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SERIES "PULMONARY HYPERTENSION: BASIC CONCEPTS FOR PRACTICAL MANAGEMENT"
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Biomarkers in pulmonary hypertension

G. Warwick*, P.S. Thomas# and D.H. Yates*

ABSTRACT: There have been significant recent advances in the understanding of the pathophysiology of pulmonary hypertension, and a growing number of therapeutic agents have become available to the treating physician. Traditional methods of diagnosing and monitoring this condition have comprised echocardiography and right heart catheterisation, in addition to functional measures, such as estimation of functional class and the 6-min walk test. An increasing number of biomarkers have been described that are elevated in pulmonary hypertension and which may assist the clinician in diagnosis and in the assessment of disease severity and response to treatment.

The present article details the more important biomarkers, their potential applications and the evidence supporting their use.

KEYWORDS: Asymmetric dimethylarginine, biomarkers, endothelin, natriuretic peptide, pulmonary hypertension

Pulmonary arterial hypertension (PAH) is a rare progressive disease, which leads to increasing pulmonary vascular resistance, right heart failure and premature death [1]. As presenting symptoms are nonspecific, diagnosis is often delayed by, on average, 2 yrs [2]; median survival in untreated disease is 2.8 yrs with an estimated 5-yr survival of 34% [3]. Diagnosis is commonly made using trans-thoracic echocardiography, or suspected because of reduced transfer factor but relatively normal lung volumes on respiratory function testing. Right heart catheterisation is required for the definitive diagnosis. Pulmonary hypertension (PH) is defined as a mean pulmonary artery pressure of >25 mmHg at rest or >30 mmHg with exercise [4]. Subsequent investigations are instituted to further characterise the disease. Causes of PH are shown in table 1. The 6-min walk test (6MWT), a measure of exercise capacity, and the New York Heart Association (NYHA)/World Health Organization (WHO) functional classification, a measure of severity, are used to follow the clinical course while receiving treatment, and these both correlate with disease severity and prognosis [5]. The aetiology of PAH is incompletely understood; several factors are implicated in its pathogenesis, including genetic predisposition and exposure to toxins and/or inflammatory mediators [6]. Pulmonary vascular tone is maintained by a balance of neural stimuli, oxygen tension, potassium channels and endogenous vasoactive substances, some of which, *e.g.* the natriuretic peptides and endothelin (ET)-1, provide the basis both for therapeutic targets and diagnostic tests, as will be discussed in the present manuscript.

Current guidelines recommend serological testing for connective tissue disease and testing for HIV in cases of unexplained PAH [7]. Otherwise, blood tests are not routinely used in the diagnosis or follow-up of PAH. Recently, however, a range of biomarkers in PAH have been described, which may be of diagnostic and prognostic significance in the future. These include markers of heart failure, endothelial and/or platelet dysfunction, cardiac myocyte damage and oxidative stress. In the present article, those of most relevance are outlined and their clinical application discussed.

MARKERS

Natriuretic peptides

Atrial natriuretic peptide (ANP) and brain (B-type) natriuretic peptide (BNP) are peptide

AFFILIATIONS
*Dept of Thoracic Medicine, St Vincent's Hospital, and
#Inflammatory Diseases Research Unit, University of New South Wales, Sydney, Australia.

CORRESPONDENCE
D.H. Yates
Dept of Thoracic Medicine
St Vincent's Hospital
390 Victoria Street
Darlinghurst
NSW 2010
Australia
Fax: 61 283822359
E-mail: Deborahy88@hotmail.com

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hormones that are released from cardiac myocytes in response to cardiac pressure and volume overload [8]. ANP is released from storage granules in atrial tissue, while BNP is secreted from ventricular tissue in a constitutive fashion. ANP secretion is stimulated by atrial stretch caused by atrial volume overload; BNP is released in response to ventricular stretch. Natriuretic peptides act on the kidney, causing natriuresis and diuresis, and relax vascular smooth muscle, causing arterial and venous dilatation, leading to reduced blood pressure and ventricular preload [9]. ANP and BNP are released as prohormones and then cleaved into the active peptide and an inactive N-terminal fragment (fig. 1).

TABLE 1 The 2003 Venice clinical classification of pulmonary hypertension [1]

PAH
IPAH
FPAH
APAH
Collagen vascular disease
Congenital systemic-to-pulmonary shunts
Portal hypertension
HIV infection
Drugs and toxins
Other (thyroid disorders, glycogen storage disease, Gaucher's disease, hereditary haemorrhagic telangiectasia, haemoglobinopathies, myeloproliferative disorders, splenectomy)
Associated with significant venous or capillary involvement
PVOD
PCH
Persistent PH of the newborn
PH with left heart disease
Left-sided atrial or ventricular heart disease
Left-sided valvular heart disease
PH associated with lung diseases and/or hypoxaemia
COPD
ILD
Sleep-disordered breathing
Alveolar hypoventilation disorders
Chronic exposure to high altitude
Developmental abnormalities
PH due to chronic thrombotic and/or embolic disease
Thromboembolic obstruction of proximal pulmonary arteries
Thromboembolic obstruction of distal pulmonary arteries
Nonthrombotic pulmonary embolism (tumour, parasites, foreign material)
Miscellaneous
Sarcoidosis
Histiocytosis X
Lymphangiomatosis
Compression of pulmonary vessels (adenopathy, tumour, fibrosing mediastinstis)

PAH: pulmonary arterial hypertension; IPAH: idiopathic PAH; FPAH: familial PAH; APAH: PAH related to risk factors or associated conditions; PVOD: pulmonary veno-occlusive disease; PCH: pulmonary capillary haemangiomatosis; PH: pulmonary hypertension; COPD: chronic obstructive pulmonary disease; ILD: interstitial lung disease.

BNP is elevated in PH of various classes: idiopathic (IPAH) [10]; PAH associated with connective tissue disease [11], congenital systemic-to-pulmonary shunts [12] and treatment of Gaucher's disease [13]; and PH associated with chronic obstructive pulmonary disease [14], interstitial lung disease [15], chronic thromboembolic disease [16, 17] and acute pulmonary embolus [18, 19]. BNP levels correlate with haemodynamic parameters [10, 15–17, 20–23], exercise capacity and NYHA functional class [21]. BNP is of prognostic significance in IPAH [10], in chronic lung disease [24] and in pulmonary embolism [18, 19]. In one study, baseline BNP was measured in 60 patients with IPAH, and repeated after a mean of 3 months; patients were followed for a mean of 24 months [10]. By multivariate analysis, baseline BNP was an independent predictor of mortality and, by receiver operating characteristic analysis, the prognostic power of baseline BNP was comparable, or even superior, to that of haemodynamic parameters.

