A review of pulmonary arterial hypertension Part 1. Novel insights and classification

C.T. Gan, A. Vonk Noordegraaf, K.M.J. Marques, J.G.F. Bronzwaer, P.E. Postmus, A. Boonstra

Pulmonary arterial hypertension (PAH) is a disease characterised by an increased pulmonary artery pressure. The precapillary pulmonary arteries show distinct pathobiological changes, i.e. medial hypertrophy, intimal fibrosis, microthrombi and plexiform lesions. Although the pathogenesis is not completely understood, pulmonary vascular proliferation and remodelling, due to a variety of mediators, is believed to play the pathogenetic key role. Genetic research reveals molecular deformities and gene mutations associated with phenotypic PAH.

This article covers novel insights into pathobiology, pathogenesis and genes of PAH, which led to a novel classification system and a diagnostic work-up, emanated from the World Health Organisation Symposium on Pulmonary Hypertension in Venice in June 2003. (*Neth Heart J* 2004;12:287-94.)

Key words: classification system, diagnostic work-up, genes, pathobiology, pathogenesis, pulmonary arterial hypertension

Pulmonary arterial hypertension (PAH) is a rare and fatal disease characterised by an elevated mean pulmonary artery pressure (mPap) with distinct changes in the precapillary pulmonary vascular system. This type of pulmonary hypertension (PH) is defined by the US National Institute of Health Registry as a mPap ≥25 mmHg in rest or ≥30 mmHg during exercise in the absence of another cause.[1] The disease is progressive and leads to right heart failure and death within 2.8 years of diagnosis.[2]

In this first part we view the novel insights on pathobiology, pathogenesis and genes, forming the foundation of a renewed classification system, and the diagnostic work-up in pulmonary hypertension.

C.T. Gan
A. Vonk Noordegraaf
A. Boonstra
P.E. Postmus
Department of Pulmonology
K.M.J. Marques
J.G.F. Bronzwaer
Department of Cardiology
VU Medical Centre, Institute for Cardiovascular Research (ICAR-VU), Amsterdam, De Boelelaan 1117, 1081 HV Amsterdam

Correspondence to: A. Vonk-Noordegraaf
E-mail: a.vonk@vumc.nl

Pathoblology, pathogenesis and genes

Pathobiology

Pulmonary arterial hypertension (PAH), which occurs in more than 40 diseases, is characterised by obstruction of small pulmonary arteries in association with plexiform lesions, (i.e. arterial lumen occlusion, aneurysmal dilatation, proliferation of interconnected vascular channels and endothelial and smooth muscle cell proliferation), medial hypertrophy, concentric laminar intimal fibrosis, fibrinoid degeneration, and thrombotic lesions. Pathological classification of patients with either plexiform or thrombotic arteriopathy seems to be arbitrary, because these are not distinct entities but polymorphic manifestations of a similar disease seen in different forms of PAH.[3]

The speculation about the pathogenic substrate for the origin of plexiform lesions has led to several theories among investigators.

According to some investigators proliferation of smooth muscle cells and transformation into myofibroblasts are the two mechanisms leading to formation of plexiform lesions. Phenotypic disease induced by endothelial cells responding to cytokines, growth factors or vascular stress is a theory proposed by other investigators.[4,5]

The bottom line in the pathogenesis of PAH is in general believed to be pulmonary vascular proliferation and remodelling, rather than vasoconstriction.

Pathogenesis

In pulmonary arterial hypertension a variety of factors interact with the vasoactive balance and smooth muscle cell proliferation.

Vasoactive factors

A dysbalance in vasoactive factors provokes vasoconstriction. Several different studies conclude the following regarding vasoactive factors and PAH.

