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REVIEW

Frontiers in cardiovascular medicine

Early detection of pulmonary vascular disease in pulmonary arterial hypertension: time to move forward

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Pulmonary arterial hypertension (PAH) can be a rapidly progressive disorder and is associated with high rate of mortality, despite medical intervention. With the availability of effective therapy, early disease detection is an important strategic objective to improve treatment outcomes. Resting echocardiography is currently the recommended screening modality for high-risk population groups. However, it is clear that derangements in resting haemodynamics (and symptoms) are late sequelae of the pathobiological processes that begin in the distal pulmonary arteries. Exercise stress may unmask early pulmonary vascular dysfunction but the definition, clinical significance, and natural history of 'exercise PAH' remain undefined. We will review the currently available and potential future strategies aimed at early disease detection, and propose that ultimately the way forward is to detect disease at a stage prior to the rise in resting pulmonary artery pressure.

Keywords Pulmonary hypertension • Early detection • Screening

Introduction

Pulmonary hypertension (PH) is a pathophysiological state defined by an increase in mean pulmonary arterial pressure (PAP) $\geq$25 mmHg at rest as assessed by right heart catheterization.[1–3] According to the values of pulmonary capillary wedge pressure (PCWP), PH is defined as pre-capillary (PCWP $\leq$ 15 mmHg) or post-capillary (PCWP $>$ 15 mmHg).[3] Pulmonary hypertension can be found in multiple clinical conditions (at least 37 separate disease entities according to the most recent clinical classification)[4] and is classified into six groups according to similar pathophysiological and therapeutic characteristics (*Table 1*). Pulmonary hypertension owing to left heart disease (post-capillary PH) is distinct from other PH groups which represent pre-capillary PH. Despite comparable elevations in PAP and pulmonary vascular resistance (PVR) in the different pre-capillary PH groups, the underlying disease mechanisms, diagnostic approaches, prognosis, and therapeutic implications are completely different.

Pulmonary arterial hypertension (PAH) (Group 1) is a distinct clinical group of uncommon conditions characterized by the presence of pre-capillary PH in the absence of other causes of PH such as lung diseases (Group 3), chronic thrombo-embolic PH (Group 4), or other rare diseases (Group 5). Although PAH (Group 1) consists of different forms [such as idiopathic, heritable, drug and toxin induced, associated with connective tissue diseases, HIV infection, portal hypertension, and congenital heart disease (CHD)],[3] they all share a similar clinical picture and virtually identical pathological changes in the distal pulmonary arteries ($<$500 μm), which we will refer to as pulmonary vascular disease (PVD). The pathological changes of established PVD are characterized by medial hypertrophy, intimal proliferative changes (concentric and/or eccentric), adventitial thickening with moderate peri-vascular inflammatory infiltrates, complex lesions (plexiform, dilated), and thrombotic lesions.[5]

The exact processes that initiate PVD are largely unknown. It is hypothesized that the interaction between genetic predisposition

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Table 1 Definition and clinical groups of pulmonary hypertension

	Haemodynamic definition[a]	Clinical group
Pre-capillary PH	Mean PAP ≥25 mmHg PCWP ≤ 15 mmHg	(Group 1) PAH (Group 3) PH due to lung disease of hypoxia (Group 4) Chronic thromboembolic PH (Group 5) PH with unclear and/or multifactorial mechanisms
Post-capillary PH	Mean PAP ≥25 mmHg PCWP > 15 mmHg	(Group 2) PH due to left heart disease
Passive	TPG ≤ 12 mmHg	
Reactive (out of proportion)	TPG > 12 mmHg	

PH, pulmonary hypertension; PAP, pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure.; TPG, transpulmonary gradient (TPG = mean PAP - PCWP).
[a]All haemodynamic values are at rest.

and environmental risk factors may be involved in the initial stages of the disease.[6] It appears that a specific injury on the vessel wall of the distal pulmonary arteries may trigger, in the predisposed individual, a pathobiological cascade of events which leads to a common final pathological obstructive condition.

The initial phases of the disease is clinically silent and detectable pathophysiological changes in the pulmonary circulation and the heart usually appear when the pathological lesions are fully developed.[7] The length of this pre-clinical phase of the disease is currently unknown.[6,8] Furthermore, if we consider that late symptom reporting and delayed diagnosis are common in PAH, it is clear how distant the early stages of the disease may be from the initiation of effective therapies. On the other hand, the data available from PAH trials support the hypothesis that earlier intervention may improve the efficacy of current therapies.[9,10] The EARLY study,[10] which included mildly symptomatic patients in WHO functional Class II, demonstrated that therapy with the endothelin receptor antagonist bosentan delayed the time to clinical worsening.

Therefore, it is imperative to reduce the time between the beginning of the pathobiological processes leading to PAH and the initiation of effective medical therapy. The present paper will discuss the possible strategies devoted to the early detection of PVD in PAH patients.

The paradigm of early disease detection

Pulmonary artery pressure rise is a late event

The normal pulmonary circulation is unique in its ability to accommodate the entire cardiac output at low arterial pressure, even during condition of maximal exercise. This is accomplished by its high-capacitance and low-resistance circuit,[11] with large microcirculatory reserves which are 'unrecruited' at rest.[12] During exercise, the pulmonary microcirculation is progressively recruited, resulting in the maintenance of relatively low arterial pressure despite increasing flow. The recruitment of the microcirculation also serves to increase the capillary surface area available for gas exchange during exercise.

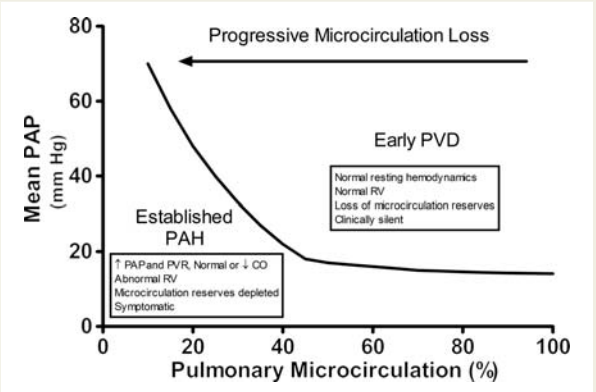

Figure 1 Schematic representation of the relationship between pulmonary microcirculation loss and PAP. The high capacitance of the pulmonary circulation implies that early microcirculation loss is not accompanied by a change in resting PAP. Many of the current screening modalities are dependent on detecting a rise in PAP, and thus will fail to detect the early stages of PVD. PAP, pulmonary artery pressure; PVD, pulmonary vascular disease; PAH, pulmonary arterial hypertension; PVR, pulmonary vascular resistance; CO, cardiac output; RV, right ventricle.

The high capacitance of the pulmonary circulation means that early PVD can be well compensated for. In fact, >50% of the pulmonary circulation must be obstructed before a rise in resting PAP is detected.[13] Single-lung transplantation may be successful in the treatment of PAH patients, and patients who have undergone pneumonectomy do not usually develop severe PH. Thus, a rise in resting PAP is a *late* marker of the obliterative and remodelling processes occurring in the distal pulmonary arteries (*Figure 1*).

This situation presents major challenges for the early detection of PVD: first, it is clinically silent until vascular damage is advanced;

and second, our current screening strategies are devoted to detecting disease at a stage when resting PAP is already elevated. Therefore, using the current haemodynamic definition of PH (resting PAP $\geq$25 mmHg),[14] PVD is necessarily identified at a relatively late stage, potentially reducing the efficacy of any current or future anti-remodelling therapeutic strategies.

Exercise pulmonary arterial hypertension: a precursor to resting pulmonary arterial hypertension?

Many diagnostic tests utilize 'stress' to elicit symptoms or abnormal physiological responses to facilitate disease detection. If alterations in resting haemodynamics are inadequate to identify early PVD, can we 'stress the system' to enable earlier disease detection? The earliest symptom in PAH is usually exertional dyspnoea, and it is thus rational that examination of the status of the pulmonary circulation during exercise stress may provide insights into early PVD. However, the exercise haemodynamics of the pulmonary circulation is a complex issue with many unresolved questions to be addressed.

Resting and exercise haemodynamics in normal individuals

Recent re-evaluation of available data has shown that the mean PAP in normal individuals at rest is 14 $\pm$ 3 mmHg with an upper limit of normal of $\sim$20 mmHg.[15,16] As the current definition of PAH is characterized by a mean PAP $\geq$25 mmHg, the significance of mean PAP values between 21 and 24 mmHg is unclear. Patients who present with PAP in this range need further evaluation in epidemiological studies to detect a possible progression to higher values (or regression to lower values) and to clinically detectable disease.