More recently, interest has turned to the N-terminal fragment of BNP (NT-proBNP) as an alternative biomarker to BNP, as it appears to provide the same information [25], while having advantages over BNP in terms of stability of the marker and high internal accuracy of the assay [26]. There have been a number of recent studies investigating the role of this marker in various classes of PH. ANDREASSEN *et al.* [27] measured NT-proBNP in 61 patients with PH of different classes undergoing right heart catheterisation and cardiopulmonary exercise testing. Plasma levels were higher in patients across the spectrum of PH compared with controls, and correlated with haemodynamic parameters and peak oxygen uptake. On multivariate analysis, NT-proBNP and peak oxygen uptake were independent predictors of mortality. By Kaplan–Meier analysis, patients with supramedian NT-proBNP levels had significantly lower survival than those with lower levels. Another study, also examining patients with different classes of PH, found elevated levels in patients compared with controls [28]. NT-proBNP correlated with haemodynamic parameters at right heart catheterisation, echocardiographic indices of right ventricular overload and the 6MWT distance. Baseline levels of the marker were related to a poor prognosis. Similar relationships were found when only the subgroup of patients with IPAH was analysed. This concurs with an earlier, smaller study of 22 patients with IPAH [29].

The role of serial measurement of NT-proBNP during acute vasoreactivity testing of IPAH patients has been evaluated in a small study [30]. An increase in NT-proBNP after a 60-min inhalation of nitric oxide distinguished responders from nonresponders with a 50% specificity and 100% sensitivity (positive predictive value 38%, negative predictive value 100%). NT-proBNP has also been evaluated as a tool to stratify disease severity. Results of a small study found significantly different NT-proBNP levels between patients in each functional class, suggesting a potential role for the marker in stratifying patients according to disease severity [31]. These results are yet to be validated in a larger population.

In systemic sclerosis, NT-proBNP is elevated in patients with PAH and correlates with pulmonary haemodynamics [32, 33]. Levels are elevated in patients with sickle cell disease compared with healthy, black controls, and are higher still in those patients with PAH [34]. This retrospective analysis showed NT-proBNP to be an independent predictor of mortality.

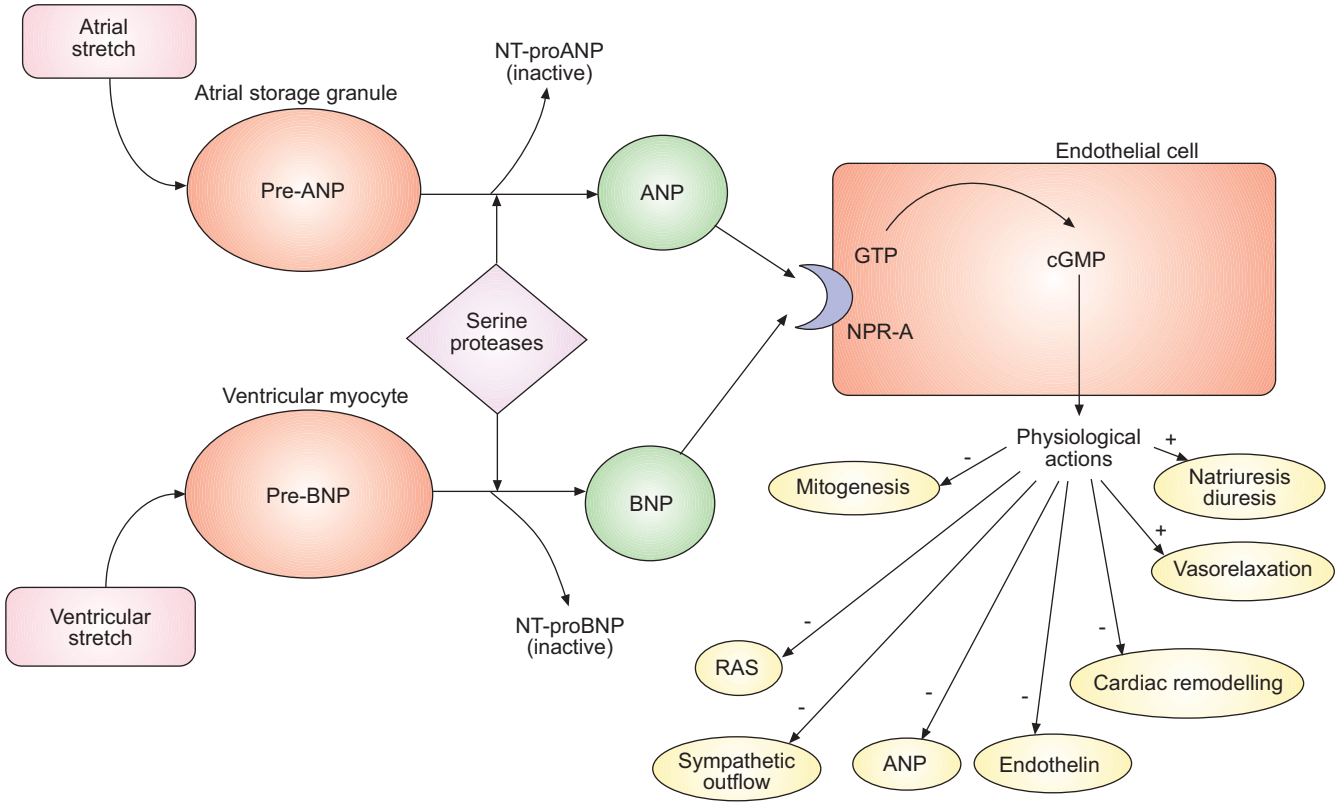

FIGURE 1. Simplified schematic of the natriuretic peptide system. Natriuretic peptide precursors are released in response to atrial and ventricular stretch, cleaved into active molecules and inactive precursors and convert guanosine 5′-triphosphate (GTP) to cyclic guanosine monophosphate (cGMP), leading to their various physiological actions. ANP: atrial natriuretic peptide; NT-proANP: N-terminal pro-ANP; BNP: brain natriuretic peptide; NT-proBNP: N-terminal pro-BNP; NPR-A: natriuretic peptide receptor A; RAS: renin–angiotensin system.

Plasma ANP is significantly increased in PH of varying aetiologies and, like BNP, correlates with haemodynamic variables [35–38]. ANP may better reflect short-term haemodynamic changes, owing to different patterns of synthesis, secretion and clearance than BNP [23].

NT-proBNP has been evaluated as a marker of early PAH in 40 patients with systemic sclerosis, using echocardiography for diagnosis [32]. Here, a high negative predictive value of 96% was found but the positive predictive value was lower at 69.2%, suggesting that NT-proBNP may be of more use in excluding rather than diagnosing the condition. However, a more recent study in a larger number of scleroderma patients, in whom right heart catheterisation was performed, found a higher positive predictive value of 95%, and lower negative predictive value of 56.5% [33]. In a screening study of 39 patients with pulmonary fibrosis of different aetiologies, BNP level predicted moderate–severe PH with 100% sensitivity and 89% sensitivity [15]. A recently published study of 230 patients with sickle cell disease compared NT-proBNP levels with echocardiography for the diagnosis of concomitant PH [34]. The investigators found a sensitivity of 57%, a specificity of 91% and a positive predictive value of 78%.

