Early detection of pulmonary arterial hypertension

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Abstract | Pulmonary arterial hypertension (PAH) remains an incurable disease associated with an unacceptably high early mortality, despite advances in therapeutic options. The disease is clinically silent until late in its natural history, when most of the distal pulmonary arteries have been obliterated. Early diagnosis of PAH is associated with improved long-term survival, and screening of at-risk populations is, therefore, a rational strategy to improve outcomes in this condition. Doppler echocardiography is the most widely used screening tool in current clinical practice. The role of evidence-based screening strategies has been clarified by research such as the DETECT study in patients with systemic sclerosis. A multimodal approach, using a range of noninvasive tests, improves the performance of screening algorithms. Right heart catheterization is mandatory to confirm a diagnosis of PAH. Uncertainties exist about the definition and prognostic relevance of pulmonary hypertension during exercise, but accumulating evidence suggests that stress testing of the pulmonary circulation can unmask clinically important early disease. Novel tools for the early detection of pulmonary vascular disease are urgently needed, given the substantial limitations of currently available techniques.

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Introduction

Pulmonary arterial hypertension (PAH) comprises a group of uncommon conditions characterized by obliterative vasculopathy of the small pulmonary arteries. Under the current classification system, PAH is termed idiopathic when no aetiological factors are identified, but can also be heritable, induced by drugs or toxins, or be related to conditions such as connective tissue diseases, congenital heart diseases, portal hypertension, or HIV infection (Box 1).[1] PAH forms a subset of precapillary pulmonary hypertension (PH) under the current clinical classification, which is defined haemodynamically by a resting mean pulmonary artery pressure (P_{pa}) ≥25 mmHg, pulmonary artery wedge pressure (P_{pw}) ≤15 mmHg, and pulmonary vascular resistance (PVR) >3 Wood units (equivalent to mmHg·min/l) at right heart catheterization (RHC).[2]

Early mortality from PAH remains unacceptably high, despite substantial therapeutic advances. In the current management era, 1-year mortality is 9–14% for idiopathic, heritable, or anorexigen-induced PAH,[3,4] and even higher for systemic-sclerosis-associated PAH (SSc-PAH; 10–30%).[5–7] Current guidelines state that early detection in populations at risk of PAH is an important objective to improve outcomes. In this Review, we summarize the current knowledge on screening for PAH in high-risk populations, and the areas that remain uncertain or unresolved in this field.

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Why is screening necessary?

Early detection of PAH is challenging

Early detection of PAH remains a clinical challenge, despite increasing awareness of this devastating condition in the medical community. Detection is particularly challenging in instances of sporadic disease, in which patients have no identifiable risk factor to alert clinicians to the possibility of underlying PAH, and systematic screening for early detection of disease is not possible. In a report from the UK and Ireland PAH registry covering the period 2001–2009, ~85% of patients with incident idiopathic PAH presented in NYHA functional class III or IV at diagnosis.[8] Similarly, investigators in the French PAH Network reported that 79% of new cases of SSc-PAH between 2006 and 2009 were diagnosed in NYHA class III–IV,[7] a rather surprising finding given the emphasis and recommendation from guidelines to screen for PAH in patients with systemic sclerosis. Therefore, if we rely on individuals seeking medical attention when symptoms related to PAH are established, most patients would be diagnosed at a very advanced stage of the illness.

Early symptoms related to PAH are often vague and nonspecific. Fatigue and exertional dyspnoea are the major symptoms and are often attributed to more common respiratory conditions, such as asthma or simply

Key points

- Pulmonary arterial hypertension (PAH) remains a devastating disease without a cure, despite therapeutic innovations
- Most patients are diagnosed at a very advanced stage of the disease
- Specific populations of patients, such as those with systemic sclerosis and carriers of PAH-causing mutations (such as in the *BMPR2* gene) are at high risk of developing PAH
- Screening of high-risk populations for PAH is recommended by current guidelines and is an important strategy to improve clinical outcomes
- Optimal screening algorithms for the early detection of PAH will continue to evolve with ongoing research

Box 1 | Clinical classification of PAH[1]

1.1. Idiopathic
1.2. Heritable (*BMPR2*, *ACVRL1*, *CAV1*, *ENG*, *KCNK3*, *SMAD9*)
1.3. Drug-induced or toxin-induced
1.4. Associated with connective-tissue disease, HIV infection, portal hypertension, congenital heart diseases, or schistosomiasis

Abbreviation: PAH, pulmonary arterial hypertension.

'lack of fitness'. Patients with systemic sclerosis or other connective-tissue diseases have multifactorial causes that contribute to effort intolerance including musculoskeletal conditions. All these factors contribute to the difficulty of detecting PAH early. Therefore, delays from time of first symptom onset to definitive diagnosis by RHC remain substantial. Investigators in an Australian study demonstrated an average time delay of 47 ± 35 months for idiopathic PAH, with patients attending 5.3 ± 3.8 general practitioner visits and 3.0 ± 2.1 specialist visits before a formal diagnosis was made.[9]

A further challenge of early detection of PAH is that discordance often exists between the severity of haemodynamic derangement and the degree of functional impairment, particularly in younger patients. In the EARLY study,[10] a randomized controlled trial involving exclusively patients with mildly symptomatic PAH in NYHA class II, the mean PVR at enrolment was ~10 Wood units, a level that is barely indicative of early disease. The presence of even mild symptoms might, therefore, already herald advanced obliterative disease of the pulmonary vascular bed in patients who have adequate adaptation to the chronic increase in right ventricular afterload.

Early diagnosis improves survival

Consistent data from multiple large, national PAH registries support the notion that diagnosis at an early stage of disease is associated with improved survival. Clinicians in the French Network on PH reported significantly prolonged survival in idiopathic, heritable, and anorexigen-induced PAH for patients in NYHA class I or II compared with those in NYHA class III or IV.[4] Patients with SSc-PAH in the UK registry had a more than twofold increase in mortality for patients in NYHA class III or IV compared with those in NYHA class I or II.[5] These findings are mirrored by data from the large North American REVEAL registry, into which 2,716 patients with any form of PAH were enrolled; NYHA functional class was one of the most powerful predictors of prognosis.[11]

A randomized controlled trial of screening for early intervention in at-risk populations would be difficult to perform and justify ethically. Screening is supported by a sound biological rationale; evidence from PAH registries indicates that early diagnosis confers a survival advantage, and early intervention even at a mildly symptomatic stage can potentially lead to improved clinical outcomes (Figure 1).[10]

A case–control study was conducted to compare the baseline characteristics and long-term survival of two cohorts of patients with incident SSc-PAH: one group diagnosed through a detection programme and the other via routine clinical care.[12] Both groups comprised patients from the same management era (between 2002 and 2003), so biases from therapeutic disparities were minimized. The detection cohort had significantly less severe pulmonary vascular disease at diagnosis measured by NYHA functional class (50.0% vs 12.5% in class I or II; $P = 0.036$) and pulmonary haemodynamics (PVR index: 734 ± 486 vs 1,299 ± 428 dyn·s·cm^{-5}·m^2; $P = 0.01$), compared with the routine clinical care cohort.[12] Importantly, significantly higher survival rates were observed in the patients with SSc-PAH who were diagnosed through a detection programme than in those who were diagnosed via routine care. In the detection cohort, the 1-year, 3-year, 5-year, and 8-year survival rates were 100%, 81%, 73%, and 64%, respectively.[12] By contrast, the survival rates were 75%, 31%, 25%, and 17%, respectively ($P = 0.0037$), in the routine-care cohort.[12] Although this study is inherently subject to the limitations of lead-time and length-time biases, it provides the most direct evidence that screening of patients with systemic sclerosis for PAH identifies milder forms of the disease, enabling earlier intervention and improved survival.

Who warrants screening?

In medicine, screening refers to identifying the presence of disease at a preclinical stage.[13] Medical screening has existed for >60 years and has become an important component and success story of modern medicine. Although the strict definition of screening refers to detection of disease in individuals who are completely asymptomatic, screening programmes for PAH are sometimes broadened to include those who might be mildly symptomatic. For screening to be worthwhile, early intervention should be demonstrated to alter the natural history of the disease and improve prognosis. Furthermore, the screening tools should be simple, widely available, noninvasive, and acceptable to patients. As with any diagnostic tests, instruments used for screening must also be tested for reproducibility, sensitivity, specificity, and accuracy against the gold-standard investigation. Box 2 outlines the WHO principles for early detection of disease.

PAH is a rare disease, with an estimated population prevalence of 15–50 cases per million, and an incidence of 2–7 cases per million.[14,15] Therefore, screening programmes must target well-defined populations that are at sufficiently high risk of developing PAH. Although

many aetiological factors are implicated in the development of PAH (such as genetics, exposure to drugs and toxins, infections, and autoimmune conditions), only a few of these risk factors accrue an absolute risk that is high enough to merit the adoption of a targeted screening strategy. A population group without a sufficiently high prevalence of disease will increase not only the economic cost, but also the number of false–positive results from screening. Given that no screening test has 100% specificity, the positive predictive value is dependent on the pretest probability (or disease prevalence) based upon Bayesian principles, with the positive predictive value decreasing with decreasing disease prevalence for any given population.