Thromboxane and endothelin-1, both vasoconstrictors and the latter also a mitogen for smooth muscle cells, are increased and prostacyclin, a vasodilator, is decreased in PAH.[6] Nitric oxide (NO), also produced by endothelium and initiated by nitric oxide synthase, stimulates vasodilatation and inhibits smooth muscle cell proliferation. The endothelium of PAH patients has negligible immunohistochemical staining for nitric oxide synthase compared with healthy individuals, suggesting impediment of NO and its vasodilatory effect.[7]

The role of vascular endothelial growth factor (VEGF) in the pathogenesis of PAH remains controversial, because of contradictions in study results.[8]

Angiopoietin-1 (Ang-1), a ligand of the endothelial-specific tyrosine kinase receptor Tie-2, promotes cell survival, vascular maturation and stabilisation. In the monocrotaline (MCT) rat model gene transfer of Ang-1 to the pulmonary microvasculature prevents PAH development by inhibiting endothelial cell apoptosis, and downregulation of the Tie-2 receptor. These study results show the important role of the Ang-1/Tie-2 system in the protection of the pulmonary system.[9,10]

Serotonin signalling pathway: role of the serotonin transporter

Plasma concentration of serotonin, stored in platelets, is increased in PAH patients.[11] In animal studies induced hypoxia increased expression of serotonin transporters, which led to an increased intracellular calcium concentration with resulting vasoconstriction and smooth muscle cell proliferation.[12]

Extracellular matrix remodelling: role of tenascin and matrix metalloproteinases

The walls of small pulmonary vessels of patients with PAH show distinct changes with increased extracellular matrix deposition of collagen. The increased deposition is due to a dysbalance in matrix degradation and excessive production.

Degradation of the extracellular matrix, by vascular serine elastase and matrix metalloproteinase, causes a release in tenascin-C and matrix-bound mitogens, which induce smooth muscle cell proliferation.[13] NO is able to reduce the serine elastase activity and thus prevents vascular remodelling.[14] Rabinovitch et al. show reversal of pulmonary hypertension in the MCT rat model by a serine elastase inhibitor.[15]

Role of ion channels

Normally the electric equilibrium of the cell membrane and the intracellular calcium status is controlled by the voltage-gated potassium channels. Hypoxia disturbs the equilibrium and the intracellular calcium status by inhibiting the potassium current, which is followed by depolarisation and calcium influx.[16] Intracellular calcium functions as a trigger for vasoconstriction and smooth muscle cell hypertrophy.

Yuan et al. showed that smooth muscle cells in PAH have a low mRNA status coding for voltage-gated potassium channels, involving a disturbance in the electric equilibrium of the cell membrane and an increase in intracellular calcium concentration, leading to vasoconstriction.[17]

Coagulation (plasminogen activator inhibitor type 1 and impaired fibrinolysis)

Thrombosis leading to occlusion of the small pulmonary arteries is believed to have a role in PAH. Reports about pleomorphism in the plasminogen activator inhibitor type 1 with increased transcription resulting in decreased fibrinolytic activity support this hypothesis.[18]

Genes

Studies by Deng and Lane in 2000 led to identification of the bone morphogenic protein receptor II(BMPR2) gene on chromosome 2q33, primarily associated with the idiopathic and familiar PAH forms. In 50% of familial PAH and 26% of idiopathic PAH, the heterogeneous germ-line mutation on the BMPR2 gene is evident.[19,20]

The BMPR2 belongs to the TGFβ-receptor superfamily, which binds cytokines, bone morphogenic protein (BMP), activin, inhibin and growth differentiation factor.[21] Discovered in association with bone growth, the BMPRs bind ligands that are also important in cell proliferation and differentiation, and apoptosis. A mutation on the BMPR2 locus could lead to a decrease in bone-morphogenic-protein signalling and loss of antiproliferative and apoptotic function in the pulmonary vascular cell.[22]

However, only 10 to 20% of the individuals with detectable mutations on BMPR2 have phenotypic disease and of the patients with familial PAH only 60% have detectable BMPR2 mutations.[20,23] This suggests genetic heterogeneity, i.e. the role of modifier genes, environmental triggers such HVV8,[24] and mutations in other parts of the gene or other genes that can alter function or expression of the BMPR2 gene or may lead to clinical PAH. Based on this it is now assumed that individuals with BMPR2 mutations will present with phenotypic manifestations of disease if additional factors as mentioned are present (figure 1).[25] Mutation in another TGFβ-receptor gene, activin-receptor-like kinase 1 (ALK-1), was identified in families with hereditary haemorrhagic telangiectasia (associated with ALK-1) and severe PAH.[26] The latter provides evidence for genetic heterogeneity. ALK-1 mutation may lead to PAH, hereditary haemorrhagic telangiectasia or both.