Natriuretic peptides have also been used to monitor response to therapy. BNP levels parallel the development of pulmonary haemodynamics and exercise capacity in patients with PAH on vasodilator therapy [22, 23], and can predict response to therapy with epoprostenol [39]. BNP levels mirror haemodynamic improvements in patients with chronic thromboembolic pulmonary hypertension undergoing pre-operative prostacyclin therapy prior to endarterectomy [17], and a persistently elevated BNP following endarterectomy is indicative of persistent PH in the post-operative period [16].

There are a number of confounding factors in the interpretation of natriuretic peptide levels, including left heart disease, sex, age and renal dysfunction [40]. Since most studies exclude patients with left heart disease and renal dysfunction, there may be problems with extrapolating these results to a less-selected population. Some authors have sought to address this by calculating a normalised ratio of BNP and NT-proBNP by dividing the measured value by age- and sex-adjusted normal values [41]. A recent study assessed the effect of renal impairment on the diagnostic accuracy of NT-proBNP as a parameter of haemodynamic status [41]. It found that, while NT-proBNP was inferior to BNP as a follow-up parameter of haemodynamic variables in patients with a creatinine clearance of <60 mL·min^{-1}, it was superior to BNP as a survival parameter, because it integrated haemodynamic impairment with renal insufficiency, which was itself a sign of increased mortality. It should also be noted that, since elevated levels of natriuretic peptides signify high ventricular wall stress, they should be considered ''late'' markers of disease. A normal

BNP/NT-proBNP level cannot, therefore, be used to exclude the presence of PH.

Endothelin-1

ET-1 is a potent endogenous vasoconstrictor and proliferative cytokine. The ET-1 gene is translated to prepro-ET-1 which is then cleaved, by the action of an intracellular endopeptidase, to form the biologically inactive big ET-1. ET-converting enzymes further cleave this to form functional ET-1 (fig. 2). There are two ET receptor isoforms, termed type A (ET_A), located predominantly on vascular smooth muscle cells, and type B (ET_B), predominantly expressed on vascular endothelial cells but also on arterial smooth muscle. Activation of both receptor subtypes, when located on vascular smooth muscle, results in vasoconstriction and cell proliferation. In addition, the endothelial ET_B receptor mediates vasodilatation and clearance of ET-1.

ET levels are elevated in the plasma of patients with PH [42–45] and there is increased expression of ET-1 protein and mRNA in the endothelial cells of affected vessels in patients with PH [46]. In 21 patients with IPAH, elevated ET-1 levels correlated with right atrial pressure and pulmonary artery oxygen saturation [37]. It has been proposed that big ET-1 may be a more reliable indicator of activity of the ET-1 system, owing to a longer half-life and less tissue extraction [47]. RUBENS *et al*. [43] found significantly elevated plasma ET-1 and big ET-1 levels in 16 patients with IPAH; plasma ET-1 and big ET-1 showed a significant positive correlation with pulmonary vascular resistance and mean pulmonary artery pressure, and a significant negative correlation with cardiac output and cardiac index and, hence, disease severity. There was also a strong negative correlation between ET-1 and big ET-1 and the 6MWT. The ratio of big ET-1 in the radial artery to the pulmonary artery decreases after inhalation of iloprost [48]: it is suggested that either increased pulmonary clearance or decreased production accounts for this. This suggests a potential role in monitoring response to therapy, though subsequent work in this area has yet to be published. There are no published data on the use of ET-1 in screening. The ratio of plasma ET-1 to its related peptide ET-3 not only correlates with haemodynamic and clinical indices in PAH, but is also associated with prognosis [49]. A number of potential confounders must be considered when interpreting ET-1 levels: African ethnicity, male sex and older age are associated with a raised plasma ET-1; while angiotensin-converting enzyme inhibitors, statins, β-blockers and vasodilators reduce the level of ET-1 in plasma [50].

Uric acid

Uric acid is the final oxidation product of purine metabolism. Serum urate levels may be increased in conditions of impaired oxidative metabolism. Elevated uric acid levels have long been known to be a poor prognostic sign in acute illness [51]. Several studies have demonstrated that an elevated urate level in PH correlates with severity of disease [52–56]. In one study, 90 patients with IPAH underwent right heart catheterisation and serum uric acid estimation, and were then followed for a mean of 31 months [54]. Serum urate was independently related to mortality on multivariate Cox proportional hazards analysis. Kaplan–Meier survival curves demonstrated that patients with high serum uric acid had a significantly higher mortality rate than those with low serum uric acid. Serum urate levels decrease on the successful treatment with prostacyclin of patients with IPAH [53]. Urate levels are dependent on age and sex, and are affected by renal impairment and diuretic therapy; hence, their interpretation may be difficult in some patients.

Troponin T

Cardiac troponins are regulatory proteins of the thin actin filaments of cardiac muscle. Disruption of the cardiac myocyte membrane causes their release and they can then be detected by highly sensitive assays in the peripheral blood. Troponin T and I are well-established prognostic markers in acute coronary syndromes [57]. One study has linked troponin T to poor prognosis of PH [58]. Patients with severe PH of varying classes, with detectable troponin T, had higher heart rates, lower mixed venous oxygen saturation, higher NT-proBNP and shorter 6MWT. They had significantly higher mortality at 6, 12 and 24 months. Troponin became undetectable with successful treatment and returned as disease progressed, although this was shown only in a small group of patients. The authors suggested that monitoring serum troponin T might aid in the timing of management decisions, such as listing for lung transplantation. As with the natriuretic peptides, elevated troponin levels represent more advanced disease, since they are indicative of myocardial ischaemia. They cannot be expected, therefore, to be a sensitive marker of early disease. Interpretation of troponin T levels in PH may be confounded by concurrent left heart disease and renal impairment.

Nitric oxide

Nitric oxide is produced by the enzyme nitric oxide synthase (NOS) in endothelial cells by the conversion of L-arginine to L-citruline and nitric oxide (NO). It diffuses into adjacent vascular smooth muscle cells and binds to soluble guanylate cyclase, stimulating the production of cyclic guanosine monophosphate (cGMP), resulting in muscle relaxation [59]. NO is measurable in exhaled air [60]; it is now accepted as reflecting airway inflammation and represents pulmonary NO production. It comes primarily from airway epithelial cells with a component from the pulmonary vasculature [61]. Exhaled NO (eNO) has been shown to be lower in patients with IPAH [62–64], although there are conflicting studies that show either no difference in eNO compared with controls [65, 66] or an increased level [67]. These divergent results could be explained by methodological differences. When measured in the bronchoalveolar lavage fluid of patients with IPAH, biochemical reaction products of NO (nitrate, nitrite and *S*-nitrosothiol proteins) are significantly lower than in control patients and correlate inversely with pulmonary artery pressures and duration of PAH [63]. eNO levels increase after initiation of intravenous [64] and inhaled [65] prostacyclin therapy, and also after established treatment with bosentan, a nonselective ET receptor antagonist [62]. In interpreting eNO results, the physician must take into account a number of potential confounding factors including age, sex, atopy, infection and some drugs, including some used in the treatment of PH, such as L-arginine [68].