One group of individuals for whom screening is required is those with mutations that predispose to PAH. Mutations in the genes encoding proteins in the transforming growth factor β (TGF-β) superfamily signalling pathways are the most common causes of heritable PAH, of which heterozygous mutations in the bone morphogenetic protein receptor type-2 (*BMPR2*) gene account for ~75% of all heritable cases.[16] Disease penetrance in carriers of the *BMPR2* mutation is incomplete, and disease onset varies widely within the same family and between unrelated individuals who harbour the same defect, ranging from early childhood to late adulthood.[17] The Vanderbilt Pulmonary Hypertension Registry in the USA has provided data on the estimated penetrance of disease in *BMPR2*-mutation carriers.[18] From a total of 1,683 at-risk siblings from affected sibships, 232 individuals were affected, with a 3:1 female-to-male ratio. Given that mutation status was not known in all at-risk siblings, assuming a 50% rate of mutation carriage, overall penetrance was estimated at ~27% (42% in females and 14% in males).[18] The cumulative incidence of disease is ~90% by the age of 57 years, although an upper limit for the age of disease onset does not seem to exist. As with *BMPR2,* other rarer PAH-causing gene mutations do not seem to be fully penetrant, but precise estimation of penetrance for other gene defects is difficult given the small number of families involved.

Several connective-tissue diseases are commonly associated with PAH, but systemic sclerosis is the highest-risk group within this population, with an estimated lifetime risk of developing PAH of 10–15%, and a yearly incidence of 0.6%.[19–21] Survival with SSc-PAH is among the lowest of all PAH subtypes,[22] and PAH accounts for almost 30% of all deaths associated with systemic sclerosis.[23] The prevalence of PAH in other types of connective-tissue diseases is not well established, but is appreciably lower than that with systemic sclerosis. Screening is not recommended for conditions such as systemic lupus erythematosus or rheumatoid arthritis, but should include patients with systemic sclerosis manifestations in the context of overlap syndromes.

PAH is a frequent complication of congenital heart disease, particularly in the setting of uncorrected systemic-to-pulmonary shunts.[24] Approximately 4–15% of individuals born with congenital heart disease are estimated to develop PAH.[25] Furthermore, in a Dutch registry, PAH prevalence was 5.7%, even in adults who had undergone corrective surgery for congenital heart disease.[26] This finding reinforces the concept that adults with congenital heart disease require appropriate long-term follow-up—even those who have undergone corrective surgery.

Among patients undergoing assessment for liver transplantation, the prevalence of portopulmonary hypertension is around 2–6%.[27,28] Severe portopulmonary hypertension is a contraindication to liver transplantation, and even mild disease is associated with an increased risk of perioperative complications.[29,30]

PH is not an uncommon complication of sickle-cell disease although the disease is mostly postcapillary in

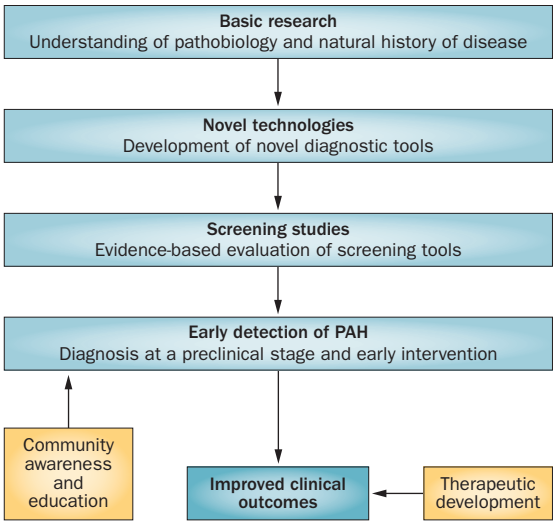

Figure 1 | The main components of how early detection of PAH might improve clinical outcomes. Abbreviation: PAH, pulmonary arterial hypertension.

Box 2 | WHO criteria for screening[124]

Condition
- The condition should be an important health problem
- A recognizable latent or early symptomatic stage should exist
- The natural history of the condition, including development from latent to declared disease, should be adequately understood

Test
- A suitable test or examination should be available
- The test should be acceptable to the population

Treatment
- An accepted treatment should exist for patients with recognized disease

Screening programme
- An agreed policy should exist on whom to treat as patients
- Facilities for diagnosis and treatment should be available
- The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole
- Case-finding should be a continued process and not a 'once-and-for-all' project

Table 1 | Risk factors for PAH and screening recommendations from guidelines

Risk factor	Recommendations	Screening in asymptomatic individuals?	Guideline society
BMPR2-mutation carriers and first-degree relatives of patients with familial PAH	Echocardiogram yearly	Yes	ACC/AHA[37]
Systemic sclerosis	Echocardiogram yearly	Yes	ACC/AHA[37] and ESC/ERS[48]
Portal hypertension	Echocardiogram if liver transplantation considered	Yes	ACC/AHA[37] and ESC/ERS[48]
Sickle-cell disease*	Echocardiogram yearly	Yes	ACC/AHA[37] and ATS[38]
Congenital heart disease	Echocardiogram and right heart catheterization at time of diagnosis; consider repair of defect	No	ACC/AHA[37]
HIV infection	Echocardiogram only if symptomatic	No	ACC/AHA[37] and ESC/ERS[48]
Previous anorexigen use	Echocardiogram only if symptomatic	No	ACC/AHA[37]

*In the current clinical classification, sickle-cell disease has been moved from group 1 (PAH) to group 5 (multifactorial mechanisms). Abbreviations: ATS, American Thoracic Society; ERS, European Respiratory Society; PAH, pulmonary arterial hypertension.

nature. The prevalence of precapillary PH in patients with sickle-cell disease is 3–4%.[31,32] Precapillary PH associated with sickle-cell disease was previously classified under group 1 PAH,[33] but the latest update of the classification system has moved sickle-cell disease to group 5 (unclear or multifactorial mechanisms) because of important differences in pathological findings and haemodynamic characteristics compared with other forms of PAH.[1]

Schistosomiasis affects 200 million people worldwide and is endemic in parts of the developing world, such as sub-Saharan Africa and regions of South America.[34] Approximately 5% of those with the hepatosplenic form of the disease can develop PAH,[35] making schistosomiasis potentially the most prevalent cause of PAH. PAH is also a fairly uncommon, but well-recognized complication of HIV infection, with an estimated prevalence of ~0.5%.[36]

The current ACC Foundation/AHA consensus document on PH recommends that asymptomatic patients at sufficient risk of developing PAH warrant periodic screening, which includes those with a known *BMPR2* mutation, scleroderma spectrum of diseases, or portal hypertension who are undergoing evaluation for liver transplantation.[37] In addition, symptomatic patients with HIV infection or previous use of appetite suppressant drugs should be investigated for PAH. The 2014 American Thoracic Society Clinical Practice Guidelines provide recommendations for periodic screening in patients with sickle-cell disease for detection of PH and as risk stratification for future mortality.[38] Table 1 provides a summary of PAH risk factors and screening recommendations from various clinical guidelines. Importantly, these recommendations comprise expert opinion, on the basis of evaluating the specific at-risk populations for disease epidemiology, economic considerations, availability of resources required for screening, and estimated net benefit of early detection of disease and intervention.

Available tools for PAH screening

Various tools are currently used in clinical practice for PAH screening in at-risk groups of individuals. These tools must be evaluated in the context of the specific at-risk population of interest, and extrapolation of data from one population to another can be misleading. For example, lung-function derangements in pateints with SSc-PAH differ substantially from those in patients with other subtypes of PAH.[39] Moreover, patients with SSc-PAH have worse right ventricular function at similar levels of afterload compared with patients with idiopathic PAH,[40,41] which will affect the performance of biomarkers such as N-terminal pro-B-type natriuretic peptide (NT-proBNP). Given that screening for SSc-PAH has received most attention and research, particular emphasis is given to this population of patients.

Resting echocardiography

Doppler echocardiography is the most widely used screening modality in clinical practice to guide referral for RHC for definitive diagnosis of PAH. Although widely accepted by the medical community as an indispensable noninvasive tool, its performance and predictive values as a stand-alone test for the detection of PAH has been questioned, particularly for screening asymptomatic or mildly symptomatic patients. Therefore, an understanding of the utility and limitations of Doppler echocardiography when used specifically to screen for PAH is crucial.

The detection of PAH by Doppler echocardiography relies principally on measuring the tricuspid regurgitation jet velocity (TRV), which can be transformed into a pressure estimate using the Bernoulli equation to assess systolic P_{pa} (systolic $P_{pa} = 4 \times TRV^2$ + right atrial pressure). Numerous studies have been performed to compare systolic P_{pa} derived using Doppler echocardiography and measurements by gold-standard RHC, performed either simultaneously or within a narrow timeframe (<1 h) of each other.[42,43] Using Bland–Altman analysis, the investigators in one study showed that Doppler echocardiography provides an accurate assessment of systolic P_{pa} (mean bias of –0.5 mmHg), but has insufficient precision as demonstrated by wide limits of agreement (–19 mmHg to 18 mmHg).[44] The lack of precision with Doppler echocardiography necessitates caution when the modality is being used to guide clinical decision-making at an individual patient level.

The performance of Doppler echocardiography is also dependent on calibrating an optimum threshold of TRV for the detection of PAH. Setting a low TRV threshold ensures maximum sensitivity, but at the expense of increasing the number of false–positive results and exposing patients to potentially unnecessary invasive RHC. Conversely, a high TRV threshold increases specificity, but the number of patients with PAH who are missed will be substantial.

In a screening study of patients with systemic sclerosis, moving the TRV threshold from 2.7 m/s to 3.4 m/s (corresponding to tricuspid gradients of 30–45 mmHg) changed the sensitivity of the test from 88% to 47%, and the specificity from 42% to 97%.[45] In a French multicentre screening study for SSc-PAH, an algorithm based on TRV value and symptoms was used to direct referral for RHC.[46] SSc-PAH was suspected and a patient referred for invasive testing if TRV was >3 m/s, or if a TRV of 2.5–3.0 m/s was present together with unexplained dyspnoea. Using this algorithm, 33 patients were referred for RHC from a total of 570 patients who underwent screening. Of the 33 patients in whom PAH was suspected, 18 had confirmed PAH, three had postcapillary PH, and 12 individuals had a mean P_{pa} <25 mmHg at rest.[46] Given that not all screened patients underwent RHC, the number of missed cases of PAH (false negatives) is unknown.