The arbitrary definition of PH on exercise (mean PAP $>$30 mmHg), as assessed by right heart catheterization, is not supported by published data and healthy individuals can reach much higher values.[15,17] While mean PAP at rest is virtually independent of age, the normal ranges of mean PAP during exercise is dependent on age, cardiac output, exercise method, and body weight. In a recent review by Kovacs *et al.*,[15] mean PAP during mild exercise was 19.4 $\pm$ 4.8 mmHg in subjects $<$50 years of age compared with 29.4 $\pm$ 8.4 mmHg in subjects $>$50 years. Mean PAP values during lower exercise levels almost always remained $<$30 mmHg in normal subjects $<$50 years, but 21% of them (and 47% of those aged 50 years or older) surpassed the threshold of 30 mmHg during maximal exercise.

The analysis of the plot between mean PAP and the cardiac index in normal individuals during exercise shows a relatively mild slope as large increases in cardiac output can be accommodated without proportional elevations of PAP. Interestingly, part of the increase of mean PAP is related to the increase of PCWP which may reach values $>$20 mmHg during supine exercise in normal subjects.[15] As a consequence, PVR, calculated as transpulmonary gradient (mean PAP$-$PCWP) divided by cardiac output, is apparently reduced on exercise in normal individuals.[15,17]

It is important to realize that our understanding of the normal haemodynamic response of the pulmonary circulation to exercise, despite the available data, remains limited. The majority of invasively measured exercise haemodynamics are derived from old studies with varying patient populations, methodologies, and exercise protocols. There are significant inconsistencies in the literature with regard to the normal ranges of PAP, PCWP, and hence PVR during exercise. Thus, at this stage, no age-specific and -sensitive right heart catheter study-based definition for exercise PH can be provided.

Exercise haemodynamics in pulmonary arterial hypertension patients

Exercise haemodynamics has been used in patients with idiopathic PAH to evaluate the efficacy of medical therapies. In the study of Castelain *et al.*,[18] the slope of the plot between the mean PAP and cardiac index during exercise at baseline is much steeper compared with normal individuals. Moreover, the extrapolated pressure intercept of the pressure–flow plot (zero-flow intercept) is positive, and consequently PVR is no longer a constant value independent of the absolute level of PAP and pulmonary blood flow.[18] In this case, PVR values calculated as the transpulmonary pressure gradient divided by cardiac output are misleading because the extrapolated pressure intercept of the linear portion of the pulmonary artery pressure–flow plot is positive and its value is not considered. Therefore, the functional state of the pulmonary circulation in patients with PAH is probably better described by the slope of multipoint mean PAP vs. cardiac index curves (true PVR) than by a single PVR determination at rest (*Figure 2*). The treatment with continuous intravenous infusion of epoprostenol for 6 weeks did not change substantially the values of mean PAP, cardiac index, and PVR (single-point measurement) at rest but a relevant increase in exercise capacity as assessed by the 6 min-walk test was detected.[18] This might be explained by a reduction in the slope of the relationship between mean PAP and cardiac index (true PVR) during the exercise haemodynamic test performed after 6 weeks of treatment. In other words, with epoprostenol therapy, higher values of cardiac index could be achieved on exercise with smaller increases in mean PAP, testifying a possible vasodilator effect of the drug detectable only with exercise stress.

This observation could be utilized in the identification of early stages of PVD in PAH, analysing not only the maximal value of mean PAP achieved during the exercise but also its relationship with the increase of cardiac index. In fact, it is possible that the maximal value of mean PAP generated by the right ventricle is not substantially different between normal individuals and patients with early PVD. However, a different behaviour of the PVR in the early stages of the disease (e.g. lack of the physiological reduction) might result in a lower rise of cardiac index for each given value of mean PAP on exercise, thus increasing the slope of the relationship. In other words, the definition of PH on exercise might require the identification of normal thresholds for the slope of the relationship between mean PAP and cardiac index rather than the absolute value of mean PAP *per se*.

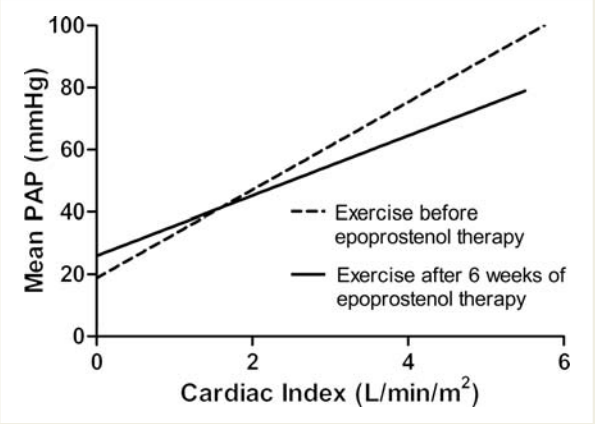

Figure 2 Exercise haemodynamics in PAH patients. The extrapolated zero flow pressure intercept is greater than PCWP and hence PVR is no longer a constant value independent of pulmonary blood flow. Furthermore, epoprostenol therapy after 6 weeks may not substantially change the values of mean PAP and cardiac index at rest, but the slope of the pressure–flow relationship during exercise (continuous line) is reduced as compared with before treatment (dotted line), explaining the improvement in exercise capacity following therapy. The functional state of the pulmonary circulation is better described by 'true PVR' or the slope of multipoint PAP vs. cardiac index relationship, rather than by a single PVR determination at rest. Redrawn from Castelain *et al.*[18] PAP, pulmonary artery pressure; PAH, pulmonary arterial hypertension; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance.

Despite the appeal of utilizing exercise in order to identify early PVD in subjects with normal resting PAP, this is constrained by the lack of uniform data on the values that define the bounds of normal physiology. Furthermore, outcome studies are needed to demonstrate that subjects with an 'abnormal' haemodynamic response during exercise will progress to resting PAH over time. Without this specific validation it is not possible, at present, to assume the atypical response to exercise as a sign of early disease.

Detecting early pulmonary vascular disease

The ideal screening test is one that is sensitive as well as specific, non-invasive, widely available, inexpensive, and able to detect disease at an early stage. For meaningful screening, it is also desirable that an effective intervention (ideally one that reduces mortality) is available when a disease is detected. Unfortunately, an ideal screening test for early PVD is lacking. In this section, we will examine: (i) the populations that a screening programme should target; (ii) the established modalities that are currently in clinical use to detect resting PH; and (iii) emerging techniques that may ultimately enable early disease detection, prior to a rise in resting PAP.

Who to screen?

Pulmonary arterial hypertension is an uncommon disease, with an estimated population prevalence of 15–50 cases per million.[19–21]

Table 2 Pulmonary arterial hypertension prevalence in the general population and at-risk polulations

Prevalence of PAH in the general population
15–50 cases per million (0.0015–0.0050%)
Prevalence of PAH in at risk populations
CHD: 4–15%
Systemic sclerosis: 8–10%
Portal hypertension: 0.5–10%
HIV: 0.5%
Sickle cell disease: 2%
BMPR2 mutation carriers: 20%

PAH, pulmonary arterial hypertension; CHD, congenital heart disease; HIV, human immunodeficiency virus; BMPR2, bone morphogenic protein receptor 2.

Any strategy aimed at early disease detection must target specific populations at high risk of PAH (*Table 2*). Although many factors including genetics, gender, drugs, toxins, and certain medical conditions are known to be risk factors for the development of PAH, only a few of these associated factors confer an absolute risk high enough to merit the adoption of a screening strategy. Germline mutations in bone morphogenic protein receptor type 2 (BMPR2) are found in around 60–70% of patients with familial PAH [22,23]. Interestingly, BMPR2 mutations are also detected in 11–40% of patients with apparently sporadic PAH without a family history,[24,25] highlighting the importance of genetic predisposition in the development of PAH. Bone morphogenic protein receptor type 2 mutation penetrance is incomplete and a carrier has an estimated lifetime risk of 20% for the development of PAH.[26] Patients with systemic sclerosis (SSc) represent a very high-risk group, with a 10–15% lifetime risk of developing clinical PAH.[27–30] Systemic sclerosis-associated PAH is also an aggressive disease, with a poorer survival compared with idiopathic PAH despite similar baseline haemodynamics.[31] Around 5–15% of adults with CHD develop PAH, usually as the result of large left-to-right shunts.[32] With advances in corrective surgery, an increasing number of children with repaired complex CHD (such as single-ventricle physiology) are surviving into adulthood with the late development of PVD. Up to 10% of patients with chronic liver disease presenting for liver transplant assessment (and 0.5% of those with less severe liver disease) have portopulmonary hypertension. Finally, 0.5% of patients with HIV infections[33] and up to 2% of subjects with sickle cell disease (as well as with other chronic haemolytic anaemias) may develop PAH.[34]