Asymmetric dimethylarginine

There has recently been increasing interest in asymmetric dimethylarginine (ADMA) as a marker and potential mediator

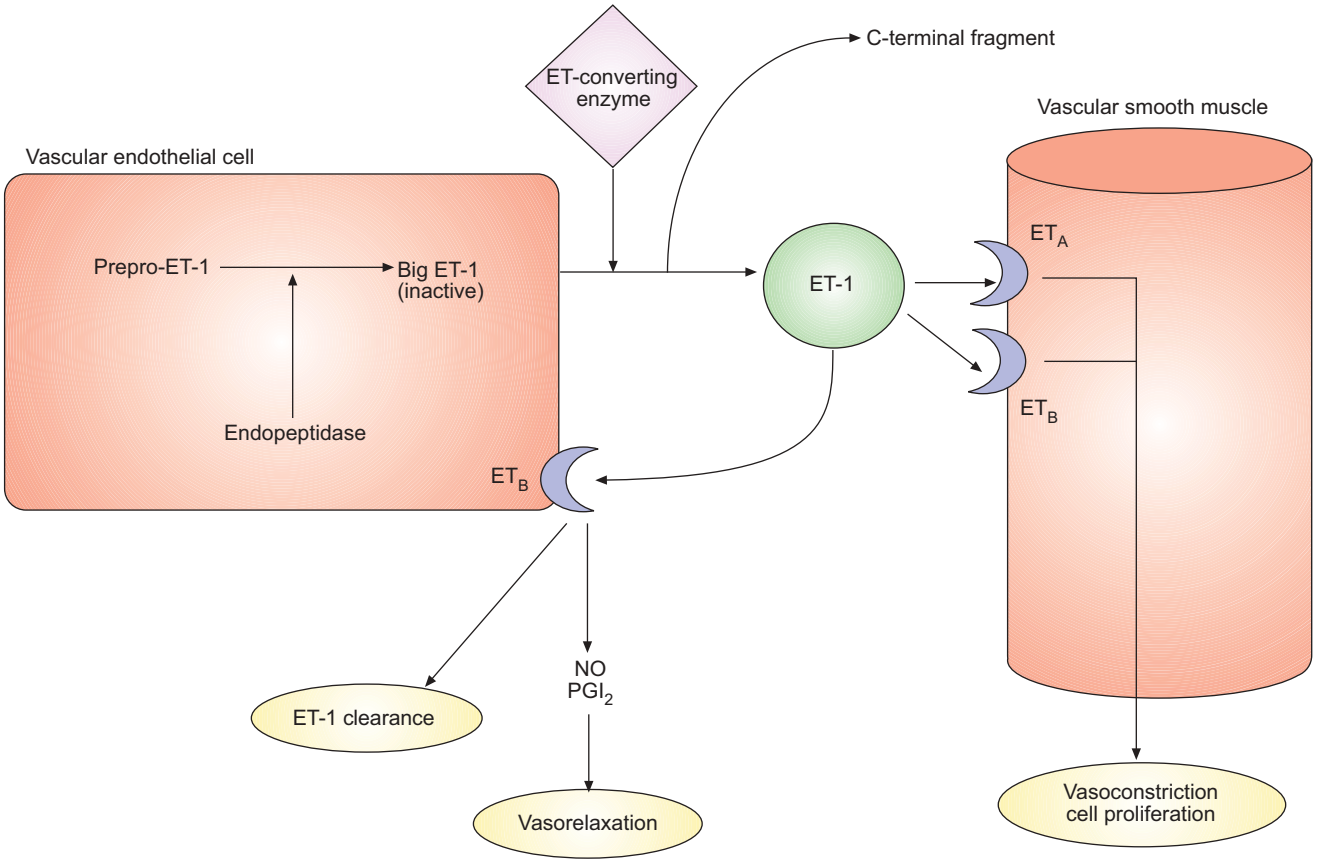

FIGURE 2. Simplified schematic of the endothelin (ET) system. Prepro-ET-1 is cleaved to inactive big ET-1 and then further cleaved to form active ET-1. This acts on vascular smooth muscle *via* the ET_A and ET_B receptors, causing vasoconstriction and cell proliferation, and on endothelial cells *via* ET_B receptors, releasing nitric oxide (NO) and prostacyclin (PGI_2), causing vasorelaxation.

of endothelial dysfunction in pulmonary vascular disease [69]. A potent competitive inhibitor of NOS, ADMA is derived from the catabolism of proteins containing methylated arginine residues. ADMA is excreted by the kidneys (and, hence, accumulates in renal failure) or is metabolised by the enzyme dimethylarginine dimethylaminohydrolase, whose activity is inhibited by homocysteine [70].

As a biomarker, ADMA has been evaluated in several different classes of PH. In IPAH, plasma levels are significantly higher than in healthy, matched controls [71]. In such patients, plasma ADMA correlates positively with right atrial pressure, and negatively with mixed venous oxygen saturation, stroke volume, cardiac index and survival. On stepwise multiple regression analysis, ADMA is an independent predictor of mortality and, using Kaplan–Meier survival curves, patients with supramedian ADMA levels have significantly worse survival than those with inframedian levels.

In patients with congenital heart disease and PH, plasma ADMA is higher when compared with patients with congenital heart disease but normal pulmonary pressures, and to controls [72].

In a recent study of 135 patients with chronic thromboembolic PH, plasma ADMA was measured at the time of initial right heart catheterisation [73] and levels were reanalysed in patients who underwent pulmonary endarterectomy. ADMA was significantly elevated in patients compared with controls and correlated with a number of haemodynamic parameters. Following pulmonary endarterectomy, ADMA levels fell to a range similar to the healthy controls. By receiver operating curve analysis, ADMA predicted death with a sensitivity of 81.1% and a specificity of 79.3% at a cut-off value of 0.64 $\mu mol \cdot L^{-1}$. The authors suggested that this value could serve as a surrogate marker for small-vessel arteriopathy in chronic thromboembolic PH.

cGMP

cGMP is an intracellular second messenger of NO, bradykinin and the natriuretic peptides [74]. It is produced by the activation of the enzyme guanylate cyclase and is an indirect marker of natriuretic peptide or NO production. Urinary cGMP levels are significantly higher in severe IPAH compared with patients with respiratory diseases without PH or normal healthy controls [75]; concentrations are higher in patients with severe haemodynamic impairment and inversely correlate with cardiac index and mixed venous oxygen saturation and, hence, may provide an indicator of disease severity [75]. Plasma cGMP levels are higher in patients with PH and decrease after inhalation of iloprost [38]. In 20 patients with PAH of differing aetiologies, a highly significant correlation between baseline plasma cGMP and pulmonary vascular resistance has been observed ($r=0.62$, $p<0.0001$) [36]. In these

patients, a marked increase in cGMP levels occurred in response to NO inhalation, but the magnitude of this response did not discriminate between responders and nonresponders. Hence, cGMP measurement could not be substituted for right heart catheterisation when testing for NO responsiveness.