The DETECT study[47] has provided additional and important insights into the use of Doppler echocardiography as a screening tool. This investigation was the first multinational, prospective study in which RHC was mandated in all patients with systemic sclerosis who were screened for PAH. Consistent with a screening population, patients with confirmed SSc-PAH in the DETECT study[47] had mild pulmonary vascular disease, with a mean P_{pa} of 33 ± 8 mmHg and PVR of 371 ± 226 dyn·s·cm^{-5}. In these patients with confirmed SSc-PAH, 7.1% did not have a measurable tricuspid regurgitation jet, 20% had a TRV <2.5 m/s, and 35.7% had a TRV ≤2.8 m/s.[47] Therefore, applying a commonly used[48] arbitrary TRV threshold of 2.8 m/s would have resulted in a large number of patients with PAH going undetected. The performance of echocardiography is also dependent on the severity of disease. For example, a population with a high number of patients with advanced disease (that is, with highly elevated P_{pa}), will be biased towards increased detection of PAH. Conversely, if most patients with PAH in a study population have only mild elevation of P_{pa}, the imprecision of echocardiography will lead to an increased risk of misclassification.

Although the assessment of TRV has been the main echocardiographic parameter used in PAH screening studies, other echocardiographic indices can be employed to provide a comprehensive assessment of the right heart–pulmonary circulation unit in clinical practice. The use of such complementary indices is particularly helpful in patients in whom the TRV signal is inadequate. Abnormal dimension and function of right-sided chambers should raise suspicion of PAH, even in the absence of an elevated TRV. However, the sensitivity of abnormal right heart dimension or function for the early detection of disease is questionable, because dilatation of right-sided chambers usually signifies fairly advanced disease with evolution towards right ventricular failure. Furthermore, accurate volume assessment of the right ventricle using ultrasonography is very difficult, owing to the irregular shape of the chamber.

In the absence of an adequate TRV signal, mean P_{pa} can be estimated using the pulse-wave Doppler profile of the right ventricular outflow tract (RVOT). The acceleration time from flow onset to peak velocity is inversely related to mean P_{pa}. An acceleration time >100 ms indicates the absence of PH, whereas an acceleration time <70 ms greatly increases the likelihood of PH.[49] In addition, the presence of mid-systolic notching in the Doppler profile of the RVOT is a specific sign of elevated PVR, and is physiologically related to enhanced wave reflection from impedance mismatch resulting in mid-systolic flow deceleration.[50]

Elevation in P_{pa} can be the result of either high cardiac output or high left atrial pressure without an increase in PVR related to pulmonary vascular disease. PVR can be estimated using a simple, validated equation (PVR = TRV/velocity time integral$_{RVOT}$ × 10 + 0.16). Moreover, a simple ratio of TRV/velocity time integral$_{RVOT}$ at a cut-off of 0.175 has a sensitivity of 77% and a specificity of 81% to determine PVR >2 Wood units.[51]

Heart failure with preserved ejection fraction is a frequent cause of PH and must be distinguished from precapillary forms of the disease, particularly in patients with systemic sclerosis, in whom both PAH and heart failure with preserved ejection fraction are common complications. Indices of left ventricular diastolic dysfunction (E/A ratio and E/e') and left atrial dimension can indicate the possibility of postcapillary PH. In one study, a scoring system based on five echocardiographic variables (right heart chamber larger than the left, left ventricular eccentricity index >1.2, dilated inferior vena cava without inspiratory collapse, E/e' ratio ≤10, and the right ventricle forming the heart apex) had positive predictive value of 67.9% and a negative predictive value of 77.5% for distinguishing between precapillary and postcapillary PH.[52] Therefore, invasive assessment of P_{pw} or left ventricular end-diastolic pressure is mandatory to diagnose precapillary and postcapillary disease accurately in clinical practice. Importantly, echocardiography is an operator-dependent procedure, which can critically influence the reliability of the measurements.

B-type natriuretic peptide

B-type natriuretic peptide (BNP) is released from the ventricle in response to increased wall tension and is a marker of ventricular strain. In patients with established PAH, both BNP and NT-proBNP (the inactive precursor N-terminal fragment) have been shown to correlate with exercise capacity and haemodynamics,[53] predict mortality,[54,55] and indicate right ventricular systolic impairment.[56] Results of numerous case–control studies have consistently shown that the NT-proBNP level is significantly elevated in patients with SSc-PAH compared with patients with systemic sclerosis but no PAH.[57–59] However, NT-proBNP is unlikely to be sensitive enough as a stand-alone marker for the detection of mild PAH. In one study, an NT-proBNP cut-off level of 395 pg/ml had sensitivity of 56% and specificity of 95% for predicting the presence of SSc-PAH. [57] The low sensitivity of NT-proBNP as a stand-alone test is probably not surprising given that it is a marker of ventricular dysfunction (a late event in the natural history of pulmonary vascular

disease), and a current treatment goal in patients with PAH is normalization or near-normalization of the NT-proBNP level.[60] Given that NT-proBNP is released in response to either left or right ventricular wall stress, its measurement cannot be used to differentiate between PAH and left heart disease. NT-proBNP clearance is dependent on glomerular filtration, and blood levels are, therefore, influenced by kidney function.[61]

Lung-function tests

Standard lung-function measurements include spirometry, diffusing capacity for carbon monoxide (DL_{CO}), and lung volumes. Of these parameters, DL_{CO} and the ratio of forced vital capacity (FVC) to DL_{CO} (FVC/DL_{CO}) have emerged as the most useful for the detection of PAH in patients with systemic sclerosis.

Patients with SSc-PAH have a significantly lower DL_{CO} than patients with systemic sclerosis but no PAH. In one study, patients with SSc-PAH had an average DL_{CO} of 52% of predicted at the time of diagnosis, compared with 80% of predicted in control individuals.[62] Furthermore, in patients with SSc-PAH who had longitudinal measurements, DL_{CO} declined before the development of overt PAH. Similar findings have been confirmed by other investigators.[45,46] In the French ItinérAIR screening study,[46] a DL_{CO} of <60% of predicted was found to be associated with a markedly increased probability of PAH (OR 9.23, 95% CI 2.73–31.15). The combined testing of lung function and NT-proBNP measurement to diagnose PAH in patients with systemic sclerosis has been studied.[63] Using an algorithm based on DL_{CO}, FVC/DL_{CO} ratio, and NT-proBNP level, a sensitivity of 94% and specificity of 55% for PAH was reported.[63] Although all patients in the study underwent RHC, the study population was highly selected and enriched with a very high prevalence of PAH (~35%), because entry criteria into the study required an echocardiography-estimated systolic P_{pa} ≥40 mmHg, a DL_{CO} ≤50% of predicted with FVC >85%, a fall in DL_{CO} ≥20% from the previous year, or unexplained dyspnoea.

Measurement of DL_{CO} comprises the composite of two components: conductance across the alveolar–capillary membrane (D_M) and pulmonary capillary blood volume (V_C).[64,65] Therefore, a reduction in DL_{CO} is not specific to PAH, and can be caused by diffuse parenchymal lung disease. DL_{CO} can be partitioned into D_M and V_C by the additional measurement of the diffusing capacity for nitric oxide (DL_{NO}), because DL_{NO} indicates almost exclusively the D_M component of gas transfer. However, the investigators of a small study ($n = 34$) to examine the utility of partitioning DL_{CO} into its two components did not demonstrate improved discrimination for the presence of PH compared with lone DL_{CO} measurement in patients with systemic sclerosis.[66]

No evidence exists to support the use of a reduced DL_{CO} as a screening tool in other subtypes of PAH, such as heritable or portopulmonary hypertension. These patients can present with advanced haemodynamic disease, despite having only mildly reduced or even normal values of DL_{CO}. Indeed, *BMPR2*-positive patients with PAH have higher DL_{CO} values than *BMPR2*-negative patients with PAH, despite more severe haemodynamic impairment in the *BMPR2*-positive group.[67] The pathophysiological reasons behind this finding are uncertain. In patients with SSc-PAH, the low DL_{CO} has been suggested to indicate a high frequency of pulmonary veno-occlusive disease, because a severe reduction in DL_{CO} is one distinguishing feature of this disease compared with idiopathic or heritable PAH.[68,69]

Cardiopulmonary exercise testing

Cardiopulmonary exercise testing (CPET) is used in clinical practice for the evaluation of effort dyspnoea and has been shown in numerous studies to be a powerful predictor of prognosis in PAH. Patients with PAH commonly show a pattern of CPET response characterized by reduced peak oxygen consumption, early anaerobic threshold, reduced oxygen pulse, high minute ventilation to carbon dioxide production (V_E/V_{CO2}) ratio, low end-tidal pCO_2, and variable oxygen desaturation.[70] These abnormalities are caused by cardiac output limitation to exercise, increased dead-space ventilation, and altered chemosensitivity leading to hyperventilation.[71] A typical CPET response in the appropriate clinical context can be highly indicative of pulmonary vascular disease.

CPET has not been widely used or studied in screening studies because this testing is a complex investigation, and interpretation can be difficult. However, some researchers have suggested CPET as a potential complementary tool for the detection of PAH.[72]

Screening algorithms

Systemic sclerosis

The DETECT study[47] was the first multinational, prospective investigation aimed at deriving an evidence-based PAH screening algorithm using a combination of clinical and laboratory variables in patients with systemic sclerosis. All participants underwent RHC, which enabled, for the first time, the number of false–negative results in a screening study to be determined. Given the invasive nature of the protocol, the investigators felt it necessary to enrich the study population with individuals who were at particularly high risk of PAH, and inclusion criteria stipulated disease duration of >3 years and a DL_{CO} of <60% of predicted. Therefore, the number of incident SSc-PAH cases was 19%, which is much higher than the rate expected in an unselected population of patients with systemic sclerosis.