The observation of mutations on two different genes but with a common outcome, i.e. clinical pulmonary arterial hypertension, indicates the

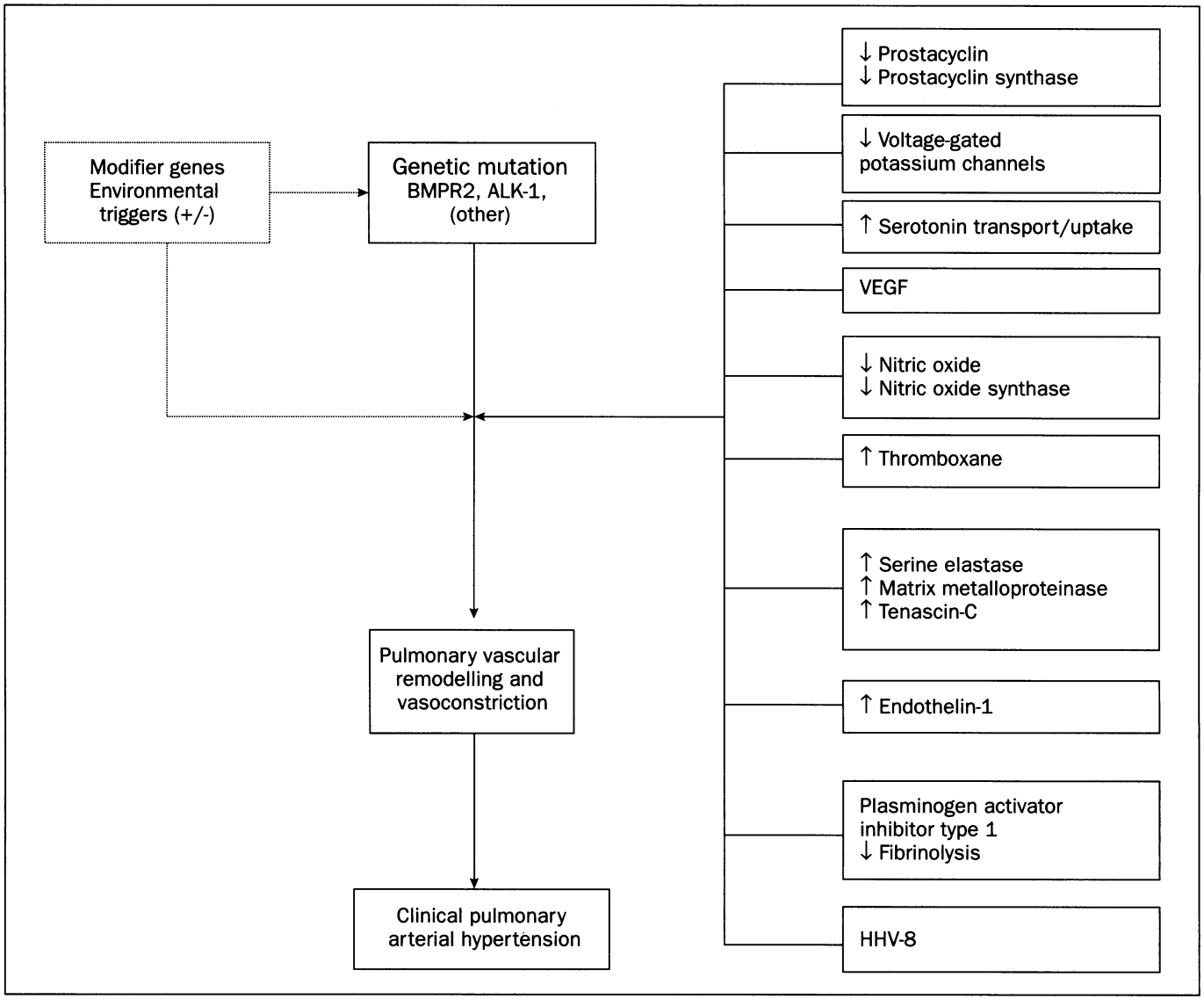

Figure 1. Pathogenesis leading to clinical PAH (see text Pathobiology, pathogenesis and genes). Clinical PAH characterised by pulmonary vascular remodelling is the result of genetic predisposition (BMPR-2), genetic heterogenity and factors identified as potentiators in the pathogenesis of PAH such as prostacyclin, prostacyclin synthase, voltage-gated potassium channels, serotonin transport and uptake, nitric oxide (NO), nitric oxide synthase (NOs), thromboxane, serine elastase, metalloproteinases, tenascin-C, endothelin-1, plasminogen activator inhibitor type 1, fibrinolysis and human herpes virus 8 (HHV-8) and perhaps other as yet undiscovered factors.

importance of TGFβ-receptors as a molecular pathway at the base of vascular remodelling.

The 'Venice' classification of 2003 WHO consensus meeting

Although the old PAH classification has worn well for clinical practice and research, i.e. drug evaluation and basic science, the World Health Organisation (WHO) organises a consensus meeting to revise this classification every five years. This so-called 'Venice Task Force on Pulmonary Arterial Hypertension' proposed an adapted clinical classification in June 2003. The discrepancy between the old classification and today's insight into the treatment of pulmonary hypertension and the progression of the insight into pathogenesis is the basis for the proposed alterations.

The most distinct change in nomenclature is to abandon the term primary pulmonary hypertension (PPH). Although the titles and the content of some of the categories have changed, the subdivision of pulmonary hypertension into five categories remains unaltered.

Pulmonary arterial hypertension (PAH) is defined according to the 1998 WHO classification as a group

Table 1. Proposal for an adapted clinical classification, The 'Venice' classification (see text *The 'Venice' classification of 2003 WHO consensus meeting*).

Pulmonary arterial hypertension	**Box 1**
Idiopathic/sporadic	
Familial	
Related to:	
- Collagen vascular disease (CVD), sclerodermia, CREST syndrome	