Currently, international guidelines support the screening for PAH in (i) family members of idiopathic/familial PAH, (ii) patients with connective tissue disease, and (iii) patients with portal hypertension being evaluated for liver transplantation.[35,36]

Established modalities for the screening of resting pulmonary arterial hypertension

The current approach to PAH screening is centred on the use of echocardiography in high-risk patients. Complementary use of

Table 3 Summary of key echocardiographic indices used in the estimate of pulmonary haemodynamics

Index	Formula	Comments
TRV	$sPAP = 4 \times TRV^2 + RAP$[a]	*PH unlikely if* TRV ≤ 2.8 m/s, sPAP ≤ 36 mmHg and no additional echocardiographic variable suggestive of PH *PH possible if* TRV ≤ 2.8 m/s, sPAP ≤ 36 mmHg with additional echocardiographic variable suggestive of PH TRV 2.9–3.4 m/s, sPAP 37–50 mmHg *PH likely if* TRV > 3.4 m/s, sPAP > 50 mmHg[14]
Pulmonary flow acceleration time		<93 ms suggests presence of PH[40]
Tei index	(IVRT + IVCT)/RVET	>0.36 suggests the presence of PH[41]
Right ventricular IVRT by tissue Doppler		>65 ms suggests sPAP > 40 mmHg[42]
Ratio of TRV to time velocity integral of right ventricular outflow tract	TRV/TVI_{RVOT}	>0.2 suggests PVR > 2 woods unit[38]
IVC size and collapsibility		Various algorithms are available to estimate RAP based on IVC size and collapsibility[37]

sPAP, systolic pulmonary artery pressure; TRV, tricuspid regurgitation velocity; RAP, right atrial pressure; IVRT, isovolumic relaxation time; IVCT, isovolumic contraction time; RVET, right ventricular ejection time, TVI_{RVOT}, time velocity integral of RV outflow tract; PVR, pulmonary vascular resistance; IVC, inferior vena cava.
[a]Assuming an RAP of 5 mmHg.

biomarkers and pulmonary function testing may also support the presence of PAH.

Resting Doppler echocardiography

Resting Doppler echocardiography (DE) is the most widely used screening method for the detection of PH. It provides a reasonably reliable and comprehensive assessment of the right heart and the pulmonary circulation, and can often indicate the underlying cause of PH.[37] Doppler echocardiography can also provide an estimation of left-sided filling pressure to support the suspicion of post-capillary PH which is common.

The main DE parameter of interest in the detection of PH is the tricuspid regurgitation velocity (TRV). Right ventricular systolic pressure (RVSP) is estimated using the modified Bernoulli equation ($RVSP = 4 \times TRV^2 +$ right atrial pressure). Right atrial pressure (RAP) can be assessed by the size and respiratory variation of the inferior vena cava.[37] Pulmonary vascular resistance may be estimated indirectly by taking into account pulmonary blood flow using the time velocity integral of the right ventricular outflow tract (TVI_{RVOT}). The related equation ($PVR = 10 \times TRV/TVI_{RVOT}$) has been shown to correlate with invasively measured PVR.[38,39] Other complementary indices, such as pulmonary acceleration time, and right ventricular isovolumetric relaxation time, can be used to support the presence of PH[40–42] (*Table 3*). These indices are particularly useful if a good-quality TRV jet is not obtainable. However, the majority of these complementary indices are based on small studies without prospective validation, and their sensitivities in detecting early PAP elevation are also questionable.

All these parameters should be integrated with echocardiographic variables that might raise or reinforce the suspicion of PH. These include increased dimensions and abnormal function of the right heart chambers, abnormal shape and function of interventricular septum, and dilated main PA. Right ventricular systolic function can be evaluated by several simple methods such as fractional area change, tricuspid annular plane systolic excursion, and right ventricular index of myocardial performance from pulsed Doppler examination of the tricuspid annulus. However, these changes tend to occur later in the course of the disease and their sensitivity for early PVD is thus poor.

The 'performance' of resting DE as a screening tool in populations at risk of PAH is entirely based on the chosen threshold of TRV. If it is low, the rate of false positive increases dramatically, while too high a threshold will increase the number of false-negative cases. The reliability of several TRV cut-off values, using right heart catheterization as reference, has been reported in large screening studies in high-risk populations. A trial evaluating the reliability of prospective screening of patients with SSc based on TRV > 2.5 m/s in symptomatic patients, or >3.0 m/s irrespective of symptoms, found that 45% of cases of DE diagnoses of PH were falsely positive.[28] In symptomatic patients with HIV infection, PH criterion based on TRV > 2.5 and 2.8 m/s was found to be a false positive in 72 and 29%, respectively.[33] At present, a TRV > 2.8 m/s is considered to be elevated, except in the elderly and

the obese where the normal range of systolic PAP is higher.[43] Recent European guidelines suggest that a TRV > 3.4 m/s is indicative of 'likely PH', and 'possible PH' when the TRV is between 2.8 and 3.4 m/s.[14]

An interpretable TRV jet may not be obtainable in about 10–20% of patients presenting for the assessment of PH,[44] although signal quality can be improved by the injection of agitated saline.[45] The precision of DE estimates of systolic PAP is also debatable. In studies that have compared DE values with heart catheter-determined values, the mean difference ranged from 3 to 38 mmHg, and systolic PAP was underestimated by >20 mmHg in 31% of all patients studied.[46] A recent study by Fisher *et al.*[47] demonstrated that up to 48% of cases have a discrepancy of >10 mmHg compared with invasive measurements. Underestimation can be particularly problematic when TRV jet quality is poor, potentially leading to delayed or incorrect diagnosis. As DE estimate of systolic PAP is a summation of the TRV-derived gradient and RAP, the imprecise estimation of RAP by echocardiography introduces further potential error into this calculation.[48]

Therefore, invasive haemodynamic confirmation of PAH is mandatory when treatment of this condition is considered. Pulmonary vascular resistance must be calculated as high cardiac output states may be associated with an elevated PAP without a true increase in PVR. During right heart catherization, confrontational testing with fluid challenge may be used to unmask diastolic dysfunction when baseline PCWP is ≤15 mmHg but this condition is clinically suspected.[49,50] However, there is no consensus about fluid challenge protocols and the age-related normal response has not been defined.

Pulmonary function testing

The classic pulmonary function abnormality seen in PAH is an isolated reduction in diffusing capacity for carbon monoxide (DLCO) in the presence of normal spirometry and lung volumes. Diffusing capacity for carbon monoxide is reduced in PAH as a result of a reduction in pulmonary capillary blood volume. Studies examining the utility of DLCO as a screening tool have been performed mainly in the SSc population. Longitudinal data from the large Pittsburgh Scleroderma Databank demonstrated that the development of PAH was strongly associated with a DLCO <55% predicted, and a forced vital capacity (FVC)-to-DLCO ratio >1.4 at the time of initial evaluation. Among those with an FVC/DLCO ratio of >1.4, ~20% developed PAH within 5 years.[51] A serial decline in DLCO was also a powerful predictor of future PAH[52] (*Figure 3*).

It should be stressed that the measurement of DLCO represents the composite of two components: conductance across the alveolar-capillary membrane (D_M) and pulmonary capillary blood volume (V_C). Therefore, a reduction in DLCO is not specific for PAH, and may represent the presence of diffuse parenchymal lung disease. However, a small study examining the utility of partitioning DLCO into its two components (D_M and V_C) failed to demonstrate improved discrimination for the presence of PH compared with a lone DLCO measurement.[53]

Biomarkers

Brain natriuretic peptide (BNP) is released from the ventricles in response to volume and pressure overload, and serve as a non-invasive marker of ventricular strain. In patients with established

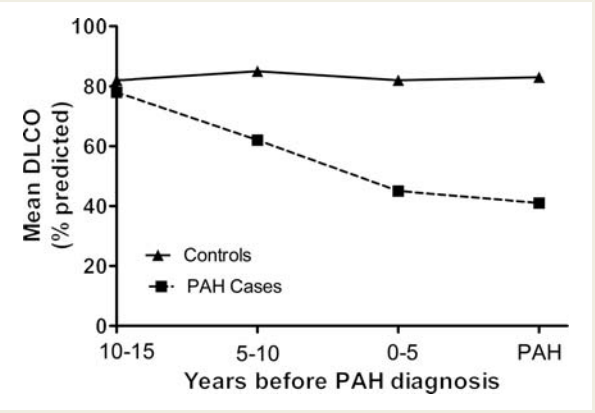

Figure 3 The relationship between DLCO and the development of SSc-associated PAH. Serial falls in DLCO are predictive of the development of future PAH, suggesting that DLCO monitoring could form part of a screening strategy for PAH in SSc. Redrawn from Steen *et al.*[51] DLCO, diffusion capacity for carbon monoxide; PAH, pulmonary arterial hypertension; SSc, systemic sclerosis.