The DETECT study[47] involved a two-step scoring algorithm: in step 1, six simple parameters were used to determine the need to refer for Doppler echocardiography; in step 2, the score from step 1 was combined with two echocardiographic variables to determine the need for confirmatory RHC. Clinical and laboratory variables used in the DETECT algorithm are summarized in Box 3. The DETECT algorithm was set at a high sensitivity of 96% to minimize false–negative cases, resulting in a specificity of 48%. Therefore, 62% of the DETECT population required referral for RHC, of whom 35% were confirmed to have SSc-PAH. The strength of the

DETECT study was that, using this algorithm, the rate of missed diagnosis was only 4% ($n = 3$). Furthermore, the majority of the DETECT population was in NYHA class I or II, and 18% of patients with confirmed SSc-PAH were asymptomatic.

The inclusion criteria of DETECT mean that the algorithm cannot be applied to patients with a DL_{CO} ≥60% of predicted. Although most patients with SSc-PAH have a DL_{CO} below this threshold, perhaps 10% of patients with SSc-PAH have a DL_{CO} ≥60% of predicted.[39] Therefore, how many patients with PAH in the overall systemic-sclerosis population who might have been missed owing to the enrichment strategy employed in the study is unknown. Conversely, inclusion of patients with systemic sclerosis and a DL_{CO} ≥60% of predicted would have resulted in an increased number of false–positive findings and referral for unnecessary RHC. The DETECT algorithm was designed to screen for PAH and not other forms of PH associated with left heart disease or lung disease, which are also common in patients with systemic sclerosis. However, 6% of individuals in the DETECT study were deemed to have PH related to lung disease, and 37% of these patients would have been missed using the DETECT algorithm. Despite the limitations, the results of the DETECT study clearly demonstrate that the use of a stand-alone test is insufficient, and a multimodal approach involving a range of noninvasive tools is required for accurate screening for PAH.

Portopulmonary hypertension

Screening is recommended in patients being assessed for liver transplantation owing to the increased morbidity and mortality associated with performing transplantation in the presence of portopulmonary hypertension. Moderate-to-severe portopulmonary hypertension (mean P_{pa} >35 mmHg) is considered a contraindication for transplantation in most centres. Doppler echocardiography is the only screening modality that has been systematically evaluated in portopulmonary hypertension. In a study of 165 patients presenting for liver transplantation assessment who underwent both Doppler echocardiography and RHC, using an estimated systolic P_{pa} cut-off value of 30 mmHg had sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of 100%, 96%, 59%, 100%, and 96%, respectively.[28] However, a subsequent study demonstrated that using a systolic P_{pa} cut-off value of >30 mmHg had a poor specificity (54%), whereas calibration of the cut-off value to >38 mmHg maintained sensitivity at 100% and improved specificity to 82%.[73] In this study, patients did not undergo RHC during pretransplantation work-up if echocardiography-derived systolic P_{pa} was ≤30 mmHg, but missing values were obtained at the time of transplantation, when all patients received RHC.

Many patients with liver disease have low systemic vascular resistance and a high cardiac output state. Consequently, the absolute value of P_{pa} can be misleading, and PVR (as an indication of the severity of pulmonary vascular disease) might not be significantly elevated when cardiac output is taken into account.

Box 3 | DETECT study[47] algorithm*

Step 1
- FVC/DL_{CO}
- Current or past telangiectasia
- Serum anti-centromere antibody
- Serum level of N-terminal pro-B-type natriuretic peptide
- Serum level of urate
- Electrocardiogram: right-axis deviation

Step 2
- Tricuspid regurgitation velocity
- Right atrial area

*The DETECT study had a scoring system for steps 1 and 2 to guide referral for echocardiography and right heart catheterization, respectively. Abbreviations: DL_{CO}, diffusing capacity for carbon monoxide; FVC, forced vital capacity.

Sickle-cell disease

Of the haemolytic anaemias associated with PH, only sickle-cell disease has been characterized in terms of disease prevalence. Although sickle-cell disease is no longer classified under group 1 PAH, precapillary PH is a known complication, and off-label therapy with PAH drugs is used in those with significantly elevated PVR, despite the lack of efficacy data to support this practice. Furthermore, a trial of sildenafil therapy in patients with sickle-cell disease and an elevated TRV ≥2.7 m/s was prematurely terminated owing to an increased rate of hospitalization for painful crises in the sildenafil-treated group.[74]

Historically, the prevalence of PH in sickle-cell disease has probably been overestimated in echocardiography-based studies.[75,76] In two new studies from France[31] and Brazil,[32] similar methodologies were employed and all patients with a screening TRV ≥2.5 m/s on echocardiography were referred for confirmatory RHC. The prevalence of PH was found to 6.2% and 10.0% in the French and Brazilian studies, respectively. Postcapillary PH was the most frequent cause, with a prevalence of 3.3% and 6.2%, respectively, whereas the prevalence of precapillary PH was only 2.9% and 3.8%.

An exploratory *post-hoc* analysis of the French study found that calibrating TRV to ≥2.9 m/s, or a TRV of 2.5–2.8 m/s plus either an NT-proBNP level >164.5 pg/ml or a 6-min walking distance of <333 m, reduced the number of RHC referrals from 63 to 21, compared with a single TRV threshold of ≥2.5 m/s.[31] Exact false–negative rates cannot be determined, because RHC was not performed in all study participants.

Congenital heart disease

Particular congenital heart diseases are recognized to predispose to PAH. The most common of these are large unrepaired post-tricuspid left-to-right shunt lesions, such as ventricular septal defects and persistent ductus arteriosus. Rarely, atrial septal defects are associated with PAH, especially those associated with anomalous pulmonary venous drainage. Furthermore, postcapillary PH can complicate congenital heart diseases such as supramitral membrane or cor triatriatum sinister.

The most extreme form of PAH in congenital heart disease is Eisenmenger syndrome, in which PVR rises to

the point of shunt reversal and deoxygenated blood flows from 'right to left', leading to cyanosis and erythrocytosis. Most patients with PAH associated with congenital heart disease have Eisenmenger syndrome.[25] Although no specific guidelines exist, screening for PAH in the context of congenital heart disease should be performed in specialized paediatric and adult congenital heart disease centres, because a wide variety of conditions exist in which PH can complicate an already complex medical situation. For example, echocardiography in the presence of abnormal cardiac connections can be confusing for inexperienced sonographers, calculation of PVR can be technically difficult in patients with asymmetrical pulmonary perfusion, and particular conditions cause postcapillary rather than precapillary PH. Most congenital heart disease units screen for PAH in patients whose lesions are known to be frequently associated with PAH (such as ventricular septal defects or persistent ductus arteriosus). If feasible, patients with haemodynamically relevant left-to-right shunts should be considered for closure, with ongoing follow-up, because development of PAH remains a substantial risk even after corrective surgery.

Heritable PAH and *BMPR2* mutation

Occurrence of familial PAH emphasizes the need for familial investigation of patients presenting with apparently sporadic disease. Moreover, ~20% of patients with sporadic PAH carry a *BMPR2* mutation and, therefore, have heritable disease.[77] Genetic testing of relatives in families known to have a PAH-causing mutation can identify individuals at high risk of developing PAH. For example, *BMPR2*-mutation carriers have a lifetime risk of developing PAH of ~20%.[16] However, the incomplete penetrance of *BMPR2* mutations suggests that the majority of asymptomatic mutation carriers will not develop PAH, and predicting those who will ultimately develop the disease is not currently possible, although women are at increased risk compared with men.[18]

Genetic testing should be conducted in the context of a multidisciplinary team, with appropriate counselling on the implications of positive and negative results. At present, individuals who test positive for PAH-causing mutations are offered a yearly screening echocardiogram and immediate clinical evaluation if new symptoms suggestive of pulmonary vascular disease develop. The same screening algorithm applies to first-degree relatives of patients with heritable PAH when no causal mutations are identified.

Currently, screening in the context of heritable PAH is based on expert consensus rather than evidence from screening studies. In France, an ongoing longitudinal study should clarify issues such as optimal screening modalities and predictors of progression to PAH in asymptomatic carriers of a *BMPR2* mutation.[78] In this study, a cohort of >50 individuals with a *BMPR2* mutation undergo yearly echocardiography, cardiopulmonary exercise testing, and measurement of serum biomarkers (as well resting and exercise RHC in volunteers). This screening approach remains investigational, but will hopefully provide information to refine future guidelines.

Areas of uncertainty

Frequency and duration of screening

Currently, asymptomatic carriers of PAH-causing mutations and patients with scleroderma spectrum diseases are recommended to undergo yearly screening. However, the optimal frequency and duration of screening in these at-risk individuals is uncertain. Screening studies, such as DETECT,[47] have a cross-sectional design and, therefore, do not inform clinicians how to conduct screening during longitudinal follow-up of patients. Furthermore, how a previous negative screening result influences the performance of future testing, if the same screening algorithm is applied repeatedly, is unknown. Current guidelines and data from randomized, controlled trials support PAH therapy only in symptomatic patients in NYHA class II or above. Therefore, individual clinicians must weigh up the risks and benefits associated with off-label PAH therapy versus an observational approach for asymptomatic patients who screen positive.