- Congenital heart disease (CHD)	
- Portal hypertension (PoH),	
- HIV infection	
- Drugs and toxins	
Other PAH with significant venous and/or capillary involvement:	
- Pulmonary veno-occlusive disease	
- Pulmonary capillary haemangiomatosis	
Persistent pulmonary hypertension of the newborn (PHN)	
PH with left heart disease	**Box 2**
Atrial or ventricular heart disease	
Valvular heart disease	
PH with lung disease and/or hypoxaemia	**Box 3**
Chronic obstructive pulmonary disease	
Interstitial lung disease	
Sleep disorders:	
- Alveolar hypoventilation	
- Chronic exposure to high altitude	
Developmental abnormalities	
PH due to chronic thrombotic and/or embolic disease	**Box 4**
Thromboembolic obstruction of proximal pulmonary arteries	
Thromboembolic obstruction of distal pulmonary arteries	
Pulmonary embolism (tumour, parasites, foreign material)	
Miscellaneous	**Box 5**
Sarcoidosis	
Histiocytosis X	
Lymphangiomatosis	
Compression of pulmonary vessels (adenopathies and tumour, fibrosing mediastinitis)	

On the basis of novel insights into pathobiology and pathogensis, *PAH with significant venous and/or capillary involvement* has been added to box 1. The term 'primary pulmonary hypertension' (PPH) has been abandoned and idiopathic and familial PAH are distinct diagnostic classes. *Compression of pulmonary vessels (adenopathies and tumour, fibrosing mediastinitis)* has been added to box 5. Boxes 2 to 4 remain unaltered. Adapted from the 3rd World Symposium on Pulmonary Arterial Hypertension, Venice, 23 to 25 June 2003.

characterised by identical obstructive pathological changes of the pulmonary vascular system and a response to long-term infusion of prostacyclin.[13,27,28] In this group, the primary pulmonary hypertension (PPH) class is replaced by the idiopathic and familiar classes. Each is a class in itself (see table 1, box 1).

PAH with significant venous and/or capillary involvement, veno-occlusive disease and pulmonary capillary haemangiomatosis have been added to the PAH category, on account of clinical interpretation and aetiological insights. Both are regularly seen with PAH and have the same clinical presentation, although in both the disease is much more progressive and rapidly fatal.