D-dimer

In situ thrombosis is a prominent finding in patients with PAH [76]. D-dimer, as measured by the ELISA method, is a specific marker for cross-linked fibrin and may represent microvascular thrombosis. In patients with IPAH, D-dimer has been shown to be elevated compared with controls [77, 78] and correlates with disease severity, as measured by NYHA class, resting oxygen saturation and pulmonary artery pressure. D-dimer also correlated inversely with survival at 1 yr. However, these results remain to be validated in a larger cohort of patients.

Serotonin

Serotonin is a pulmonary vasoconstrictor and vascular smooth muscle mitogen [79]. It is released from pulmonary neuroendocrine cells and neuroepithelial bodies distributed throughout the airways. Elevated levels have been demonstrated in patients with IPAH [80] with a concomitant decrease in the serotonin content of platelets, giving a normal whole blood serotonin level. This may be explained by abnormal handling of serotonin by platelets. KÉREVEUR *et al*. [81] also found an elevated serotonin level in patients with IPAH compared with controls, and this positively correlated with total pulmonary resistance. However, it was not found to be a predictive marker of PAH severity, and its evolution was independent of clinical and haemodynamic status. Despite a therapeutic benefit, treatment with the potent anti-aggregating agent epoprostenol did not prevent further increases in plasma serotonin.

Plasma von Willebrand factor

Plasma von Willebrand factor (vWF) is a large glycoprotein synthesised mainly in endothelial cells. As the carrier for coagulation factor VIII, it plays a role in platelet aggregation and adhesion at sites of vascular injury. Elevated plasma vWF and its antigen (vWF:Ag) have been used as markers of endothelial cell injury in a variety of conditions. Levels of vWF:Ag are elevated in PH [82, 83] and baseline vWF:Ag correlates with the risk of death in the subsequent year [83]. In another study, vWF was also found to be elevated in severe PAH, and paralleled improvements in haemodynamics on prostacyclin therapy, although this was only in 10 patients [84].

APPLICATIONS

Screening

One of the requirements of a screening programme is that, once the condition is found, an effective treatment or intervention exists. This is now the case with recent therapeutic advances in PAH. The incidence of PAH in the general population is so low that this obviates the need for a general screening programme. However, there are certain high-risk groups in which the likelihood of PAH is greater, and in these individuals there is a need for screening. The gold standard for diagnosis of PH is right heart catheterisation, which remains mandatory in establishing the presence of disease. However, it is an invasive procedure and is impractical as a screening test. Doppler echocardiography is less sensitive and specific than cardiac catheterisation but is more practicable and less invasive. The sensitivity of echocardiography can be improved by exercise during the examination, and by the additional measurement of a biomarker. Current recommendations advise Doppler echocardiography for patients in recognised high-risk groups in circumstances where diagnosis could lead to further evaluation and/or change in management [4]. One such high-risk group is patients with limited scleroderma, in whom the prevalence of PAH is estimated to be $\geqslant$12% [26]. NT-proBNP has been evaluated as a marker of early PAH in patients with systemic sclerosis [32, 33]. NT-proBNP has also been used to screen for PH in patients with sickle cell disease [34]. BNP may be useful in predicting PH in patients with pulmonary fibrosis [15].

Prognosis

Assessment of prognosis in patients with PH is important, as it influences both medical therapy and, where appropriate, timing of referral for lung transplantation. A combination of different factors must be taken into consideration when estimating survival, including the NYHA functional class, the 6MWT and cardiac catheter or echocardiographic indices [85]. A number of studies have sought to define the prognostic value of biomarkers in PH. Several markers have been shown to be independent predictors of mortality in PAH, including serum uric acid [54], BNP [10] and NT-proBNP [28]. Cardiac troponin T is an independent predictor of mortality in patients with PH of various classes [58], and in sickle cell disease elevated NT-proBNP is a major risk factor for death [34]. Plasma vWF [83] and D-dimer [77] have also been shown to predict mortality in patients with PH. A recent retrospective study of patients with IPAH demonstrated that a low arterial carbon dioxide tension ($<$4.25 kPa) was an independent prognostic marker with similar predictive value to 6MWT and right atrial pressure [86]. Growth differentiation factor-15, a member of the transforming growth factor-β superfamily, has been shown to be of prognostic value in patients with non-ST elevation acute coronary syndrome [87] and heart failure [88]. Preliminary results show that this marker may be of similar value in patients with PH (M.M. Hoeper, Hanover Medical School, Hanover, Germany; personal communication).

Response to therapy

Instead of frequent repetition of right heart catheterisation and echocardiography, it is more practicable to use functional measures such as estimation of NYHA/WHO functional class, or the 6MWT, to follow the course of a patient's disease. However, in some conditions there may be coexistent features of the disease that may influence these measures and thus they may not truly represent a patient's clinical state, *e.g.* musculoskeletal involvement in systemic sclerosis [33]. Biomarkers can potentially offer additional information to the clinician for monitoring the efficacy of treatment and the patient's clinical course. Serum uric acid levels decrease with successful treatment with prostacyclin of patients with IPAH [54]. Exhaled NO may be useful in the long-term monitoring of patients on prostacyclin [89] and bosentan [62].

LIMITATIONS

For a biomarker to become accepted in clinical use, its utility should be consistently demonstrated in large, prospective

studies. This has been achieved for biomarkers in the context of acute coronary syndrome [90] and heart failure [91], in which studies typically involve hundreds or even thousands of subjects. However, such data do not exist for biomarkers in PH: most of the studies presented have <50 subjects. Some studies suggest cut-off values, with reference to receiver operating characteristic analysis, but extrapolation of these figures to the wider PH population must be attempted with caution, given the small numbers of patients from which they have been derived. In view of the small numbers of patients with PH available for any one unit to study, relative to acute coronary syndrome or left heart failure, for example, it may be that a collaborative, multicentre approach to biomarker analysis will become the established future means of validating biomarkers in what remains an uncommon disease.

Many of the biomarkers are subject to multiple confounding variables, *e.g.* renal failure or left heart disease, and studies typically exclude such patients. This may affect the applicability of these results to a broader, ''real-life'' population.

The temporal characteristics of biomarkers need to be taken into consideration when assessing their utility. Many of the currently available markers, *e.g.* BNP/NT-proBNP and troponin, could be considered to be ''late'' markers of disease, since they signify high ventricular wall stress and ischaemia, respectively. A ''normal'' level of these markers would not, therefore, exclude the presence of early disease.