Borderline PH and PH with exercise

Normal resting P_{pa} is 14 ± 3 mmHg, with an upper limit of 20 mmHg.[79] Therefore, a resting P_{pa} of 21–24 mmHg forms a grey zone in which haemodynamics fall outside normal limits, but do not fulfil the formal criteria for diagnosis of resting PH (defined as P_{pa} ≥25 mmHg). The natural history and clinical relevance of patients with a resting P_{pa} of 21–24 mmHg requires clarification. However, a study involving patients with systemic sclerosis showed that those with 'borderline' PH (21–24 mmHg) had an increased risk of developing resting PH on follow-up (HR 3.7, P <0.001) compared with those with a normal resting P_{pa}.[80] In this study, 76 patients with systemic sclerosis underwent repeat catheterization, and a transpulmonary gradient (mean $P_{pa} - P_{pw}$) ≥11 mmHg at baseline also predicted future PH (HR 7.9, P <0.001).[80] Importantly, incident cases of PAH at follow-up were not benign, with a mortality of 18% within 3 years. Therefore, a borderline level of resting P_{pa} in patients with systemic sclerosis requires close monitoring for development of overt PAH.

Compelling pathophysiological mechanisms indicate that stress testing of the pulmonary circulation can unmask early or latent pulmonary vascular disease. The majority (>60%) of the pulmonary circulation must be obstructed before resting mean P_{pa} rises above 25 mmHg, supported by the observation that pneumonectomy or unilateral balloon occlusion of the pulmonary artery do not result in PH in individuals with normal cardiopulmonary reserve.[81–83] In numerous studies, an exaggerated pulmonary hypertensive response during exercise has been associated with decreased exercise capacity.[84,85] PH on exercise might, therefore, be an intermediate pathophysiological stage of PAH, with greater vascular reserve and less severe pulmonary vascular disease.[86] We also observe that patients with chronic thromboembolic disease, fatigue–dyspnoea symptoms, and normal pulmonary haemodynamics at rest, derive major functional improvements after pulmonary endarterectomy.[87,88] Therefore, patients with clinically important pulmonary

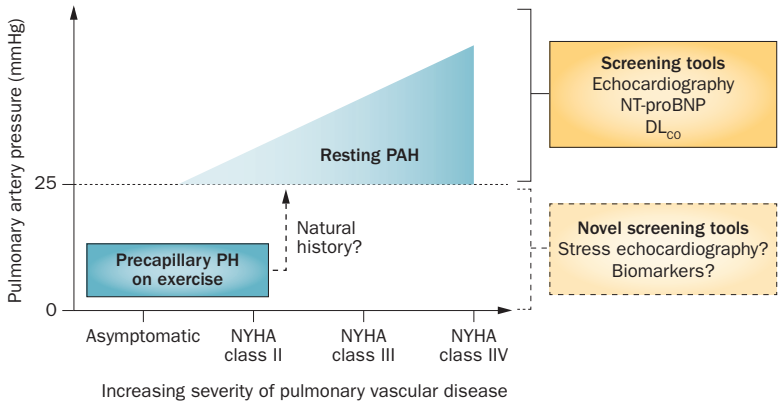

Figure 2 | Relationship between PH on exercise and resting PAH. PH on exercise as a precursor to resting PAH is a physiologically rational concept, but data on the natural history of this entity are scarce. Currently, no consensus exists on the haemodynamic definition of this condition. The efficacy of PAH therapy for improving functional capacity or delaying disease progression is also unknown for patients with PH on exercise. Abbreviations: DL_{CO}, diffusing capacity for carbon monoxide; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PAH, pulmonary arterial hypertension; PH, pulmonary hypertension.

vascular disease exist who do not fulfil the current diagnostic criteria for resting PH.

The old exercise definition of PH (mean P_{pa} >30 mmHg on exercise) was abolished in 2008, because this cut-off was arbitrary, and even healthy individuals can have a mean P_{pa} >30 mmHg during exercise.[89] The removal of the exercise definition was owing to the paucity of data on how best to define a pathological pulmonary haemodynamic response during exercise, rather than because exercise testing of the pulmonary circulation is without clinical value. After the 5th World Symposium on PH in 2013, exercise criteria for PH have still not been defined.[2]

The classic Ohmic equation of the pulmonary circulation is given by: mean $P_{pa} = PVR \times CO + P_{la}$. At a given level of incremental PVR during exercise, mean P_{pa} is influenced by both cardiac output (CO) and left atrial pressure (P_{la}). Therefore, a single value of P_{pa} cannot easily be used to define PH at exercise. A large augmentation in CO (as can occur in healthy individuals) or a brisk rise in P_{la} (in elderly patients or those with left heart disease) can result in mean P_{pa} >30 mmHg without the presence of pulmonary vascular disease. Any proposed criteria for PH on exercise must, therefore, take into account the level of flow or CO.[90,91] An evaluation of P_{la} response during exercise is also crucial, to delineate precapillary or postcapillary contributions to any abnormal P_{pa} rise during exercise.

Very limited data exist on the natural history of precapillary PH on exercise. In a longitudinal study involving 42 patients with systemic sclerosis and precapillary PH on exercise, survival was significantly better than in those with resting SSc-PAH, but eight patients (19%) developed overt resting PAH during follow-up (mean 30 ± 16 months).[5] In another study, only two out of 24 patients with systemic sclerosis and precapillary PH on exercise developed resting disease during follow-up (mean 26 ± 16 months).[92] Therefore, not all patients with precapillary PH on exercise invariably develop resting PAH, at least within the medium-term duration of follow-up in these studies. In our clinical experience, we have also observed that some patients with precapillary PH on exercise remain stable over a long period without developing resting PAH. Furthermore, whether any predictors of developing resting PAH exist, and whether the phenotype of precapillary PH on exercise varies according to underlying aetiology, is unknown (Figure 2).

Data on the effect of targeted PAH therapy in exercise PH is scarce. An observational study of the use of ambrisentan in 12 patients with systemic sclerosis and precapillary PH on exercise demonstrated improvements in exercise haemodynamics at 24 weeks, with peak mean P_{pa} falling from 42 ± 5 mmHg to 37 ± 8 mmHg (P = 0.02), and peak CO improving from 8.4 ± 1.6 l/min to 9.8 ± 2.2 l/min (P = 0.006).[93] The 6-min walking distance also increased significantly by 45 ± 10 m.[93]

Alternative diagnostic tools

Given the limitations of current noninvasive tools used for the detection of PAH, development and validation of alternative and novel diagnostic techniques are required. As with any diagnostic methods, rigorous evaluation of reproducibility and diagnostic performance (sensitivity, specificity, and accuracy) are required before implementation into clinical practice. Also, any new potential screening tool should be widely available without prohibitive costs.

Stress echocardiography

Echocardiography using exercise or pharmacological stress is widely used in clinical practice for the evaluation of coronary artery disease, valvular heart disease, and myocardial disease. The study of the pulmonary circulation using exercise echocardiography has been demonstrated to be feasible,[94] and a number of investigators have reported, using this technique, abnormal systolic P_{pa} response during exercise in at-risk populations. In a large, multicentre study of stress echocardiography, relatives of patients with idiopathic or heritable PAH had an exaggerated pulmonary hypertensive response to stress compared with control individuals.[95] During exercise, 32% of relatives, but only 10% of controls, had TRV augmentation to >3.08 m/s. Furthermore, relatives with *BMPR2* mutations had the highest likelihood of developing a hypertensive response to stress. Similarly, studies of exercise Doppler echocardiography in the patients with systemic sclerosis have demonstrated a high prevalence of pulmonary hypertensive response in 40–60% of patients, depending on the threshold of systolic P_{pa} used to define abnormality.[96–99]

The reported upper limits of systolic P_{pa} in healthy individuals from exercise echocardiography studies are 40–45 mmHg, but can be up to ~55–60 mmHg in highly-conditioned athletes.[100,101] Given the flow-dependency of P_{pa}, an attempt should be made to measure CO at a given level of P_{pa} such that pulmonary vascular resistance can be estimated. Protocols for exercise echocardiography vary, and no standardization currently exists (treadmill

versus bicycle; upright versus recumbent). Ideally, measurements of systolic P_{pa} and CO should be made at incremental workloads, so that the multipoint pressure–flow response of the pulmonary circulation can be inferred. Measurements must also be taken during exercise, because haemodynamics change rapidly and sometimes unpredictably during recovery. Therefore, a protocol using a semirecumbent cycle ergometer seems most suitable for exercise echocardiography-based assessment of pulmonary haemodynamics. As with resting echocardiography, precise estimation of P_{la} is difficult, and studies have shown poor correlation between echocardiography-derived and invasively-measured P_{la} during exercise.[102] Results from a pilot study have shown that dobutamine stress can be used to augment CO and construct noninvasive pressure–flow relationships using echocardiography.[103]

Despite the physiological appeal of stress echocardiography as a noninvasive tool to probe the functional state of the pulmonary circulation, the performance of this technique remains largely unvalidated for the early detection of PAH. Robust validation studies are required before stress echocardiography can be incorporated into routine clinical practice for screening at-risk patients.

CT

Dilatation of the main pulmonary artery is commonly used by radiologists as an indication of the presence of PH. Results from multiple studies have confirmed at least a moderate correlation between pulmonary artery diameter measured on CT scan and invasively-derived P_{pa}.[104,105] The main pulmonary artery to aorta ratio might be a better index than absolute pulmonary artery diameter, because it is less dependent on body surface area. In patients with a wide range of cardiovascular conditions, investigators showed that a pulmonary artery to aorta ratio >1 had sensitivity and specificity of 70% and 92%, respectively, for the detection of PH.[106]

Investigators have also used CT pulmonary angiography and sophisticated fractal branching geometry to quantify the degree of vascular pruning in paediatric PAH.[107] The pulmonary arterial bed was segmented and skeletonized using advanced postprocessing imaging software. The severity of PAH could be assessed by fractal dimension as a surrogate measure of vascular pruning. Fractal dimension was significantly correlated with 6-min walking distance, NYHA functional class, and PVR.