The second category (box 2) is reserved for pulmonary hypertension with left-sided heart disease, which requires a specific treatment of its own.

Except for abandoning the neonatal lung disease and alveolar-capillary dysplasia classes, category 3 (pulmonary hypertension with lung disease and/or hypoxaemia) and category 4 (pulmonary hypertension due to chronic thrombotic and/or embolic disease) remain intact. The fifth category is named miscellaneous, and compression of pulmonary vessels from category 2 has been added to this category.[29]

Pulmonary arterial hypertension diagnostic work-up

Similarity between the clinical presentation of PAH and other diseases is a cause of delayed diagnosis, but it remains the starting point of the path leading to the diagnosis.[30] Detection of PAH might also be due to incidental findings by means of diagnostic tools, i.e. chest X-ray, ECG and TT echo, assessed for other clinical purposes. As mentioned clinical presentation, history, physical examination, and a suspicion of PAH may lead to the first step in the diagnostic work-up, as follows from figure 2. The diagnostic work-up is emanated from the classification system and to diagnose PAH other causes of pulmonary hypertension have to be excluded and distinguished from PAH.

Pulmonary function testing and high-resolution computer tomoghraphy (HRCT) are helpful in excluding parenchymal lung disorders. In the majority of PAH cases the function test is normal but slightly reduced in lung volumes and mildly reduced diffusing capacity for carbon monoxide.[31]

A more sensitive tool to detect pulmonary hypertension is a submaximal exercise test on a cycle ergometer. Sun et al. showed that patients with mild PH already have an abnormal ventilatory equivalent for CO_2 during exercise.[32]

The six-minute walk test is a standard method to assess exercise performance, response to therapy and predicting prognosis.[33] A sleep study is done to exclude obstructive sleep apnoea syndrome (OSAS).

Typical findings on chest X-ray are enlarged pulmonary arteries, right atrial and ventricular dilatation and clear lung fields.[34] If parenchymal lung disease