PAH, both BNP and NT-proBNP (the inactive precursor N-terminal fragment) have been shown to correlate with exercise capacity and haemodynamics,[54] predict mortality,[55,56] and indicate right ventricular systolic impairment.[57] Recent attention has focused on these biomarkers as a potential screening tool for early PAH in high-risk populations. Using NT-proBNP and a cut-off value of 365 pg/mL, the sensitivity and specificity in detecting PAH in SSc patients ranges from 56–69 and 95–100%, respectively.[58,59] The results of these studies suggest that elevation in BNPs is a relative late event related to RV dysfunction, and lacks sensitivity to be used as a stand-alone test to detect early PAH.

Angiopoietins are circulating angiogenic factors which control the formation and maturation of blood vessels. The role of aberrant regulation of the angiopoeitin–Tie2 receptor system in the pathogenesis of PAH remains incompletely understood, with conflicting results in the literature.[60,61] In a recent study by Kumpers *et al.*,[62] angiopoietin-2 levels demonstrated a surprisingly good correlation with disease severity, pulmonary haemodynamics, and treatment response after 3 months of PAH-targeted therapy. These preliminary observations suggest that angiopoeitins may be promising new biomarkers in the assessment of PAH.

Emerging modalities which may detect pulmonary vascular disease prior to a rise in resting pulmonary arterial pressure

Many promising techniques are emerging which may ultimately enable disease detection at a stage prior to the rise in PAP. These techniques, however, require further validation before implementation into clinical practice can be recommended.

Stress Doppler echocardiography

Stress DE employs either exercise or hypoxia as the 'stressors' of the pulmonary circulation. Exercise DE is usually performed on a semi-recumbent cycle ergometer at left tilt position to optimize

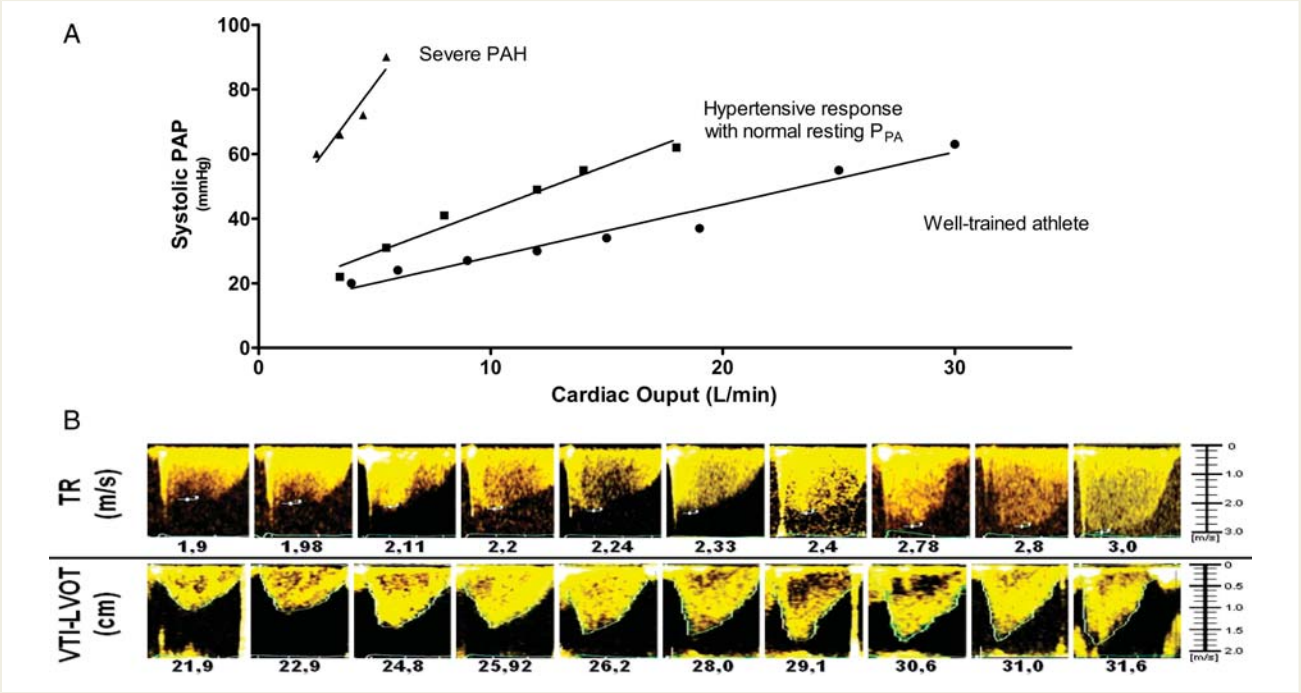

Figure 4 (*A*) Exercise echocardiography data represented as multipoint pressure–flow relationship of the pulmonary circulation. (*B*) Systolic PAP is estimated by TRV, and CO by VTI of the LVOT. It is important to take cardiac output into consideration when interpreting exercise echocardiographic data, as a well-trained athlete can reach 'hypertensive range' of systolic PAP due to large augmentation in CO. Reproduced from Argiento *et al.*[66] with permission from European Respiratory Society. PAP, pulmonary artery pressure; TRV, tricuspid regurgitation velocity; CO, cardiac output, VTI, velocity time integral; LVOT, left ventricular outflow tract.

echocardiographic windows, with PAP and cardiac output estimated at each stage of incremental workload. For hypoxic stress, an inspired oxygen concentration of 12–13% is delivered for 90–120 min. This time period is necessary to produce a maximal hypoxic pressor response.[63]

Although the normal values of exercise systolic PAP have been reported to remain at <45 mmHg,[64] well-conditioned athletes are capable of reaching systolic PAP >60 mmHg at peak exercise.[65] This is explained mostly by their ability to generate large augmentations in cardiac output (and thus pulmonary flow) during exercise. This highlights the importance of taking cardiac output into consideration when interpreting the systolic PAP. A similar systolic PAP may be reached during peak exercise by a well-conditioned athlete and a subject with early PVD; however, if cardiac output is taken into consideration, it is clear that the estimated PVR will be substantially different. Therefore, analysis of multipoint pressure–flow relationships during incremental exercise is the ideal method to assess the status of the pulmonary circulation,[66] and a compromised pulmonary circulation should display a steeper slope than normal in the pressure–flow line[67] (*Figure 4*).

In a recent large multi-centre stress DE study, a high proportion of relatives of patients with idiopathic and familial PAH displayed a 'pulmonary hypertensive' response to stress. During exercise, 32% of relatives but only 10% of controls had TRV values >3.08 m/s; and during hypoxia, 26% of relatives had TRV values >3.08 m/s compared with 10% of controls.[68] Furthermore, relatives with BMPR2 mutation had the highest likelihood of developing a hypertensive response to stress. Similarly, exercise DE studies in the SSc population have demonstrated a high-prevalence exercise hypertensive response in 40–60% of patients, using systolic PAP >40 mmHg as the cut-off.[69–72]

Exercise DE is potentially an elegant and physiological (although technically challenging) tool to uncover early PVD. However, the application of exercise DE is hampered by the fact that the exact natural history of exercise-induced 'pulmonary hypertensive response' remains to be elucidated. Furthermore, there remains a paucity of data on the normal ranges of TRV during exercise, and in particular, the influence of age, gender, and obesity on these values has not been clearly defined. There is also a lack of standardization for the performance of exercise DE in the assessment of the pulmonary circulation. These unresolved issues imply that exercise DE, based on the currently available evidence, cannot be recommended as a routine method for the early detection of PVD.[14,73]

Invasive cardiopulmonary exercise testing

Cardiopulmonary exercise testing (CPET) with invasive measurements of central haemodynamics remains the 'gold standard' in the assessment of the functional status of the pulmonary circulation during exercise. Invasive CPET can also uncover

exercise-related diastolic dysfunction not present at rest, which is an important and common cause of exertional dyspnoea and exercise-induced PH.[74,75] In the study by Tolle *et al.*,[75] patients with 'exercise PAH' (defined arbitrarily as mean PAP >30 mmHg and PCWP <20 mmHg during exercise) demonstrated a reduced cardiac index and higher PVR at peak exercise, suggesting that 'exercise PAH' may potentially represent an early and clinically relevant phase of the PAH spectrum. However, the invasive nature of this modality and the arbitrary nature of the definition of 'exercise PAH' limits its role as a screening tool in the detection of PVD in high-risk populations.