MRI

MRI has emerged as a unique tool in the assessment of PAH, with applications in diagnosis, evaluation of disease severity, and monitoring of treatment response. At present, the clinical use of MRI is restricted mainly to the examination of patients with established PAH and, in particular, for the evaluation of right ventricular structure and function for which it is regarded as the gold standard.[108,109]

Pulmonary artery stiffness assessed using MRI might have utility in the identification of early pulmonary vascular disease. In one study, indices of pulmonary artery stiffness (compliance and capacitance), derived from MRI and right heart catheter measurements, were reduced in patients with exercise PH compared with healthy controls.[110] Quantitative calculation of regional lung perfusion is also possible with 3D gadolinium-enhanced magnetic resonance perfusion analysis. Investigators demonstrated a marked difference in pulmonary perfusion in patients with PAH compared with controls, but whether this technique is sensitive for the early detection of pulmonary vascular disease is unknown.[111] One drawback of MRI is that a direct estimation of P_{pa} is not possible. Various prediction models have been evaluated using MRI indices (such as flow and right ventricular mass) to predict P_{pa}, with conflicting results.[112–114] At present, the high cost, lack of widespread availability, and special expertise required for MRI limit its potential application as a screening method for asymptomatic patients.

Functional nuclear imaging

Pulmonary vascular cells in the remodelled arteries of patients with PAH are hyperproliferative and have altered metabolism.[115] This is similar to the Warburg effect observed in cancer cells, in which glucose oxidation is impaired, with a shift to glycolytic metabolism. Therefore, *in vivo* assessment of glucose metabolism might provide insights into the activity of pulmonary vascular remodelling. Using ^{18}F-labelled deoxyglucose (FDG) PET, many investigators have demonstrated increased lung FDG uptake in patients with idiopathic PAH compared with controls.[116,117] However, in one study, patients with idiopathic PAH who were receiving PAH drugs had similar lung FDG uptake compared with patients who had coronary artery disease but no PH.[118] Furthermore, FDG uptake did not correlate with parameters of disease severity. In patients with systemic sclerosis, increased lung FDG uptake occurs in the presence of interstitial lung disease, which confounds the potential applicability of PET for detecting PAH in this population.

Ventilation and perfusion scintigraphy is a routine investigation in the diagnostic evaluation for PH to exclude thromboembolic disease. With single-photon emission computed tomography, 3D quantitative perfusion analysis is possible. In healthy individuals, a cranial–caudal (apex–base) perfusion gradient exists in the upright posture owing to the gravitational influence on blood-flow distribution. By assessing the degree of perfusion shift between upright and supine postures, investigators have demonstrated that patients with PAH can be distinguished from control individuals with an area under the curve of 0.94 in receiver operating characteristic analysis.[119] However, all patients in this study had established PAH, and those with very mild disease were not included.

Exhaled breath biomarkers

The analysis of biological compounds in exhaled breath might have potential as novel biomarkers of PAH. Given that the pulmonary microcirculation is in close contact with the alveolar membrane, specific biomarkers of underlying disease pathobiology might be detected in exhaled breath.

Sensor arrays based on nanoparticle technology have enabled detection of disease-specific patterns of volatile organic compounds via an artificial nose device.[120] These devices have already been tested for detection of cancers and other diseases.[121,122] Investigators in a preliminary, proof-of-concept study used this technology to test the hypothesis that patients with PAH have a unique breath fingerprint of volatile organic compounds compared with controls.[123] Excellent discrimination between patients with PAH and controls was found, with an area under the curve of 0.91 in receiver operating characteristic analysis. Interestingly, this device could also be used to discriminate between individuals with idiopathic PAH and those with heritable PAH associated with *BMPR2* mutations. A larger validation study is currently being conducted.

Conclusions

Early detection of pulmonary vascular disease is now an important aim to allow early management and potentially reduce morbidity and mortality from advanced PAH and right heart failure. Multimodality screening algorithms in high-risk populations are currently best placed to optimize disease detection. The concept of PH on exercise and its relationship to early disease are still evolving. Experts must continue their efforts to increase awareness of PAH, and disseminate new knowledge to the wider medical community.

1. Simonneau, G. *et al.* Updated clinical classification of pulmonary hypertension. *J. Am. Coll. Cardiol.* **62** (Suppl.), D34–D41 (2013).
2. Hoeper, M. M. *et al.* Definitions and diagnosis of pulmonary hypertension. *J. Am. Coll. Cardiol.* **62** (Suppl.), D42–D50 (2013).
3. Thenappan, T. *et al.* Survival in pulmonary arterial hypertension: a reappraisal of the NIH risk stratification equation. *Eur. Respir. J.* **35**, 1079–1087 (2010).
4. Humbert, M. *et al.* Survival in patients with idiopathic, familial, and anorexigen-associated pulmonary arterial hypertension in the modern management era. *Circulation* **122**, 156–163 (2010).
5. Condliffe, R. *et al.* Connective tissue disease-associated pulmonary arterial hypertension in the modern treatment era. *Am. J. Respir. Crit. Care Med.* **179**, 151–157 (2009).
6. Mathai, S. C. *et al.* Survival in pulmonary hypertension associated with the scleroderma spectrum of diseases: impact of interstitial lung disease. *Arthritis Rheum.* **60**, 569–577 (2009).
7. Launay, D. *et al.* Survival in systemic sclerosis-associated pulmonary arterial hypertension in the modern management era. *Ann. Rheum. Dis.* **72**, 1940–1946 (2013).
8. Ling, Y. *et al.* Changing demographics, epidemiology, and survival of incident pulmonary arterial hypertension: results from the pulmonary hypertension registry of the United Kingdom and Ireland. *Am. J. Respir. Crit. Care Med.* **186**, 790–796 (2012).
9. Strange, G. *et al.* Time from symptoms to definitive diagnosis of idiopathic pulmonary arterial hypertension: the delay study. *Pulm. Circ.* **3**, 89–94 (2013).
10. Galie, N. *et al.* Treatment of patients with mildly symptomatic pulmonary arterial hypertension with bosentan (EARLY study): a double-blind, randomised controlled trial. *Lancet* **371**, 2093–2100 (2008).
11. Benza, R. L. *et al.* Predicting survival in pulmonary arterial hypertension: insights from the Registry to Evaluate Early and Long-Term Pulmonary Arterial Hypertension Disease Management (REVEAL). *Circulation* **122**, 164–172 (2010).
12. Humbert, M. *et al.* Screening for pulmonary arterial hypertension in patients with systemic sclerosis: clinical characteristics at diagnosis and long-term survival. *Arthritis Rheum.* **63**, 3522–3530 (2011).
13. Rose, G. & Barker, D. J. Epidemiology for the uninitiated. Screening. *Br. Med. J.* **2**, 1417–1418 (1978).
14. Peacock, A. J., Murphy, N. F., McMurray, J. J., Caballero, L. & Stewart, S. An epidemiological study of pulmonary arterial hypertension. *Eur. Respir. J.* **30**, 104–109 (2007).
15. Humbert, M. *et al.* Pulmonary arterial hypertension in France: results from a national registry. *Am. J. Respir. Crit. Care Med*. **173**, 1023–1030 (2006).
16. Machado, R. D. *et al.* Mutations of the TGF-beta type II receptor BMPR2 in pulmonary arterial hypertension. *Hum. Mutat.* **27**, 121–132 (2006).
17. Machado, R. D. *et al.* BMPR2 haploinsufficiency as the inherited molecular mechanism for primary pulmonary hypertension. *Am. J. Hum. Genet.* **68**, 92–102 (2001).
18. Larkin, E. K. *et al.* Longitudinal analysis casts doubt on the presence of genetic anticipation in heritable pulmonary arterial hypertension. *Am. J. Respir. Crit. Care Med.* **186**, 892–896 (2012).
19. Pope, J. E. *et al.* Prevalence of elevated pulmonary arterial pressures measured by echocardiography in a multicenter study of patients with systemic sclerosis. *J. Rheumatol.* **32**, 1273–1278 (2005).
20. Stupi, A. M. *et al.* Pulmonary hypertension in the CREST syndrome variant of systemic sclerosis. *Arthritis Rheum.* **29**, 515–524 (1986).
21. Mukerjee, D. *et al.* Prevalence and outcome in systemic sclerosis associated pulmonary arterial hypertension: application of a registry approach. *Ann. Rheum. Dis.* **62**, 1088–1093 (2003).
22. Benza, R. L. *et al.* An evaluation of long-term survival from time of diagnosis in pulmonary arterial hypertension from the REVEAL Registry. *Chest* **142**, 448–456 (2012).
23. Tyndall, A. J. *et al.* Causes and risk factors for death in systemic sclerosis: a study from the EULAR Scleroderma Trials and Research (EUSTAR) database. *Ann. Rheum. Dis.* **69**, 1809–1815 (2010).
24. D'Alto, M. & Mahadevan, V. S. Pulmonary arterial hypertension associated with congenital heart disease. *Eur. Respir. Rev.* **21**, 328–337 (2012).
25. Duffels, M. G. *et al.* Pulmonary arterial hypertension in congenital heart disease: an epidemiologic perspective from a Dutch registry. *Int. J. Cardiol.* **120**, 198–204 (2007).
26. van Riel, A. C. *et al.* Contemporary prevalence of pulmonary arterial hypertension in adult congenital heart disease following the updated clinical classification. *Int. J. Cardiol.* **174**, 299–305 (2014).
27. Hadengue, A., Benhayoun, M. K., Lebrec, D. & Benhamou, J. P. Pulmonary hypertension complicating portal hypertension: prevalence and relation to splanchnic hemodynamics. *Gastroenterology* **100**, 520–528 (1991).
28. Colle, I. O. *et al.* Diagnosis of portopulmonary hypertension in candidates for liver transplantation: a prospective study. *Hepatology* **37**, 401–409 (2003).
29. Krowka, M. J. *et al.* Pulmonary hemodynamics and perioperative cardiopulmonary-related mortality in patients with portopulmonary hypertension undergoing liver transplantation. *Liver Transpl.* **6**, 443–450 (2000).
30. Murray, K. F., Carithers, R. L. Jr & Aasld. AASLD practice guidelines: evaluation of the patient for liver transplantation. *Hepatology* **41**, 1407–1432 (2005).
31. Parent, F. *et al.* A hemodynamic study of pulmonary hypertension in sickle cell disease. *N. Engl. J. Med.* **365**, 44–53 (2011).
32. Fonseca, G. H., Souza, R., Salemi, V. M., Jardim, C. V. & Gualandro, S. F. Pulmonary hypertension diagnosed by right heart catheterisation in sickle cell disease. *Eur. Respir. J.* **39**, 112–118 (2012).
33. Simonneau, G. *et al.* Updated clinical classification of pulmonary hypertension. *J. Am. Coll. Cardiol* **54** (Suppl.), S43–S54 (2009).
34. Chitsulo, L., Engels, D., Montresor, A. & Savioli, L. The global status of schistosomiasis and its control. *Acta Trop.* **77**, 41–51 (2000).
35. Lapa, M. *et al.* Cardiopulmonary manifestations of hepatosplenic schistosomiasis. *Circulation* **119**, 1518–1523 (2009).
36. Sitbon, O. *et al.* Prevalence of HIV-related pulmonary arterial hypertension in the current antiretroviral therapy era. *Am. J. Respir. Crit. Care Med.* **177**, 108–113 (2008).
37. McLaughlin, V. V. *et al.* 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. *J. Am. Coll. Cardiol.* **53**, 1573–1619 (2009).
38. Klings, E. S. *et al.* An official American Thoracic Society clinical practice guideline: diagnosis, risk stratification, and management of pulmonary hypertension of sickle cell disease. *Am. J. Respir. Crit. Care Med.* **189**, 727–740 (2014).
39. Schwaiger, J. P., Khanna, D. & Gerry Coghlan, J. Screening patients with scleroderma for pulmonary arterial hypertension and implications for other at-risk populations. *Eur. Respir. Rev.* **22**, 515–525 (2013).
40. Tedford, R. J. *et al.* Right ventricular dysfunction in systemic sclerosis-associated pulmonary arterial hypertension. *Circ. Heart Fail.* **6**, 953–963 (2013).