CONCLUSION

In recent years there have been significant advances in the understanding of the pathophysiology of pulmonary arterial hypertension, and there have been dramatic improvements in available therapies [92]. There is increasing interest in the use of biomarkers as a means of screening and diagnosis, for delineating the severity and prognosis of disease, and for monitoring the course of pulmonary arterial hypertension and its response to therapy. An ideal biomarker would be quick, inexpensive and easy to measure; it would be highly reproducible and would be broadly applicable across a range of different disease classes, with no confounding effect from comorbidities, age, sex, *etc*. It would be highly sensitive and specific and a change in the value of the marker would represent a predictable change in the patient's clinical condition. None of the markers discussed in the present article can be described as ideal, but there is an increasing volume of evidence in support of their use in clinical practice. Some of them, particularly brain natriuretic peptide, N-terminal pro-brain natriuretic peptide and, perhaps, troponin T, may soon become part of the standard work-up and follow-up of patients with pulmonary arterial hypertension.

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REFERENCES

1 Simonneau G, Galie N, Rubin LJ, *et al*. Clinical classification of pulmonary hypertension. *J Am Coll Cardiol* 2004; 43: Suppl. 12, 5S–12S.
2 Peacock AJ. Treatment of pulmonary hypertension. *BMJ* 2003; 326: 835–836.
3 D'Alonzo GE, Barst RJ, Ayres SM, *et al*. Survival in patients with primary pulmonary hypertension. Results from a national prospective registry. *Ann Intern Med* 1991; 115: 343–349.
4 Barst RJ, McGoon M, Torbicki A, *et al*. Diagnosis and differential assessment of pulmonary arterial hypertension. *J Am Coll Cardiol* 2004; 43: Suppl. 12, 40S–47S.
5 Hoeper MM, Oudiz RJ, Peacock A, *et al*. End points and clinical trial designs in pulmonary arterial hypertension: clinical and regulatory perspectives. *J Am Coll Cardiol* 2004; 43: Suppl. 12, 48S–55S.
6 Martin KB, Klinger JR, Rounds SI. Pulmonary arterial hypertension: new insights and new hope. *Respirology* 2006; 11: 6–17.
7 McGoon M, Gutterman D, Steen V, *et al*. Screening, early detection, and diagnosis of pulmonary arterial hypertension: ACCP evidence-based clinical practice guidelines. *Chest* 2004; 126: Suppl. 1, 14S–34S.
8 Yap LB, Mukerjee D, Timms PM, Ashrafian H, Coghlan JG. Natriuretic peptides, respiratory disease, and the right heart. *Chest* 2004; 126: 1330–1336.
9 de Lemos JA, McGuire DK, Drazner MH. B-type natriuretic peptide in cardiovascular disease. *Lancet* 2003; 362: 316–322.
10 Nagaya N, Nishikimi T, Uematsu M, *et al*. Plasma brain natriuretic peptide as a prognostic indicator in patients with primary pulmonary hypertension. *Circulation* 2000; 102: 865–870.
11 Wilkins MR, Paul GA, Strange JW, *et al*. Sildenafil *versus* Endothelin Receptor Antagonist for Pulmonary Hypertension (SERAPH) study. *Am J Respir Crit Care Med* 2005; 171: 1292–1297.
12 Nagaya N, Nishikimi T, Uematsu M, *et al*. Secretion patterns of brain natriuretic peptide and atrial natriuretic peptide in patients with or without pulmonary hypertension complicating atrial septal defect. *Am Heart J* 1998; 136: 297–301.
13 Elstein D, Nir A, Klutstein M, Rudensky B, Zimran A. C-reactive protein and NT-proBNP as surrogate markers for pulmonary hypertension in Gaucher disease. *Blood Cells Mol Dis* 2005; 34: 201–205.
14 Bozkanat E, Tozkoparan E, Baysan O, Deniz O, Ciftci F, Yokusoglu M. The significance of elevated brain natriuretic peptide levels in chronic obstructive pulmonary disease. *J Int Med Res* 2005; 33: 537–544.
15 Leuchte HH, Neurohr C, Baumgartner R, *et al*. Brain natriuretic peptide and exercise capacity in lung fibrosis and pulmonary hypertension. *Am J Respir Crit Care Med* 2004; 170: 360–365.
16 Nagaya N, Ando M, Oya H, *et al*. Plasma brain natriuretic peptide as a noninvasive marker for efficacy of pulmonary thromboendarterectomy. *Ann Thorac Surg* 2002; 74: 180–184.
17 Nagaya N, Sasaki N, Ando M, *et al*. Prostacyclin therapy before pulmonary thromboendarterectomy in patients with chronic thromboembolic pulmonary hypertension. *Chest* 2003; 123: 338–343.
18 Kucher N, Printzen G, Goldhaber SZ. Prognostic role of brain natriuretic peptide in acute pulmonary embolism. *Circulation* 2003; 107: 2545–2547.