Novel invasive haemodynamic evaluation

Endothelial dysfunction is thought to be an early event in the pathogenesis of PVD, and may precede the development vascular remodelling and rise in PAP. Celermajer *et al.*[76] studied the response of acetylcholine infusion (an endothelium-dependent vasodilator) in the pulmonary arteries of children with congenital left-to-right shunts without established PAH. Compared with controls, children with left-to-right shunts demonstrated impairment of acetylcholine-mediated vasodilatation, indicating that the *in vivo* identification of endothelial dysfunction may be an early marker of PVD.

More recently, there has been extensive interest on the principle of 'flow reserve' in the assessment of the integrity of a microcirculation bed. The concept of 'coronary flow reserve' is a well-established physiological index in the evaluation of the coronary microcirculation. Ilsar *et al.*[77] applied this concept of flow reserve to the pulmonary microcirculation in an animal model of early pulmonary vascular embolic disease. The pulmonary microcirculation of baboons was obstructed with microspheres embolization to a point prior to the development of a rise in PAP. 'Pulmonary flow reserve' was assessed via the measurement of blood flow augmentation after local infusions of high-dose vasodilator agents in order to achieve maximal hyperaemia (and microcirculation recruitment). Progressive microspheres embolization, to an extent insufficient to cause an elevation of PAP, led to a reduction in pulmonary flow reserve, suggesting that the measurement of this index may have potential utility in the early detection of PVD.

Advanced imaging

Magnetic resonance imaging (MRI) has emerged as a power tool in the assessment of PAH with applications in diagnosis, determining disease severity, and monitoring of treatment response. The ability of MRI to comprehensively assess the heart and the pulmonary circulation as an integrated functional unit makes it a useful non-invasive tool in the evaluation of PVD. At present, MRI has been restricted to the examination of patients with established PAH and its utility as a screening modality remains undefined.

Owing to the complex three-dimensional geometry of the RV, MRI is the gold standard in the study of RV structure and function. In particular, RV mass can be measured accurately by multiplying the volume of RV muscle to the specific weight of the myocardium.[78] An increase in RV mass may be an early marker of PVD as adaptive muscle hypertrophy represents the initial compensated state resulting from an increase in ventricular afterload.[50] However, RV mass may also be influenced by factors such as the degree of physical activity in apparently healthy individuals. Thus, distinguishing a physiological adaptation to high physical activity against the early pathological compensation to disease may be difficult. Pulmonary artery stiffness assessed by MRI may have utility in the identification of early PVD. In the study by Sanz *et al.*,[79] indices of pulmonary artery stiffness (compliance and capacitance), derived from MRI and right heart catheter measurements, were reduced in subjects with exercise-induced PH compared with normal controls. Further studies are required to evaluate the normal ranges of MRI-derived indices of RV mass and PA stiffness and their predictive value in identifying PVD.

Quantitative calculation of regional lung perfusion can be performed with three-dimensional gadolinium-enhanced MR perfusion analysis, based on the principles of the indicator-dilution technique. Ohno *et al.*[80] demonstrated a marked difference in pulmonary perfusion in controls vs. patients with PAH (71 mL/100 mL/min vs. 130 mL/100 mL/min), but whether this technique is sensitive enough for the early detection of PVD remains to be seen. In addition, the high cost of MRI may limit its use as a screening method for asymptomatic or mildly symptomatic subjects.

Similarly, current CT technology can enable regional quantitative lung perfusion analysis. Electron beam CT is a specific technique which enables ultrafast rotational times in order to reduce motion artefacts. The principle is based on lung density changes following the passage of contrast in a region of interest (ROI), usually in the lung periphery to eliminate the influence of major blood vessels. Jones *et al.*[81] demonstrated a loss of gravity-dependent vertical gradient of lung perfusion, and markedly reduced overall perfusion in patients with PAH compared with controls. Electron beam CT is not widely available, but the advent of fast multi-detector CT should allow pulmonary perfusion analysis to be feasible using conventional CT technology.[82]

Improvements in the technology of MRI and CT imaging may allow visualization of the lung microcirculation in the future, and in particular the distal pulmonary arteries (*Figure 5*). This sort of 'virtual histology' may fill the gap between the sites of disease initiation (distal pulmonary arteries) and the late pathophysiological consequences observed on cardio-pulmonary haemodynamics.

The challenges ahead

Early detection of PVD in PAH is an important strategic objective in the battle against this terrible disease in which mortality remains high, despite recent therapeutic progress. Unfortunately, the length of time between the initiation of the disease and the early clinical and haemodynamic consequences detectable with current methods is largely unknown. The future paradigm of early disease detection in high-risk patients should ideally be aimed at detecting disease prior to a rise in resting PAP. Further studies in the haemodynamic response of the pulmonary circulation during exercise with non-invasive and invasive methods, including the response of normal individuals of different ages, and outcome studies in high-risk subjects, are required before adopting such methods in clinical practice. The assessment of aspects of the abnormal pathophysiology of the lung microcirculation (such as endothelial dysfunction) may help the identification of early disease, as demonstrated in preliminary investigational studies. Future progress in non-invasive imaging such as

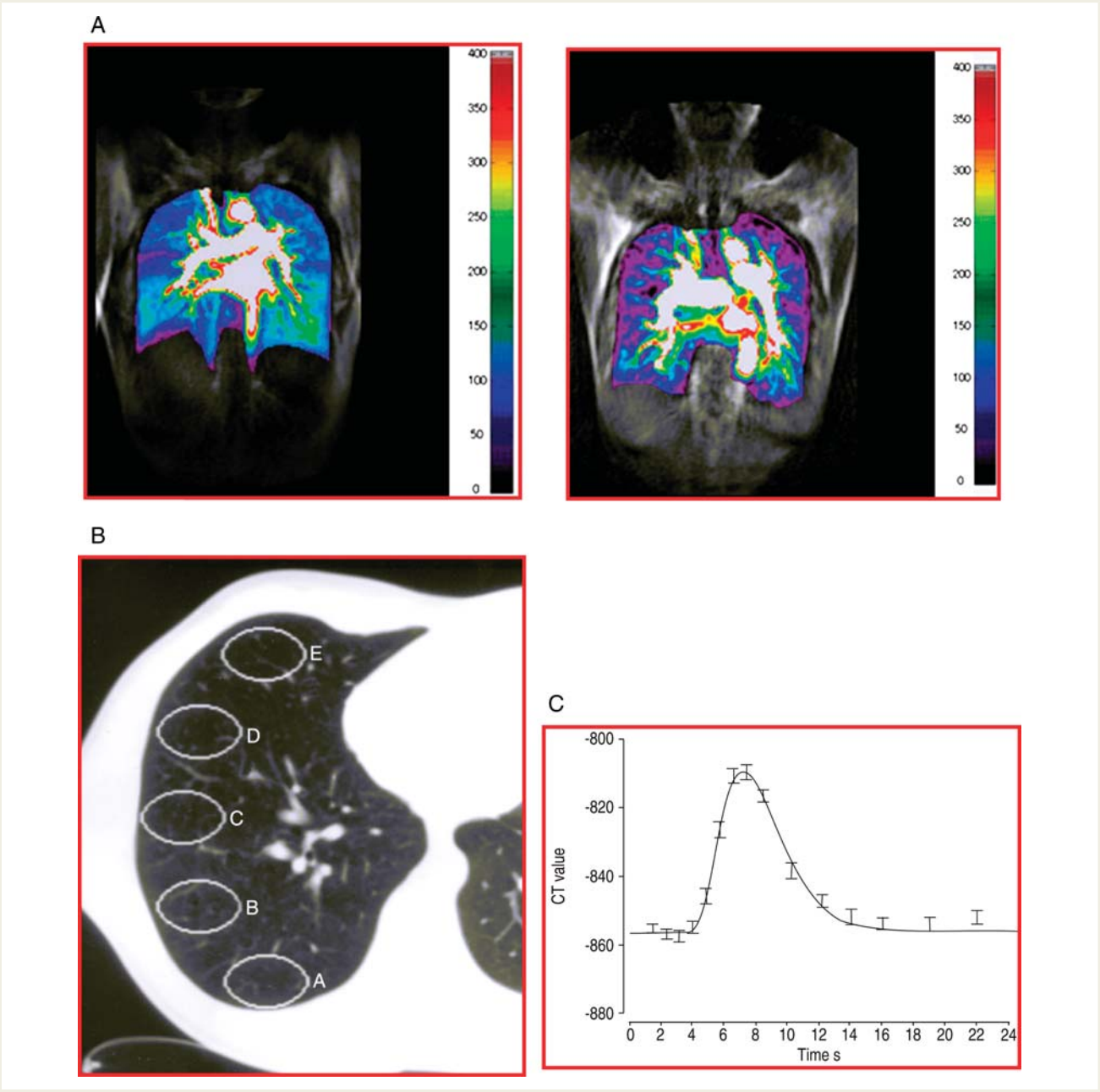

Figure 5 Quantitative lung perfusion analysis with advanced magnetic resonance imaging and computed tomography imaging techniques. (*A*) Pulmonary blood flow map using contrast-enhanced magnetic resonance imaging. Patient with pulmonary arterial hypertension (right image) demonstrating reduced pulmonary blood flow compared with normal control (left image). Reproduced from Ohno *et al.*[80] with permission from American Roentogen Ray Society. (*B*) Electron beam computed tomography showing region of interest in the lung section (white circles). Time density curves are constructed for each region of interest. Reproduced from Jones *et al.*[81] with permission from European Respiratory Society.