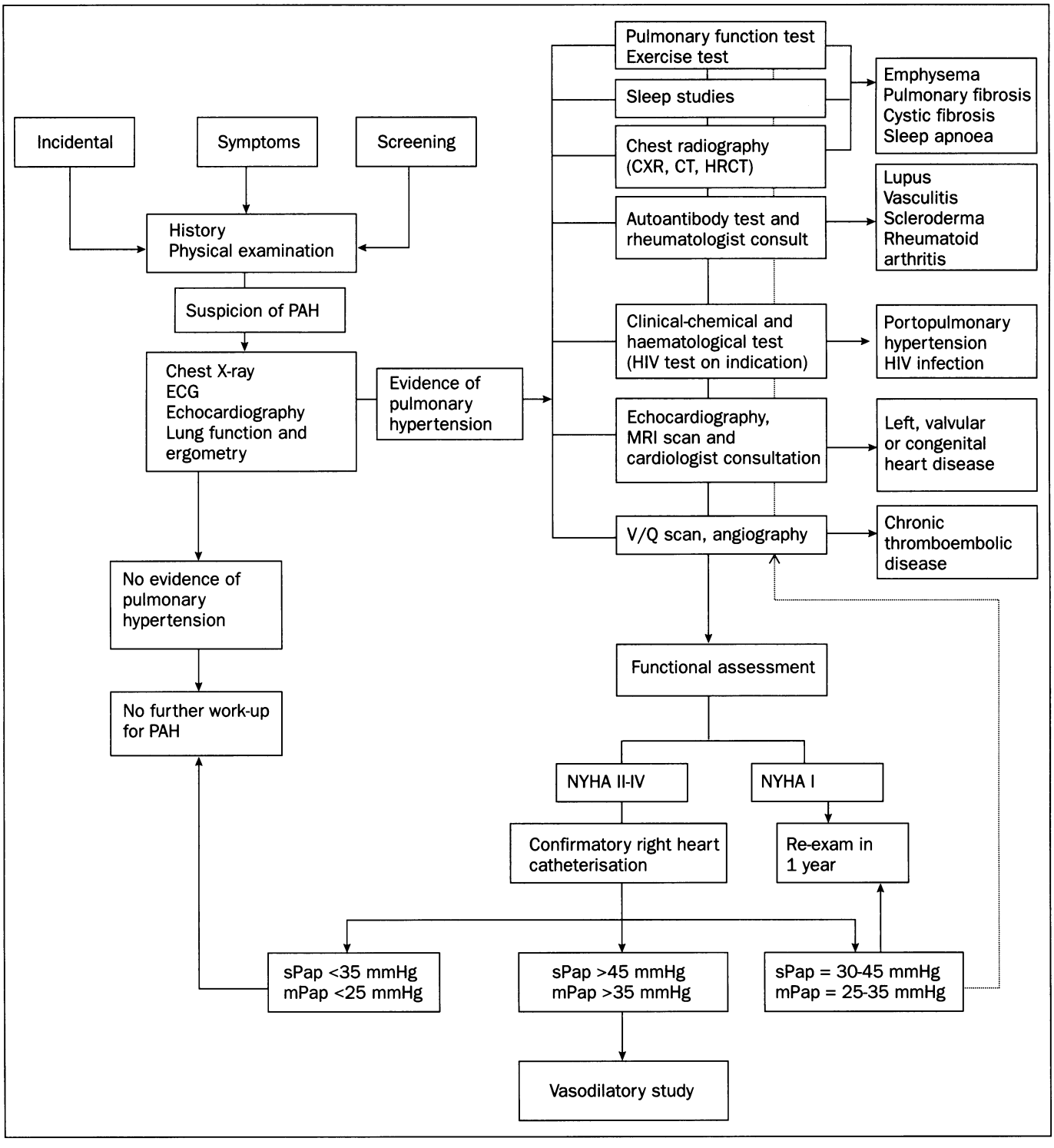

Figure 2. Diagnostic flow chart of PAH in the VU Medical Centre (see text Pulmonary Arterial Hypertension Diagnostic work-up). Clinical symptoms and a suspicion of PAH will start the diagnostic work-up. Chest X-ray, ECG, echocardiography, lung function and ergometry decide the next step. Evidence of pulmonary hypertension leads to intensive diagnostic work-up. Following the right side of the flow chart, causes of PH and studies which can differentiate are shown. Eventually functional assessment will demand confirmatory right heart catheterisation for NYHA class II-IV. Functional class NYHA I will be re-examined in one year. Systolic pulmonary artery pressure sPap >45 mmHg and/or a mean pulmonary artery pressure mPap >35 mmHg measured by catheterisation is conclusive for PAH and requires vasodilatory study to assess vasoreactivity (see text). When sPap = 30-40 mmHg and/or mPap = 25-35 mmHg, re-examination in one year or with clinical deterioration, is required (dashed line). No further work-up is done when sPap <35 mmHg and/or mPap <25 mmHg.

Table 2. Standard right heart catheterisation measurements (see text *Pulmonary Arterial Hypertension Diagnostic work-up).* The following parameters should be standard measurements during right heart catheterisation.

Right atrial pressure
Right ventricle pressure
Pulmonary artery pressure
Pulmonary capillary wedge pressure
Pulmonary arterial vasoreactivity

or mediastinal fibrosis is suspected to be the underlying cause of PH, HRCT might help to discriminate.[8]

The next step in the diagnostic work-up is echocardiography. Flow peak jet velocity in tricuspidalis regurgitation can determine the systolic and diastolic pulmonary artery pressure and the RV systolic pressure. Right atrial pressure (RAP), right ventricular function and hypertrophy, interventricular septum and left ventricular function can be estimated.[35] It can also exclude congenital heart disease or left-sided heart disease, such as mitral valve disease or left ventricular dysfunction.[8] Echocardiography with Doppler is commonly used in the diagnosis and follow-up of patients with pulmonary hypertension to monitor progression of the disease and the response to therapy.[36,37] Although echocardiography will remain the most used noninvasive technique for the diagnosis and follow-up in pulmonary hypertension in the coming years, the considerable false positive and negative diagnostic value of this technique, even in experienced hands, means that the diagnosis of PH should not be based on echocardiographic findings only, nor should it be excluded on these grounds if there is other clinical evidence for this diagnosis.[38]

Clinical chemical testing is nonspecific but a liver function test may be helpful in excluding portopulmonary hypertension.[1] Haematological and immunological testing may help differentiate forms of PAH, i.e. PAH associated with HIV.[8] Consultation of a rheumatologist must be considered when interpreting laboratory results. The role of brain natriuretic peptide in the follow-up of PAH is currently under investigation.

