl):213S-24S.
14. Deng Z, Morse JH, Slager SL, et al. Familial primary pulmonary hypertension (gene PPH1) is caused by mutations in the bone morphogenic protein receptor-II gene. *Am J Hum Genet* 2000;67:737-44.
15. Lane KB, Machado RD, Pauculo MW, et al. Heterozygous germline mutations in BMPR2, encoding a TGF-beta receptor, cause familial primary pulmonary hypertension. The International PPH Consortium. *Nat Genet* 2000;26:81-4.
16. Loscalzo J. Genetic clues to the cause of primary pulmonary hypertension. *N Engl J Med* 2001;345:367-71.
17. Archer S, Rich S. Primary pulmonary hypertension: A vascular biology and translational research "work in progress". *Circulation* 2000;102:2781-91.
18. Tuder RM, Cool CD, Geraci MW, et al. Prostacyclin synthase gene expression is decreased in lungs from patients with severe pulmonary hypertension. *Am J Respir Crit Care Med* 1999;159:1925-32.
19. Christman BW, McPherson CD, Newman JH, et al. An imbalance between the excretion of thromboxane and prostacyclin metabolites in pulmonary hypertension. *N Engl J Med* 1992;327:70-5.
20. Stewart DJ, Levy RD, Cernacek P, Langleben D. Increased plasma endothelin-1 in pulmonary hypertension: Marker or mediator of disease? *Ann Intern Med* 1991;114:464-9.
21. Herve P, Launay JM, Scrobahaci ML, et al. Increased plasma serotonin in primary pulmonary hypertension. *Am J Med* 1995;99:249-54.
22. Humbert M, Morrell NW, Archer SL, et al. Cellular and molecular pathobiology of pulmonary arterial hypertension. *J Am Coll Cardiol* 2004;43(12 Suppl S):13S-24S.
23. Nagaya N, Nishikimi T, Uematsu M, et al. Plasma brain natriuretic peptide as a prognostic indicator in patients with primary pulmonary hypertension. *Circulation* 2000;102:865-70.
24. Torbicki A, Kurzyna M, Kuca P, et al. Detectable serum cardiac troponin T as a marker of poor prognosis among patients with chronic precapillary pulmonary hypertension. *Circulation* 2003;109:844-8.
25. Nagaya N, Uematsu M, Satoh T, et al. Serum uric acid levels correlate with the severity and the mortality of primary pulmonary hypertension. *Am J Respir Crit Care Med* 1999;160:487-92.
26. Sitbon O, Humbert M, Jagot JL, et al. Inhaled nitric oxide as a screening agent for safely identifying responders to oral calcium channel blockers in primary pulmonary hypertension. *Eur Respir J* 1998;12:265-70.
27. Schrader BJ, Inbar S, Kaufmann L, Vestal RE, Rich S. Comparison of the effects of adenosine and nifedipine in pulmonary hypertension. *J Am Coll Cardiol* 1992;19:1060-4.
28. Groves BM, Badesch DB, Turkevitch D, et al. Correlation of acute prostacyclin response in primary (unexplained) pulmonary hypertension with efficacy of treatment with calcium channel blockers and survival. In: Weir K, ed. *Ion Flux in Pulmonary Vascular Control*. New York: Plenum Press, 1993:317-30.
29. Sitbon O, Humbert M, Jais X, et al. Acute vasodilator responsiveness and long-term response to calcium channel blockers in different forms of PAH. *Am J Respir Crit Care Med* 2004;169:A210. (Abst)
30. McLaughlin VV, Genthner DE, Panella MM, Rich S. Reduction in pulmonary vascular resistance with long-term epoprostenol (prostacyclin) therapy in primary pulmonary hypertension. *N Engl J Med* 1998;338:273-7.
31. Barst RJ, Rubin LJ, McGoon MD, et al. A comparison of continuous intravenous epoprostenol (prostacyclin) with conventional therapy for primary pulmonary hypertension: The Primary Pulmonary Hypertension Study Group. *N Engl J Med* 1996;334:296-302.
32. Sitbon O, Humbert M, Nunes H, et al. Long-term intravenous epoprostenol infusion in primary pulmonary hypertension: Prognostic factors and survival. *J Am Coll Cardiol* 2002;40:780-8.
33. McLaughlin VV, Shillington A, Rich S. Survival in primary pulmonary hypertension: The impact of epoprostenol therapy. *Circulation* 2002;106:1477-82.
34. Badesch DB, Tapson VF, McGoon MD, et al. Continuous intravenous epoprostenol for pulmonary hypertension due to the scleroderma spectrum of disease. A randomized, controlled trial. *Ann Intern Med* 2000;132:425-34.
35. Simonneau G, Barst RJ, Galie N, et al. Continuous subcutaneous infusion of treprostinil, a prostacyclin analogue, in patients with pulmonary arterial hypertension. A double-blind, randomized, placebo-controlled trial. *Am J Respir Crit Care Med* 2002;165:800-4.
36. Fedullo PF, Auger WR, Channick RN, Kerr KM, Rubin LJ. Chronic thromboembolic pulmonary hypertension. *Clin Chest Med* 2001;22:561-81.
37. Rubin LJ, Badesch DB, Barst RJ, et al. Bosentan therapy for pulmonary arterial hypertension. *N Engl J Med* 2002;346:896-903.
38. Sitbon O, Badesch DB, Channick RN, et al. Effects of the dual endothelin receptor antagonist bosentan in patients with pulmonary arterial hypertension: A 1-year follow-up study. *Chest* 2003;124:247-54.
39. Galie N, Seeger W, Naeije R, Simonneau G, Rubin LJ. Comparative analysis of clinical trials and evidence-based treatment algorithm in pulmonary arterial hypertension. *J Am Coll Cardiol* 2004;43(12 Suppl S):81S-88S.
40. Rich S, Kaufmann E, Levy PS. The effect of high doses of calcium-channel blockers on survival in primary pulmonary hypertension. *N Engl J Med* 1992;327:76-81.
41. Rich S, Brundage BH. High-dose calcium channel-blocking therapy for primary pulmonary hypertension: Evidence for long-term reduction in pulmonary arterial pressure and regression of right ventricular hypertrophy. *Circulation* 1987;76:135-41.
42. Fuster VF, Steele PM, Edwards WD, Gersh BJ, McGoon MD, Frye RL. Primary pulmonary hypertension: Natural history and the importance of thrombosis. *Circulation* 1984;70:580-7.
43. Valentini V, Seiditz M, Dodin E, et al. Acute effects of digoxin on hemodynamics, neurohormones and baroreceptor response in pulmonary hypertension. *Circulation* 1996;94:123-4. (Abst)
44. Barst RJ, Langleben D, Frost A, et al. Sitaxsentan therapy for pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2004;169:441-7.
45. Rubin L, Galie N, Badesch D, et al. Ambrisentan improves exercise capacity and clinical measures in pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2004;169:A210. (Abst)