MRI and CT may give further insight into the pathophysiological aspects of the distal pulmonary circulation in the early stages of PVD. At present, resting echocardiography, despite its multiple limitations, remains the most widely available method for the screening and initial diagnosis of PAH.

Conflict of interest: D.S.C. has been on the Speaker's Bureau and Advisory Boards for Actelion, a company which makes medications for the treatment of Pulmonary Vascular Disease, and has also received research funding from Actelion for investigator-initiated work. N.G. has been involved with Steering Committee

activities for Eli Lilly and company, Actelion, Pfizer, Bayer-Schering, and GlaxoSmithKline; he has also been a paid lecturer, for Actelion, Eli Lilly and company, Pfizer, Bayer-Schering, and GlaxoSmithKline; he has done contract research for Actelion, Pfizer, United Therapeutics, Bayer-Schering, and GlaxoSmithKline. A.M. has been a paid lecturer, for Actelion and Eli Lilly and company.

References

1. Hatano S, T. S. World health organization 1975. *Primary Pulmonary Hypertension*. Geneva: WHO; 1975.
2. D'Alonzo GE, Barst RJ, Ayres SM, Bergofsky EH, Brundage BH, Detre KM, Fishman AP. Survival in patients with primary pulmonary hypertension. Results from a national prospective registry. Ann Intern Med 1991;**115**:343–349.
3. Galie N, Hoeper MM, Humbert M, Torbicki A, Vachiery JL, Barbera JA, Beghetti M, Corris P, Gaine S, Gibbs JS, Gomez-Sanchez MA, Jondeau G, Klepetko W, Opitz C, Peacock A, Rubin L, Zellweger M, Simonneau G, Vahanian A, Auricchio A, Bax J, Ceconi C, Dean V, Filippatos G, Funck-Brentano C, Hobbs R, Kearney P, McDonagh T, McGregor K, Popescu BA, Reiner Z, Sechtem U, Sirnes PA, Tendera M, Vardas P, Widimsky P, Al Attar N, Andreotti F, Aschermann M, Asteggiano R, Benza R, Berger R, Bonnet D, Delcroix M, Howard L, Kitsiou AN, Lang I, Maggioni A, Nielsen-Kudsk JE, Park M, Perrone-Filardi P, Price S, Domenech MT, Vonk-Noordegraaf A, Zamorano JL. Guidelines for the diagnosis and treatment of pulmonary hypertension: the task force for the diagnosis and treatment of pulmonary hypertension of the European society of cardiology (esc) and the European respiratory society (ers), endorsed by the international society of heart and lung transplantation (ishlt). Eur Heart J 2009;**30**:2493–2537.
4. Simonneau G, Robbins IM, Beghetti M, Channick RN, Delcroix M, Denton CP, Elliott CG, Gaine SP, Gladwin MT, Jing ZC, Krowka MJ, Langleben D, Nakanishi N, Souza R. Updated clinical classification of pulmonary hypertension. J Am Coll Cardiol 2009;**54**:S43–S54.
5. Pietra GG, Capron F, Stewart S, Leone O, Humbert M, Robbins IM, Reid LM, Tuder RM. Pathologic assessment of vasculopathies in pulmonary hypertension. J Am Coll Cardiol 2004;**43**:25S–32S.
6. Galie N, Palazzini M, Manes A. Pulmonary arterial hypertension: from the kingdom of the near-dead to multiple clinical trial meta-analyses. Eur Heart J 2010;**31**:2080–2086.
7. Galie N, Palazzini M, Manes A. Pulmonary hypertension and pulmonary arterial hypertension: a clarification is needed. *Eur Respir J* 2010;**36**:986–990.
8. Galie N, Manes A, Palazzini M, Negro L, Romanazzi S, Branzi A. Pharmacological impact on right ventricular remodelling in pulmonary arterial hypertension. *Eur Heart J* 2007;**9**(suppl H):H68–H74.
9. Sitbon O, Humbert M, Nunes H, Parent F, Garcia G, Herve P, Rainisio M, Simonneau G. Long-term intravenous epoprostenol infusion in primary pulmonary hypertension: prognostic factors and survival. J Am Coll Cardiol 2002;**40**:780–788.
10. Galie N, Rubin L, Hoeper M, Jansa P, Al-Hiti H, Meyer G, Chiossi E, Kusic-Pajic A, Simonneau G. Treatment of patients with mildly symptomatic pulmonary arterial hypertension with Bosentan (early study): a double-blind, randomised controlled trial. Lancet 2008;**371**:2093–2100.
11. Naeije R. Pulmonary vascular function. In: Peacock A, Rubin L, eds. *Pulmonary Circulation—Diseases and their Treatment*. London: Edward Arnold; 2004.
12. Reeves J, Dempsey JA, Grover RF. Pulmonary circulation during exercise. In: Wier EK, Reeves JT, eds. *Pulmonary Vascular Physiology and Pathophysiology*. New York: Mercel Dekker; 1989.
13. Peacock A. Prevention and early diagnosis of pulmonary hypertension. In: Demedts M, Delcroix M, Verhaege R, Verleden GM, eds. *Pulmonary Vascular Pathology: A Clinical Update*. Sheffield: European Respiratory Society Monograph; 2003.
14. Galie N, Hoeper MM, Humbert M, Torbicki A, Vachiery JL, Barbera JA, Beghetti M, Corris P, Gaine S, Gibbs JS, Gomez-Sanchez MA, Jondeau G, Klepetko W, Opitz C, Peacock A, Rubin L, Zellweger M, Simonneau G. Guidelines for the diagnosis and treatment of pulmonary hypertension. Eur Respir J 2009;**34**:1219–1263.
15. Kovacs G, Berghold A, Scheidl S, Olschewski H. Pulmonary arterial pressure during rest and exercise in healthy subjects: a systematic review. Eur Respir J 2009;**34**:888–894.
16. Badesch DB, Champion HC, Sanchez MA, Hoeper MM, Loyd JE, Manes A, McGoon M, Naeije R, Olschewski H, Oudiz RJ, Torbicki A. Diagnosis and assessment of pulmonary arterial hypertension. J Am Coll Cardiol 2009;**54**:S55–S66.
17. Naeije R, Melot C, Niset G, Delcroix M, Wagner PD. Mechanisms of improved arterial oxygenation after peripheral chemoreceptor stimulation during hypoxic exercise. J Appl Physiol 1993;**74**:1666–1671.
18. Castelain V, Chemla D, Humbert M, Sitbon O, Simonneau G, Lecarpentier Y, Herve P. Pulmonary artery pressure–flow relations after prostacyclin in primary pulmonary hypertension. Am J Respir Crit Care Med 2002;**165**:338–340.
19. Peacock AJ, Murphy NF, McMurray JJ, Caballero L, Stewart S. An epidemiological study of pulmonary arterial hypertension. Eur Respir J 2007;**30**:104–109.
20. Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, Yaici A, Weitzenblum E, Cordier JF, Chabot F, Dromer C, Pison C, Reynaud-Gaubert M, Haloun A, Laurent M, Hachulla E, Simonneau G. Pulmonary arterial hypertension in France: results from a national registry. Am J Respir Crit Care Med 2006;**173**:1023–1030.
21. Peacock AJ. Treatment of pulmonary hypertension. BMJ 2003;**326**:835–836.
22. Cogan JD, Pauciulo MW, Batchman AP, Prince MA, Robbins IM, Hedges LK, Stanton KC, Wheeler LA, Phillips JA III, Loyd JE, Nichols WC. High frequency of bmpr2 exonic deletions/duplications in familial pulmonary arterial hypertension. Am J Respir Crit Care Med 2006;**174**:590–598.
23. Aldred MA, Vijayakrishnan J, James V, Soubrier F, Gomez-Sanchez MA, Martensson G, Galie N, Manes A, Corris P, Simonneau G, Humbert M, Morrell NW, Trembath RC. Bmpr2 gene rearrangements account for a significant proportion of mutations in familial and idiopathic pulmonary arterial hypertension. Hum Mutat 2006;**27**:212–213.
24. Machado RD, Aldred MA, James V, Harrison RE, Patel B, Schwalbe EC, Gruenig E, Janssen B, Koehler R, Seeger W, Eickelberg O, Olschewski H, Elliott CG, Glissmeyer E, Carlquist J, Kim M, Torbicki A, Fijalkowska A, Szewczyk G, Parma J, Abramowicz MJ, Galie N, Morisaki H, Kyotani S, Nakanishi N, Morisaki T, Humbert M, Simonneau G, Sitbon O, Soubrier F, Coulet F, Morrell NW, Trembath RC. Mutations of the tgf-beta type ii receptor bmpr2 in pulmonary arterial hypertension. Hum Mutat 2006;**27**:121–132.
25. Thomson JR, Machado RD, Pauciulo MW, Morgan NV, Humbert M, Elliott GC, Ward K, Yacoub M, Mikhail G, Rogers P, Newman J, Wheeler L, Higenbottam T, Gibbs JS, Egan J, Crozier A, Peacock A, Allcock R, Corris P, Loyd JE, Trembath RC, Nichols WC. Sporadic primary pulmonary hypertension is associated with germline mutations of the gene encoding bmpr-ii, a receptor member of the tgf-beta family. J Med Genet 2000;**37**:741–745.
26. Machado RD, Eickelberg O, Elliott CG, Geraci MW, Hanaoka M, Loyd JE, Newman JH, Phillips JA III, Soubrier F, Trembath RC, Chung WK. Genetics and genomics of pulmonary arterial hypertension. J Am Coll Cardiol 2009;**54**:S32–S42.
27. Pope JE, Lee P, Baron M, Dunne J, Smith D, Docherty PS, Bookman A, Abu-Hakima M. Prevalence of elevated pulmonary arterial pressures measured by echocardiography in a multicenter study of patients with systemic sclerosis. J Rheumatol 2005;**32**:1273–1278.
28. Hachulla E, Gressin V, Guillevin L, Carpentier P, Diot E, Sibilia J, Kahan A, Cabane J, Frances C, Launay D, Mouthon L, Allanore Y, Tiev KP, Clerson P, de Groote P, Humbert M. Early detection of pulmonary arterial hypertension in systemic sclerosis: a French nationwide prospective multicenter study. Arthritis Rheum 2005;**52**:3792–3800.
29. MacGregor AJ, Canavan R, Knight C, Denton CP, Davar J, Coghlan J, Black CM. Pulmonary hypertension in systemic sclerosis: risk factors for progression and consequences for survival. Rheumatology (Oxford) 2001;**40**:453–459.
30. Phung S, Strange G, Chung LP, Leong J, Dalton B, Roddy J, Deague J, Playford D, Musk M, Gabbay E. Prevalence of pulmonary arterial hypertension in an Australian scleroderma population: screening allows for earlier diagnosis. Intern Med J 2009;**39**:682–691.
31. Kawut SM, Taichman DB, Archer-Chicko CL, Palevsky HI, Kimmel SE. Hemodynamics and survival in patients with pulmonary arterial hypertension related to systemic sclerosis. Chest 2003;**123**:344–350.
32. Duffels MG, Engelfriet PM, Berger RM, van Loon RL, Hoendermis E, Vriend JW, van der Velde ET, Bresser P, Mulder BJ. Pulmonary arterial hypertension in congenital heart disease: an epidemiologic perspective from a Dutch registry. Int J Cardiol 2007;**120**:198–204.
33. Sitbon O, Lascoux-Combe C, Delfraissy JF, Yeni PG, Raffi F, De Zuttere D, Gressin V, Clerson P, Sereni D, Simonneau G. Prevalence of hiv-related pulmonary arterial hypertension in the current antiretroviral therapy era. Am J Respir Crit Care Med 2008;**177**:108–113.
34. Machado RF, Gladwin MT. Pulmonary hypertension in hemolytic disorders: pulmonary vascular disease: the global perspective. Chest 2010;**137**:30S–38S.
35. Rich S. *Primary Pulmonary Hypertension: Executive Summary from the World Symposium on Primary Pulmonary Hypertension*. Evian, France: World Health Organisation; 1998.
36. Murray KF, Carithers RL Jr. Aasld practice guidelines: evaluation of the patient for liver transplantation. Hepatology 2005;**41**:1407–1432.