41. Overbeek, M. J. *et al.* Right ventricular contractility in systemic sclerosis-associated and idiopathic pulmonary arterial hypertension. *Eur. Respir. J.* **31**, 1160–1166 (2008).
42. Rich, J. D., Shah, S. J., Swamy, R. S., Kamp, A. & Rich, S. Inaccuracy of Doppler echocardiographic estimates of pulmonary artery pressures in patients with pulmonary hypertension: implications for clinical practice. *Chest* **139**, 988–993 (2011).
43. Fisher, M. R. *et al.* Accuracy of Doppler echocardiography in the hemodynamic assessment of pulmonary hypertension. *Am. J. Respir. Crit. Care Med.* **179**, 615–621 (2009).
44. D'Alto, M. *et al.* Accuracy and precision of echocardiography versus right heart catheterization for the assessment of pulmonary hypertension. *Int. J. Cardiol.* **168**, 4058–4062 (2013).
45. Mukerjee, D. *et al.* Echocardiography and pulmonary function as screening tests for pulmonary arterial hypertension in systemic sclerosis. *Rheumatology (Oxford)* **43**, 461–466 (2004).
46. Hachulla, E. *et al.* Early detection of pulmonary arterial hypertension in systemic sclerosis: a French nationwide prospective multicenter study. *Arthritis Rheum.* **52**, 3792–3800 (2005).
47. Coghlan, J. G. *et al.* Evidence-based detection of pulmonary arterial hypertension in systemic sclerosis: the DETECT study. *Ann. Rheum Dis.* **73**, 1340–1349 (2014).
48. Galie, N. *et al.* 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.* **30**, 2493–2537 (2009).
49. Kitabatake, A. *et al.* Noninvasive evaluation of pulmonary hypertension by a pulsed Doppler technique. *Circulation* **68**, 302–309 (1983).
50. Arkles, J. S. *et al.* Shape of the right ventricular Doppler envelope predicts hemodynamics and right heart function in pulmonary hypertension. *Am. J. Respir. Crit. Care Med.* **183**, 268–276 (2011).
51. Abbas, A. E. *et al.* A simple method for noninvasive estimation of pulmonary vascular resistance. *J. Am. Coll. Cardiol.* **41**, 1021–1027 (2003).
52. D'Alto, M. *et al.* Echocardiographic prediction of pre- versus postcapillary pulmonary hypertension. *J. Am. Soc. Echocardiogr.* http://dx.doi.org/10.1016/j.echo.2014.09.004.
53. Leuchte, H. H. *et al.* Clinical significance of brain natriuretic peptide in primary pulmonary hypertension. *J. Am. Coll. Cardiol.* **43**, 764–770 (2004).
54. Fijalkowska, A. *et al.* Serum N-terminal brain natriuretic peptide as a prognostic parameter in patients with pulmonary hypertension. *Chest* **129**, 1313–1321 (2006).
55. Nagaya, N. *et al.* Plasma brain natriuretic peptide as a prognostic indicator in patients with primary pulmonary hypertension. *Circulation* **102**, 865–870 (2000).
56. Blyth, K. G. *et al.* NT-proBNP can be used to detect right ventricular systolic dysfunction in pulmonary hypertension. *Eur. Respir. J.* **29**, 737–744 (2007).
57. Williams, M. H. *et al.* Role of N-terminal brain natriuretic peptide (N-TproBNP) in scleroderma-associated pulmonary arterial hypertension. *Eur. Heart J.* **27**, 1485–1494 (2006).
58. Mukerjee, D. *et al.* Significance of plasma N-terminal pro-brain natriuretic peptide in patients with systemic sclerosis-related pulmonary arterial hypertension. *Respir. Med.* **97**, 1230–1236 (2003).
59. Allanore, Y. *et al.* N-terminal pro-brain natriuretic peptide as a diagnostic marker of early pulmonary artery hypertension in patients with systemic sclerosis and effects of calcium-channel blockers. *Arthritis Rheum.* **48**, 3503–3508 (2003).
60. McLaughlin, V. V. *et al.* Treatment goals of pulmonary hypertension. *J. Am. Coll. Cardiol.* **62** (Suppl.), D73–D81 (2013).
61. Luchner, A. *et al.* Effect of compensated renal dysfunction on approved heart failure markers: direct comparison of brain natriuretic peptide (BNP) and N-terminal pro-BNP. *Hypertension* **46**, 118–123 (2005).
62. Steen, V. & Medsger, T. A. Jr. Predictors of isolated pulmonary hypertension in patients with systemic sclerosis and limited cutaneous involvement. *Arthritis Rheum.* **48**, 516–522 (2003).
63. Thakkar, V. *et al.* The inclusion of N-terminal pro-brain natriuretic peptide in a sensitive screening strategy for systemic sclerosis-related pulmonary arterial hypertension: a cohort study. *Arthritis Res. Ther.* **15**, R193 (2013).
64. Borland, C. A place for $T_{L,NO}$ with $T_{L,CO}$? *Eur. Respir. J.* **31**, 918–919 (2008).
65. Roughton, F. J. & Forster, R. E. Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lung, with special reference to true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. *J. Appl. Physiol.* **11**, 290–302 (1957).
66. Overbeek, M. J. *et al.* 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.* **9**, 68 (2008).
67. Trip, P. *et al.* Diffusion capacity and *BMPR2* mutations in pulmonary arterial hypertension. *Eur. Respir. J.* **43**, 1195–1198 (2014).
68. Montani, D. *et al.* Pulmonary veno-occlusive disease. *Eur. Respir. J.* **33**, 189–200 (2009).
69. Gunther, S. *et al.* Computed tomography findings of pulmonary venoocclusive disease in scleroderma patients presenting with precapillary pulmonary hypertension. *Arthritis Rheum.* **64**, 2995–3005 (2012).
70. Sun, X. G., Hansen, J. E., Oudiz, R. J. & Wasserman, K. Pulmonary function in primary pulmonary hypertension. *J. Am. Coll. Cardiol.* **41**, 1028–1035 (2003).
71. Arena, R., Lavie, C. J., Milani, R. V., Myers, J. & Guazzi, M. Cardiopulmonary exercise testing in patients with pulmonary arterial hypertension: an evidence-based review. *J. Heart Lung Transplant.* **29**, 159–173 (2010).
72. Trip, P., Vonk-Noordegraaf, A. & Bogaard, H. J. Cardiopulmonary exercise testing reveals onset of disease and response to treatment in a case of heritable pulmonary arterial hypertension. *Pulm. Circ.* **2**, 387–389 (2012).
73. Raevens, S. *et al.* Echocardiography for the detection of portopulmonary hypertension in liver transplant candidates: an analysis of cutoff values. *Liver Transpl.* **19**, 602–610 (2013).
74. Machado, R. F. *et al.* Hospitalization for pain in patients with sickle cell disease treated with sildenafil for elevated TRV and low exercise capacity. *Blood* **118**, 855–864 (2011).
75. Ataga, K. I. *et al.* Pulmonary hypertension in patients with sickle cell disease: a longitudinal study. *Br. J. Haematol.* **134**, 109–115 (2006).
76. Gladwin, M. T. *et al.* Pulmonary hypertension as a risk factor for death in patients with sickle cell disease. *N. Engl. J. Med.* **350**, 886–895 (2004).
77. Soubrier, F. *et al.* Genetics and genomics of pulmonary arterial hypertension. *J. Am. Coll. Cardiol.* **62** (Suppl.), D13–D21 (2013).
78. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01600898 (2014).
79. Kovacs, G., Berghold, A., Scheidl, S. & Olschewski, H. Pulmonary arterial pressure during rest and exercise in healthy subjects: a systematic review. *Eur. Respir. J.* **34**, 888–894 (2009).
80. Valerio, C. J., Schreiber, B. E., Handler, C. E., Denton, C. P. & Coghlan, J. G. Borderline mean pulmonary artery pressure in patients with systemic sclerosis: transpulmonary gradient predicts risk of developing pulmonary hypertension. *Arthritis Rheum.* **65**, 1074–1084 (2013).
81. Brofman, B. L. *et al.* Unilateral pulmonary artery occlusion in man; control studies. *J. Thorac. Surg.* **34**, 206–227 (1957).
82. Reed, C. E., Spinale, F. G. & Crawford, F. A. Jr Effect of pulmonary resection on right ventricular function. *Ann. Thorac. Surg.* **53**, 578–582 (1992).
83. Sasahara, A. A. Pulmonary vascular responses to thromboembolism. *Mod. Concepts Cardiovasc. Dis.* **36**, 55–60 (1967).
84. Tolle, J. J., Waxman, A. B., Van Horn, T. L., Pappagianopoulos, P. P. & Systrom, D. M. Exercise-induced pulmonary arterial hypertension. *Circulation* **118**, 2183–2189 (2008).
85. Kovacs, G. *et al.* Borderline pulmonary arterial pressure is associated with decreased exercise capacity in scleroderma. *Am. J. Respir. Crit. Care Med.* **180**, 881–886 (2009).
86. Oudiz, R. J. & Rubin, L. J. Exercise-induced pulmonary arterial hypertension: a new addition to the spectrum of pulmonary vascular diseases. *Circulation* **118**, 2120–2121 (2008).
87. Lang, I. M. & Madani, M. Update on chronic thromboembolic pulmonary hypertension. *Circulation* **130**, 508–518 (2014).
88. Hoeper, M. M. *et al.* Chronic thromboembolic pulmonary hypertension. *Lancet Respir. Med.* **2**, 573–582 (2014).
89. Hoeper, M. M. *et al.* Diagnosis, assessment, and treatment of non-pulmonary arterial hypertension pulmonary hypertension. *J. Am. Coll. Cardiol.* **54**, S85–96 (2009).
90. Naeije, R. *et al.* Exercise-induced pulmonary hypertension: physiological basis and methodological concerns. *Am. J. Respir. Crit. Care Med.* **187**, 576–583 (2013).
91. Lewis, G. D. *et al.* Pulmonary vascular hemodynamic response to exercise in cardiopulmonary diseases. *Circulation* **128**, 1470–1479 (2013).
92. Bae, S. *et al.* Baseline characteristics and follow-up in patients with normal haemodynamics versus borderline mean pulmonary arterial pressure in systemic sclerosis: results from the PHAROS registry. *Ann. Rheum. Dis.* **71**, 1335–1342 (2012).
93. Saggar, R. *et al.* Brief report: effect of ambrisentan treatment on exercise-induced pulmonary hypertension in systemic sclerosis: a prospective single-center, open-label pilot study. *Arthritis Rheum.* **64**, 4072–4077 (2012).
94. Bossone, E. *et al.* Echocardiography in pulmonary arterial hypertension: from diagnosis to prognosis. *J. Am. Soc. Echocardiogr.* **26**, 1–14 (2013).