19 ten Wolde M, Tulevski, II, Mulder JW, *et al*. Brain natriuretic peptide as a predictor of adverse outcome in patients with pulmonary embolism. *Circulation* 2003; 107: 2082–2084.
20 Ishii J, Nomura M, Ito M, *et al.* Plasma concentration of brain natriuretic peptide as a biochemical marker for the evaluation of right ventricular overload and mortality in chronic respiratory disease. *Clin Chim Acta* 2000; 301: 19–30.
21 Leuchte HH, Holzapfel M, Baumgartner RA, *et al.* Clinical significance of brain natriuretic peptide in primary pulmonary hypertension. *J Am Coll Cardiol* 2004; 43: 764–770.
22 Leuchte HH, Holzapfel M, Baumgartner RA, Neurohr C, Vogeser M, Behr J. Characterization of brain natriuretic peptide in long-term follow-up of pulmonary arterial hypertension. *Chest* 2005; 128: 2368–2374.
23 Nagaya N, Nishikimi T, Okano Y, *et al.* Plasma brain natriuretic peptide levels increase in proportion to the extent of right ventricular dysfunction in pulmonary hypertension. *J Am Coll Cardiol* 1998; 31: 202–208.
24 Leuchte HH, Baumgartner RA, Nounou ME, *et al.* Brain natriuretic peptide is a prognostic parameter in chronic lung disease. *Am J Respir Crit Care Med* 2006; 173: 744–750.
25 Hammerer-Lercher A, Neubauer E, Muller S, Pachinger O, Puschendorf B, Mair J. Head-to-head comparison of N-terminal pro-brain natriuretic peptide, brain natriuretic peptide and N-terminal pro-atrial natriuretic peptide in diagnosing left ventricular dysfunction. *Clin Chim Acta* 2001; 310: 193–197.
26 Mukerjee D, Yap LB, Holmes AM, *et al.* Significance of plasma N-terminal pro-brain natriuretic peptide in patients with systemic sclerosis-related pulmonary arterial hypertension. *Respir Med* 2003; 97: 1230–1236.
27 Andreassen AK, Wergeland R, Simonsen S, Geiran O, Guevara C, Ueland T. N-terminal pro-B-type natriuretic peptide as an indicator of disease severity in a heterogeneous group of patients with chronic precapillary pulmonary hypertension. *Am J Cardiol* 2006; 98: 525–529.
28 Fijalkowska A, Kurzyna M, Torbicki A, *et al.* Serum N-terminal brain natriuretic peptide as a prognostic parameter in patients with pulmonary hypertension. *Chest* 2006; 129: 1313–1321.
29 Souza R, Jardim C, Martins B, *et al.* Effect of bosentan treatment on surrogate markers in pulmonary arterial hypertension. *Curr Med Res Opin* 2005; 21: 907–911.
30 Souza R, Bogossian HB, Humbert M, *et al.* N-terminal-pro-brain natriuretic peptide as a haemodynamic marker in idiopathic pulmonary arterial hypertension. *Eur Respir J* 2005; 25: 509–513.
31 Souza R, Jardim C, Julio Cesar Fernandes C, Silveira Lapa M, Rabelo R, Humbert M. NT-proBNP as a tool to stratify disease severity in pulmonary arterial hypertension. *Respir Med* 2007; 101: 69–75.
32 Allanore Y, Borderie D, Meune C, *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* 2003; 48: 3503–3508.
33 Williams MH, Handler CE, Akram R, *et al.* Role of N-terminal brain natriuretic peptide (NT-proBNP) in scleroderma-associated pulmonary arterial hypertension. *Eur Heart J* 2006; 27: 1485–1494.
34 Machado RF, Anthi A, Steinberg MH, *et al.* N-terminal pro-brain natriuretic peptide levels and risk of death in sickle cell disease. *JAMA* 2006; 296: 310–318.
35 Adnot S, Chabrier PE, Andrivet P, *et al.* Atrial natriuretic peptide concentrations and pulmonary hemodynamics in patients with pulmonary artery hypertension. *Am Rev Respir Dis* 1987; 136: 951–956.
36 Ghofrani HA, Wiedemann R, Rose F, *et al.* Lung cGMP release subsequent to NO inhalation in pulmonary hypertension: responders *versus* nonresponders. *Eur Respir J* 2002; 19: 664–671.
37 Nootens M, Kaufmann E, Rector T, *et al.* Neurohormonal activation in patients with right ventricular failure from pulmonary hypertension: relation to hemodynamic variables and endothelin levels. *J Am Coll Cardiol* 1995; 26: 1581–1585.
38 Wiedemann R, Ghofrani HA, Weissmann N, *et al.* Atrial natriuretic peptide in severe primary and nonprimary pulmonary hypertension: response to iloprost inhalation. *J Am Coll Cardiol* 2001; 38: 1130–1136.
39 Park MH, Scott RL, Uber PA, Ventura HO, Mehra MR. Usefulness of B-type natriuretic peptide as a predictor of treatment outcome in pulmonary arterial hypertension. *Congest Heart Fail* 2004; 10: 221–225.
40 Munagala VK, Burnett JC Jr, Redfield MM. The natriuretic peptides in cardiovascular medicine. *Curr Probl Cardiol* 2004; 29: 707–769.
41 Leuchte HH, El Nounou M, Tuerpe JC, *et al.* N-Terminal pro-brain natriuretic peptide and renal insufficiency as predictors of mortality in pulmonary hypertension. *Chest* 2007; 131: 402–409.
42 Cacoub P, Dorent R, Nataf P, *et al.* Endothelin-1 in the lungs of patients with pulmonary hypertension. *Cardiovasc Res* 1997; 33: 196–200.
43 Rubens C, Ewert R, Halank M, *et al.* Big endothelin-1 and endothelin-1 plasma levels are correlated with the severity of primary pulmonary hypertension. *Chest* 2001; 120: 1562–1569.
44 Stewart DJ, Levy RD, Cernacek P, Langleben D. Increased plasma endothelin-1 in pulmonary hypertension: marker or mediator of disease? *Ann Intern Med* 1991; 114: 464–469.
45 Cody RJ, Haas GJ, Binkley PF, Capers Q, Kelley R. Plasma endothelin correlates with the extent of pulmonary hypertension in patients with chronic congestive heart failure. *Circulation* 1992; 85: 504–509.
46 Giaid A, Yanagisawa M, Langleben D, *et al.* Expression of endothelin-1 in the lungs of patients with pulmonary hypertension. *N Engl J Med* 1993; 328: 1732–1739.
47 Hemsen A, Ahlborg G, Ottosson-Seeberger A, Lundberg JM. Metabolism of big endothelin-1 (1–38) and (22–38) in the human circulation in relation to production of endothelin-1 (1–21). *Regul Pept* 1995; 55: 287–297.
48 Wilkens H, Bauer M, Forestier N, *et al.* Influence of inhaled iloprost on transpulmonary gradient of big endothelin in patients with pulmonary hypertension. *Circulation* 2003; 107: 1509–1513.
49 Montani D, Souza R, Binkert C, *et al*. Endothelin-1/endothelin-3 ratio: a potential prognostic factor of pulmonary arterial hypertension. *Chest* 2007; 131: 101–108.
50 Shah R. Endothelins in health and disease. *Eur J Intern Med* 2007; 18: 272–282.