37. Milan A, Magnino C, Veglio F. Echocardiographic indexes for the non-invasive evaluation of pulmonary hemodynamics. *J Am Soc Echocardiogr* 2010;**23**: 225–239; quiz 332–224.
38. Abbas AE, Fortuin FD, Schiller NB, Appleton CP, Moreno CA, Lester SJ. A simple method for noninvasive estimation of pulmonary vascular resistance. J Am Coll Cardiol 2003;**41**:1021–1027.
39. Vlahos AP, Feinstein JA, Schiller NB, Silverman NH. Extension of Doppler-derived echocardiographic measures of pulmonary vascular resistance to patients with moderate or severe pulmonary vascular disease. J Am Soc Echocardiogr 2008; **21**:711–714.
40. Lanzarini L, Fontana A, Campana C, Klersy C. Two simple echo-Doppler measurements can accurately identify pulmonary hypertension in the large majority of patients with chronic heart failure. J Heart Lung Transplant 2005; **24**:745–754.
41. Vonk MC, Sander MH, van den Hoogen FH, van Riel PL, Verheugt FW, van Dijk AP. Right ventricle tei-index: a tool to increase the accuracy of non-invasive detection of pulmonary arterial hypertension in connective tissue diseases. Eur J Echocardiogr 2007;**8**:317–321.
42. Brechot N, Gambotti L, Lafitte S, Roudaut R. Usefulness of right ventricular isovolumic relaxation time in predicting systolic pulmonary artery pressure. Eur J Echocardiogr 2008;**9**:547–554.
43. McQuillan BM, Picard MH, Leavitt M, Weyman AE. Clinical correlates and reference intervals for pulmonary artery systolic pressure among echocardiographically normal subjects. Circulation 2001;**104**:2797–2802.
44. Borgeson DD, Seward JB, Miller FA Jr, Oh JK, Tajik AJ. Frequency of Doppler measurable pulmonary artery pressures. J Am Soc Echocardiogr 1996;**9**:832–837.
45. Jeon DS, Luo H, Iwami T, Miyamoto T, Brasch AV, Mirocha J, Naqvi TZ, Siegel RJ. The usefulness of a 10% air–10% blood–80% saline mixture for contrast echocardiography: Doppler measurement of pulmonary artery systolic pressure. J Am Coll Cardiol 2002;**39**:124–129.
46. McGoon M, Gutterman D, Steen V, Barst R, McCrory DC, Fortin TA, Loyd JE. Screening, early detection, and diagnosis of pulmonary arterial hypertension: Accp evidence-based clinical practice guidelines. Chest 2004;**126**:14S–34S.
47. Fisher MR, Forfia PR, Chamera E, Housten-Harris T, Champion HC, Girgis RE, Corretti MC, Hassoun PM. Accuracy of Doppler echocardiography in the hemodynamic assessment of pulmonary hypertension. Am J Respir Crit Care Med 2009;**179**:615–621.
48. Rich JD, Shah SJ, Swamy R, Kamp A, Rich S. The inaccuracy of Doppler echocardiographic estimates of pulmonary artery pressures in patients with pulmonary hypertension: implications for clinical practice. *Chest* 2001;**139**:988–993.
49. Hemnes AR, Forfia PR, Champion HC. Assessment of pulmonary vasculature and right heart by invasive haemodynamics and echocardiography. *Int J Clin Pract Suppl* 2009;**162**:4–19.
50. Champion HC, Michelakis ED, Hassoun PM. Comprehensive invasive and noninvasive approach to the right ventricle-pulmonary circulation unit: state of the art and clinical and research implications. Circulation 2009;**120**:992–1007.
51. Steen VD, Graham G, Conte C, Owens G, Medsger TA Jr. Isolated diffusing capacity reduction in systemic sclerosis. Arthritis Rheum 1992;**35**:765–770.
52. Steen V, Medsger TA Jr. Predictors of isolated pulmonary hypertension in patients with systemic sclerosis and limited cutaneous involvement. Arthritis Rheum 2003; **48**:516–522.
53. Overbeek MJ, Groepenhoff H, Voskuyl AE, Smit EF, Peeters JW, Vonk-Noordegraaf A, Spreeuwenberg MD, Dijkmans BC, Boonstra A. Membrane diffusion- and capillary blood volume measurements are not useful as screening tools for pulmonary arterial hypertension in systemic sclerosis: a case control study. Respir Res 2008;**9**:68.
54. Leuchte HH, Holzapfel M, Baumgartner RA, Ding I, Neurohr C, Vogeser M, Kolbe T, Schwaiblmair M, Behr J. Clinical significance of brain natriuretic peptide in primary pulmonary hypertension. J Am Coll Cardiol 2004;**43**:764–770.
55. Fijalkowska A, Kurzyna M, Torbicki A, Szewczyk G, Florczyk M, Pruszczyk P, Szturmowicz M. Serum n-terminal brain natriuretic peptide as a prognostic parameter in patients with pulmonary hypertension. Chest 2006;**129**:1313–1321.
56. Nagaya N, Nishikimi T, Uematsu M, Satoh T, Kyotani S, Sakamaki F, Kakishita M, Fukushima K, Okano Y, Nakanishi N, Miyatake K, Kangawa K. Plasma brain natriuretic peptide as a prognostic indicator in patients with primary pulmonary hypertension. Circulation 2000;**102**:865–870.
57. Blyth KG, Groenning BA, Mark PB, Martin TN, Foster JE, Steedman T, Morton JJ, Dargie HJ, Peacock AJ. Nt-probnp can be used to detect right ventricular systolic dysfunction in pulmonary hypertension. Eur Respir J 2007;**29**:737–744.
58. Williams MH, Handler CE, Akram R, Smith CJ, Das C, Smee J, Nair D, Denton CP, Black CM, Coghlan JG. Role of n-terminal brain natriuretic peptide (n-tprobnp) in scleroderma-associated pulmonary arterial hypertension. Eur Heart J 2006;**27**: 1485–1494.
59. Mukerjee D, Yap LB, Holmes AM, Nair D, Ayrton P, Black CM, Coghlan JG. Significance of plasma n-terminal pro-brain natriuretic peptide in patients with systemic sclerosis-related pulmonary arterial hypertension. Respir Med 2003; **97**:1230–1236.
60. Morrell NW, Adnot S, Archer SL, Dupuis J, Jones PL, MacLean MR, McMurtry IF, Stenmark KR, Thistlethwaite PA, Weissmann N, Yuan JX, Weir EK. Cellular and molecular basis of pulmonary arterial hypertension. J Am Coll Cardiol 2009;**54**: S20–S31.