Imaging techniques, ventilation-perfusion scintigraphy and pulmonary angiography discriminate chronic thromboembolic PH from PAH.[34,39]

In the VU Medical Centre we developed a magnetic resonance imaging (MRI) protocol to accurately evaluate the morphology and function of the right atrium, ventricle, interventricular septum and pulmonary artery in a short period of time.[40,41] This protocol not only provides diagnostic information but can also be used for the noninvasive monitoring of the patients under treatment.[41,42]

Of all diagnostic tools, right heart catheterisation with selective pulmonary vasodilator testing is the golden standard to characterise the pulmonary vascular bed and to confirm or exclude the definitive diagnosis of pulmonary hypertension.[43] Standard right heart catheterisation measurements are summarised in table 2.

According to the National Institutes of Health Registry of patients with pulmonary hypertension, the criteria are mean pulmonary artery pressure of ≥25 mmHg,[44] a pulmonary capillary wedge (PCW) pressure of ≤15 mmHg and pulmonary vascular resistance of ≥3 Woods units (240 dynes)[1,43], shown in table 3.

Of note is that even in patients with veno-occlusive disease, pulmonary wedge pressure is normal, and thus an elevated pulmonary arterial pressure excludes diagnosis of box 1, table 1.[45] A normal wedge pressure is therefore critical for the choice of therapy. If there is any doubt about the accuracy of the wedge measurement, which is often the case, left ventricle pressures should be measured.

Acute vasoreactivity testing is mandatory in all PAH forms summarised in box 1. The rationale to perform vasoreactivity testing is to identify the small group of PAH patients who have reversible pulmonary hypertension on calcium channel blockers (CCB) and might show long-term survival if treated with this medication.[46] Initial baseline haemodynamic evaluation is performed while patients are breathing room air. Subsequently patients are tested with O_2 and inhalation of a mixture of NO-air both administered through a facemask each over five minutes and with a minimum pause of five minutes in between the two tests.[47] The vasoreactivity test is continued with a prostacyclin, epoprostenol, administered invasively and over five minutes with every increasing dose. The test is ceased when patients experience adverse effects or show a vasoreactive response. CCB are not indicated for this initial vasoreactivity testing, because life-threatening adverse effects can occur in patients with no pulmonary vasoreactivity.[48]

Several definitions of vasoreactive response are in use. In our hospital Galié's definition is used.[49] Patients with no response must be treated with specific PAH medication as prostacyclin or an endothelium antagonist. Patients responding to acute pulmonary vasodilator testing by a decrease in PVR of ≥20%

Table 3. Criteria of the National Institute of Health Registry for Pulmonary Hypertension.

mPap	≥25 mmHg
PCWP	≤15 mmHg
PVR	≥ 3 Woods units (240 dynes)

mPap=mean pulmonary artery pressure, PCWP=pulmonary capillary wedge pressure, PVR=pulmonary vascular resistance.

associated with reduction of mPap ≥20% and no change or increase in cardiac index can be safely challenged by means of nifedipine 10 mg orally. If the same vasoreactive response occurs after this medication, calcium channel blockers are indicated.

The performance of vasoreactivity testing is of diagnostic and prognostic value. It has been shown that pulmonary vasoreactivity correlates well with survival in patients with PAH of the familial and idiopathic kind.[50]

Conclusion

Novel insights into the molecular deformities revealed by the genetic causes of PAH have changed the classification of PAH. Current research focussed on the role of the BMP pathway in the pathogenesis of PAH offers new effective therapeutic approaches. Although these insights will rapidly change our approach to PAH, right cardiac catheterisation remains crucial to diagnose and to offer the possibility to exclude or differentiate PAH from cardiac causes of pulmonary hypertension. ■

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