51 Woolliscroft JO, Colfer H, Fox IH. Hyperuricemia in acute illness: a poor prognostic sign. *Am J Med* 1982; 72: 58–62.
52 Bendayan D, Shitrit D, Ygla M, Huerta M, Fink G, Kramer MR. Hyperuricemia as a prognostic factor in pulmonary arterial hypertension. *Respir Med* 2003; 97: 130–133.
53 Hoeper MM, Hohlfeld JM, Fabel H. Hyperuricaemia in patients with right or left heart failure. *Eur Respir J* 1999; 13: 682–685.
54 Nagaya N, Uematsu M, Satoh T, *et al.* Serum uric acid levels correlate with the severity and the mortality of primary pulmonary hypertension. *Am J Respir Crit Care Med* 1999; 160: 487–492.
55 Voelkel MA, Wynne KM, Badesch DB, Groves BM, Voelkel NF. Hyperuricemia in severe pulmonary hypertension. *Chest* 2000; 117: 19–24.
56 Wensel R, Opitz CF, Anker SD, *et al.* Assessment of survival in patients with primary pulmonary hypertension: importance of cardiopulmonary exercise testing. *Circulation* 2002; 106: 319–324.
57 Antman EM. Decision making with cardiac troponin tests. *N Engl J Med* 2002; 346: 2079–2082.
58 Torbicki A, Kurzyna M, Kuca P, *et al.* Detectable serum cardiac troponin T as a marker of poor prognosis among patients with chronic precapillary pulmonary hypertension. *Circulation* 2003; 108: 844–848.
59 Barnes PJ, Kharitonov SA. Exhaled nitric oxide: a new lung function test. *Thorax* 1996; 51: 233–237.
60 Barnes PJ, Belvisi MG. Nitric oxide and lung disease. *Thorax* 1993; 48: 1034–1043.
61 Yates DH. Role of exhaled nitric oxide in asthma. *Immunol Cell Biol* 2001; 79: 178–190.
62 Girgis RE, Champion HC, Diette GB, Johns RA, Permutt S, Sylvester JT. Decreased exhaled nitric oxide in pulmonary arterial hypertension: response to bosentan therapy. *Am J Respir Crit Care Med* 2005; 172: 352–357.
63 Kaneko FT, Arroliga AC, Dweik RA, *et al.* Biochemical reaction products of nitric oxide as quantitative markers of primary pulmonary hypertension. *Am J Respir Crit Care Med* 1998; 158: 917–923.
64 Ozkan M, Dweik RA, Laskowski D, Arroliga AC, Erzurum SC. High levels of nitric oxide in individuals with pulmonary hypertension receiving epoprostenol therapy. *Lung* 2001; 179: 233–243.
65 Forrest IA, Small T, Corris PA. Effect of nebulized epoprostenol (prostacyclin) on exhaled nitric oxide in patients with pulmonary hypertension due to congenital heart disease and in normal controls. *Clin Sci (Lond)* 1999; 97: 99–102.
66 Riley MS, Porszasz J, Miranda J, Engelen MP, Brundage B, Wasserman K. Exhaled nitric oxide during exercise in primary pulmonary hypertension and pulmonary fibrosis. *Chest* 1997; 111: 44–50.
67 Archer SL, Djaballah K, Humbert M, *et al.* Nitric oxide deficiency in fenfluramine- and dexfenfluramine-induced pulmonary hypertension. *Am J Respir Crit Care Med* 1998; 158: 1061–1067.
68 American Thoracic Society, European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005; 171: 912–930.
69 Cooke JP. A novel mechanism for pulmonary arterial hypertension? *Circulation* 2003; 108: 1420–1421.
70 Stuhlinger MC, Tsao PS, Her J-H, Kimoto M, Balint RF, Cooke JP. Homocysteine impairs the nitric oxide synthase pathway: role of asymmetric dimethylarginine. *Circulation* 2001; 104: 2569–2575.
71 Kielstein JT, Bode-Boger SM, Hesse G, *et al.* Asymmetrical dimethylarginine in idiopathic pulmonary arterial hypertension. *Arterioscler Thromb Vasc Biol* 2005; 25: 1414–1418.
72 Gorenflo M, Zheng C, Werle E, Fiehn W, Ulmer HE. Plasma levels of asymmetrical dimethyl-L-arginine in patients with congenital heart disease and pulmonary hypertension. *J Cardiovasc Pharmacol* 2001; 37: 489–492.
73 Skoro-Sajer N, Mittermayer F, Panzenboeck A, *et al.* Asymmetric dimethylarginine is increased in chronic thromboembolic pulmonary hypertension. *Am J Respir Crit Care Med* 2007; 176: 1154–1160.
74 McDonald LJ, Murad F. Nitric oxide and cyclic GMP signaling. *Proc Soc Exp Biol Med* 1996; 211: 1–6.
75 Bogdan M, Humbert M, Francoual J, *et al.* Urinary cGMP concentrations in severe primary pulmonary hypertension. *Thorax* 1998; 53: 1059–1062.
76 Rubin LJ. Primary pulmonary hypertension. *N Engl J Med* 1997; 336: 111–117.
77 Shitrit D, Bendayan D, Bar-Gil-Shitrit A, *et al.* Significance of a plasma D-dimer test in patients with primary pulmonary hypertension. *Chest* 2002; 122: 1674–1678.
78 Shitrit D, Bendayan D, Rudensky B, *et al.* Elevation of ELISA D-dimer levels in patients with primary pulmonary hypertension. *Respiration* 2002; 69: 327–329.
79 Lee SL, Wang WW, Fanburg BL. Superoxide as an intermediate signal for serotonin-induced mitogenesis. *Free Radic Biol Med* 1998; 24: 855–858.
80 Herve P, Launay JM, Scrobohaci ML, *et al.* Increased plasma serotonin in primary pulmonary hypertension. *Am J Med* 1995; 99: 249–254.
81 Kéreveur A, Callebert J, Humbert M, *et al.* High plasma serotonin levels in primary pulmonary hypertension. Effect of long-term epoprostenol (prostacyclin) therapy. *Arterioscler Thromb Vasc Biol* 2000; 20: 2233–2239.
82 Friedman R, Mears JG, Barst RJ. Continuous infusion of prostacyclin normalizes plasma markers of endothelial cell injury and platelet aggregation in primary pulmonary hypertension. *Circulation* 1997; 96: 2782–2784.
83 Lopes AA, Maeda NY. Circulating von Willebrand factor antigen as a predictor of short-term prognosis in pulmonary hypertension. *Chest* 1998; 114: 1276–1282.
84 Veyradier A, Nishikubo T, Humbert M, *et al.* Improvement of von Willebrand factor proteolysis after prostacyclin infusion in severe pulmonary arterial hypertension. *Circulation* 2000; 102: 2460–2462.
85 McLaughlin VV, Presberg KW, Doyle RL, *et al.* Prognosis of pulmonary arterial hypertension: ACCP evidence-based clinical practice guidelines. *Chest* 2004; 126: Suppl. 1, 78S–92S.
86 Hoeper MM, Pletz MW, Golpon H, Welte T. Prognostic value of blood gas analyses in patients with idiopathic pulmonary arterial hypertension. *Eur Respir J* 2007; 29: 944–950.
87 Wollert KC, Kempf T, Peter T, *et al.* Prognostic value of growth-differentiation factor-15 in patients with non-ST-elevation acute coronary syndrome. *Circulation* 2007; 115: 962–971.

88 Kempf T, von Haehling S, Peter T, *et al.* Prognostic utility of growth differentiation factor-15 in patients with chronic heart failure. *J Am Coll Cardiol* 2007; 50: 1054–1060.
89 Machado RF, Londhe Nerkar MV, Dweik RA, *et al.* Nitric oxide and pulmonary arterial pressures in pulmonary hypertension. *Free Radic Biol Med* 2004; 37: 1010–1017.
90 Antman EM, Tanasijevic MJ, Thompson B, *et al.* Cardiac-specific troponin I levels to predict the risk of mortality in patients with acute coronary syndromes. *N Engl J Med* 1996; 335: 1342–1349.
91 Maisel AS, Krishnaswamy P, Nowak RM, *et al.* Rapid measurement of B-Type natriuretic peptide in the emergency diagnosis of heart failure. *N Engl J Med* 2002; 347: 161–167.
92 Rubin LJ, Galie N. Pulmonary arterial hypertension: a look to the future. *J Am Coll Cardiol* 2004; 43: Suppl. 12, 89S–90S.