61. Rudge JS, Thurston G, Yancopoulos GD. Angiopoietin-1 and pulmonary hypertension: cause or cure? Circ Res 2003;**92**:947–949.
62. Kumpers P, Nickel N, Lukasz A, Golpon H, Westerkamp V, Olsson KM, Jonigk D, Maegel L, Bockmeyer CL, David S, Hoeper MM. Circulating angiopoietins in idiopathic pulmonary arterial hypertension. Eur Heart J 2010;**31**:2291–2300.
63. Grunig E, Mereles D, Hildebrandt W, Swenson ER, Kubler W, Kuecherer H, Bartsch P. Stress Doppler echocardiography for identification of susceptibility to high altitude pulmonary edema. J Am Coll Cardiol 2000;**35**:980–987.
64. Vachiery F, Pavalescu A. Exercise echocardiography in pulmonary hypertension. Eur Heart J Suppl 2007;**9**:H48–H53.
65. Bossone E, Rubenfire M, Bach DS, Ricciardi M, Armstrong WF. Range of tricuspid regurgitation velocity at rest and during exercise in normal adult men: implications for the diagnosis of pulmonary hypertension. J Am Coll Cardiol 1999;**33**: 1662–1666.
66. Argiento P, Chesler N, Mule M, D'Alto M, Bossone E, Unger P, Naeije R. Exercise stress echocardiography for the study of the pulmonary circulation. Eur Respir J 2009;**35**:1273–1278.
67. Huez S, Naeije R. Exercise stress tests for detection and evaluation of pulmonary hypertension. Eur Heart J Suppl 2007;**9**:H17–H21.
68. Grunig E, Weissmann S, Ehlken N, Fijalkowska A, Fischer C, Fourme T, Galie N, Ghofrani A, Harrison RE, Huez S, Humbert M, Janssen B, Kober J, Koehler R, Machado RD, Mereles D, Naeije R, Olschewski H, Provencher S, Reichenberger F, Retailleau K, Rocchi G, Simonneau G, Torbicki A, Trembath R, Seeger W. Stress Doppler echocardiography in relatives of patients with idiopathic and familial pulmonary arterial hypertension: results of a multicenter European analysis of pulmonary artery pressure response to exercise and hypoxia. Circulation 2009;**119**:1747–1757.
69. Reichenberger F, Voswinckel R, Schulz R, Mensch O, Ghofrani HA, Olschewski H, Seeger W. Noninvasive detection of early pulmonary vascular dysfunction in scleroderma. Respir Med 2009;**103**:1713–1718.
70. Collins N, Bastian B, Quiqueree L, Jones C, Morgan R, Reeves G. Abnormal pulmonary vascular responses in patients registered with a systemic autoimmunity database: pulmonary hypertension assessment and screening evaluation using stress echocardiography (phase-i). Eur J Echocardiogr 2006;**7**:439–446.
71. Alkotob ML, Soltani P, Sheatt MA, Katsetos MC, Rothfield N, Hager WD, Foley RJ, Silverman DI. Reduced exercise capacity and stress-induced pulmonary hypertension in patients with scleroderma. Chest 2006;**130**:176–181.
72. Steen V, Chou M, Shanmugam V, Mathias M, Kuru T, Morrissey R. Exercise-induced pulmonary arterial hypertension in patients with systemic sclerosis. Chest 2008;**134**:146–151.
73. McLaughlin VV, Archer SL, Badesch DB, Barst RJ, Farber HW, Lindner JR, Mathier MA, McGoon MD, Park MH, Rosenson RS, Rubin LJ, Tapson VF, Varga J, Harrington RA, Anderson JL, Bates ER, Bridges CR, Eisenberg MJ, Ferrari VA, Grines CL, Hlatky MA, Jacobs AK, Kaul S, Lichtenberg RC, Moliterno DJ, Mukherjee D, Pohost GM, Schofield RS, Shubrooks SJ, Stein JH, Tracy CM, Weitz HH, Wesley DJ. Accf/aha 2009 expert consensus document on pulmonary hypertension: a report of the American college of cardiology foundation task force on expert consensus documents and the American heart association: developed in collaboration with the American college of chest physicians, American Thoracic Society, Inc., and the pulmonary hypertension association. Circulation 2009;**119**:2250–2294.
74. Ha JW, Choi D, Park S, Shim CY, Kim JM, Moon SH, Lee HJ, Choi EY, Chung N. Determinants of exercise-induced pulmonary hypertension in patients with normal left ventricular ejection fraction. Heart 2009;**95**:490–494.
75. Tolle JJ, Waxman AB, Van Horn TL, Pappagianopoulos PP, Systrom DM. Exercise-induced pulmonary arterial hypertension. Circulation 2008;**118**: 2183–2189.
76. Celermajer DS, Cullen S, Deanfield JE. Impairment of endothelium-dependent pulmonary artery relaxation in children with congenital heart disease and abnormal pulmonary hemodynamics. Circulation 1993;**87**:440–446.
77. Ilsar R, Chawantanpipat C, Chan KH, Dobbins TA, Waugh R, Hennessy A, Celermajer DS, Ng MK. Measurement of pulmonary flow reserve and pulmonary index of microcirculatory resistance for detection of pulmonary microvascular obstruction. PLoS One 2010;**5**:e9601.
78. Kovacs G, Reiter G, Reiter U, Rienmuller R, Peacock A, Olschewski H. The emerging role of magnetic resonance imaging in the diagnosis and management of pulmonary hypertension. Respiration 2008;**76**:458–470.

79. Sanz J, Kariisa M, Dellegrottaglie S, Prat-Gonzalez S, Garcia MJ, Fuster V, Rajagopalan S. Evaluation of pulmonary artery stiffness in pulmonary hypertension with cardiac magnetic resonance. JACC Cardiovasc Imaging 2009;**2**:286–295.
80. Ohno Y, Hatabu H, Murase K, Higashino T, Nogami M, Yoshikawa T, Sugimura K. Primary pulmonary hypertension: 3d dynamic perfusion mri for quantitative analysis of regional pulmonary perfusion. AJR Am J Roentgenol 2007;**188**:48–56.
81. Jones AT, Hansell DM, Evans TW. Pulmonary perfusion quantified by electron-beam computed tomography: effects of hypoxia and inhaled no. Eur Respir J 2003;**21**:855–861.
82. Vonk-Noordegraaf A, van Wolferen SA, Marcus JT, Boonstra A, Postmus PE, Peeters JW, Peacock AJ. Noninvasive assessment and monitoring of the pulmonary circulation. Eur Respir J 2005;**25**:758–766.