95. Grunig, E. *et al.* 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* **119**, 1747–1757 (2009).
96. Reichenberger, F. *et al.* Noninvasive detection of early pulmonary vascular dysfunction in scleroderma. *Resp. Med.* **103**, 1713–1718 (2009).
97. Collins, N. *et al.* 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.* **7**, 439–446 (2006).
98. Alkotob, M. L. *et al.* Reduced exercise capacity and stress-induced pulmonary hypertension in patients with scleroderma. *Chest* **130**, 176–181 (2006).
99. Steen, V. *et al.* Exercise-induced pulmonary arterial hypertension in patients with systemic sclerosis. *Chest* **134**, 146–151 (2008).
100. Bossone, E., Rubenfire, M., Bach, D. S., Ricciardi, M. & Armstrong, W. F. 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.* **33**, 1662–1666 (1999).
101. Argiento, P. *et al.* Exercise stress echocardiography of the pulmonary circulation: limits of normal and sex differences. *Chest* **142**, 1158–1165 (2012).
102. Maeder, M. T., Thompson, B. R., Brunner-La Rocca, H. P. & Kaye, D. M. Hemodynamic basis of exercise limitation in patients with heart failure and normal ejection fraction. *J. Am. Coll. Cardiol.* **56**, 855–863 (2010).
103. Lau, E. M. *et al.* Dobutamine stress echocardiography for the assessment of pressure-flow relationships of the pulmonary circulation. *Chest* **146**, 959–966 (2014).
104. Kuriyama, K. *et al.* CT-determined pulmonary artery diameters in predicting pulmonary hypertension. *Invest. Radiol.* **19**, 16–22 (1984).
105. Haimovici, J. B. *et al.* Relationship between pulmonary artery diameter at computed tomography and pulmonary artery pressures at right-sided heart catheterization. Massachusetts General Hospital Lung Transplantation Program. *Acad. Radiol.* **4**, 327–334 (1997).
106. Ng, C. S., Wells, A. U. & Padley, S. P. A CT sign of chronic pulmonary arterial hypertension: the ratio of main pulmonary artery to aortic diameter. *J. Thorac. Imaging* **14**, 270–278 (1999).
107. Moledina, S. *et al.* Fractal branching quantifies vascular changes and predicts survival in pulmonary hypertension: a proof of principle study. *Heart* **97**, 1245–1249 (2011).
108. Boxt, L. M., Katz, J., Kolb, T., Czegledy, F. P. & Barst, R. J. Direct quantitation of right and left ventricular volumes with nuclear magnetic resonance imaging in patients with primary pulmonary hypertension. *J. Am. Coll. Cardiol.* **19**, 1508–1515 (1992).
109. Benza, R., Biederman, R., Murali, S. & Gupta, H. Role of cardiac magnetic resonance imaging in the management of patients with pulmonary arterial hypertension. *J. Am. Coll. Cardiol.* **52**, 1683–1692 (2008).
110. Sanz, J. *et al.* Evaluation of pulmonary artery stiffness in pulmonary hypertension with cardiac magnetic resonance. *JACC Cardiovasc. Imaging* **2**, 286–295 (2009).
111. Ohno, Y. *et al.* Primary pulmonary hypertension: 3D dynamic perfusion MRI for quantitative analysis of regional pulmonary perfusion. *Am. J. Roentgenol.* **188**, 48–56 (2007).
112. Roeleveld, R. J. *et al.* A comparison of noninvasive MRI-based methods of estimating pulmonary artery pressure in pulmonary hypertension. *J. Magn. Reson. Imaging* **22**, 67–72 (2005).
113. Saba, T. S., Foster, J., Cockburn, M., Cowan, M. & Peacock, A. J. Ventricular mass index using magnetic resonance imaging accurately estimates pulmonary artery pressure. *Eur. Respir. J.* **20**, 1519–1524 (2002).
114. Laffon, E. *et al.* A computed method for noninvasive MRI assessment of pulmonary arterial hypertension. *J. Appl. Physiol. (1985)* **96**, 463–468 (2004).
115. Archer, S. L., Weir, E. K. & Wilkins, M. R. Basic science of pulmonary arterial hypertension for clinicians: new concepts and experimental therapies. *Circulation* **121**, 2045–2066 (2010).
116. Zhao, L. *et al.* Heterogeneity in lung ^{18}FDG uptake in pulmonary arterial hypertension: potential of dynamic ^{18}FDG positron emission tomography with kinetic analysis as a bridging biomarker for pulmonary vascular remodeling targeted treatments. *Circulation* **128**, 1214–1224 (2013).
117. Hagan, G. *et al.* ^{18}FDG PET imaging can quantify increased cellular metabolism in pulmonary arterial hypertension: A proof-of-principle study. *Pulm. Circ.* **1**, 448–455 (2011).
118. Ruiter, G. *et al.* Pulmonary 2-deoxy-2-[^{18}F]-fluoro-D-glucose uptake is low in treated patients with idiopathic pulmonary arterial hypertension. *Pulm. Circ.* **3**, 647–653 (2013).
119. Lau, E. M. *et al.* Pulmonary hypertension leads to a loss of gravity dependent redistribution of regional lung perfusion: a SPECT/CT study. *Heart* **100**, 47–53 (2014).
120. Hakim, M. *et al.* Volatile organic compounds of lung cancer and possible biochemical pathways. *Chem. Rev.* **112**, 5949–5966 (2012).
121. Peled, N. *et al.* Non-invasive breath analysis of pulmonary nodules. *J. Thorac. Oncol.* **7**, 1528–1533 (2012).
122. Peng, G. *et al.* Diagnosing lung cancer in exhaled breath using gold nanoparticles. *Nat. Nanotechnol.* **4**, 669–673 (2009).
123. Cohen-Kaminsky, S. *et al.* A proof of concept for the detection and classification of pulmonary arterial hypertension through breath analysis with a sensor array. *Am. J. Respir. Crit. Care Med.* **188**, 756–759 (2013).
124. Wilson, J. M. G. & Jungner, G. *Principles and Practice of Screening for Disease* (WHO, 1968).

Author contributions

All the authors researched data for the article. E.M.T.L. wrote the manuscript, and all the authors reviewed and edited the article before submission.